12th INTERNATIONAL CONGRESS OF PLANT PATHOLOGY

ICPP 2023

ONE HEALTH
for all plants, crops and trees

LYON

20-25 August, France

BOOK OF ABSTRACTS

ISPP INTERNATIONAL SOCIETY FOR PLANT PATHOLOGY

www.icpp2023.org

SFP Société Française de Phytopathologie

The International Society for Plant Pathology & the French Phytopathological Society

KEYNOTES SESSIONS

Plant Pathology in a One Health World

K1-1

PLANT HEALTH FOR ONE HEALTH IN CENTRAL & WEST AFRICA

PITA Justin. (1)

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Text

One Health is defined as the triad of human health, animal health, and the environment. While the latter is commonly considered the neglected component of the triad, plant health is usually excluded from the One Health concept. To address this important omission, the organizing committee of the 12th International Congress of Plant Pathology chose “Plant Pathology in a One Health world” as the theme of the 2023 conference, to promote the integration of plant health and plant pathology into the One Health concept. But how can this integration be achieved in Central and West Africa if plant health itself is not firmly rooted in the region? We will review the status of the One Health in 10 Central and West African countries and present the approach of the WAVE Regional Center of Excellence to boost Plant Health and One Health across Africa, through the management of transboundary plant pathogens.

K1-2

WHAT IS THE ADDED VALUE OF ONE HEALTH FOR PLANT HEALTH?

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Text

The One Health approach to understanding disease epidemiology and achieving surveillance and prevention is holistic all while currently focusing on zoonotic diseases. Plant health deploys principles of agroecology for holistic goals of plant health management. Nevertheless, One Health offers 4 knowledge challenges that could boost prospects for novel approaches to plant disease surveillance, prediction and prevention: i) uncovering reservoirs and revising pathogen/vector life histories, ii) elucidating drivers of virulence beyond direct host-pathogen interactions, iii) accounting for the natural highways of long distance dissemination, and iv) updating disease forecasts in the face of changing land use, cultivation practices and climate. The implementation of a One Health approach to

surveillance and prevention – for plant and zoonotic diseases alike - will require mobilization of tools to deal with the representation and accessibility of massive and heterogeneous data and knowledge; means for knowledge inference, data science, modelling, and pattern recognition; and multi-actor approaches that unite different sectors of society and different scientific disciplines. The key commonalities, where actors in the efforts to prevent zoonotic and plant disease can work together, are the need to build these tools and related infrastructure for a *bona fide* One Health approach to surveillance and prevention of human, animal and plant disease and sustainable management of biodiversity.

K1-3

ANTIMICROBIAL USE AND RESISTANCE IN PLANT AGRICULTURE: A ONE HEALTH PERSPECTIVE

TEMPELMAN Kim-Anh. (1), **KOUMBA Descartes. (1)**

(1) Food and Agriculture Organization of the United Nations, Rome, ITALY

Text
Antimicrobial Resistance (AMR) is among the top 10 global threats to human health with the highest burdens in low- and middle income countries. AMR in humans is linked to agricultural production of animals, fish and plants and is thereby a perfect example of an issue requiring a One Health approach.

Over the past decades, most AMR-related concerns were raised in the context of intensified animal protein production, including aquaculture, and pig and poultry production. More recently, the use of antimicrobials in crops is seen as an area that needs to be better understood and quantified.

Antimicrobials used in plant production and protection include antibiotics and fungicides. Fungicides are widely used to control fungal plant diseases and fungal resistance has triggered particular attention, due to the link with human health.

The Food and Agriculture Organization of the United Nations (FAO), is developing the International FAO Antimicrobial Resistance Monitoring platform (InFarm). This platform will collect data on antimicrobial resistance in animals, and antimicrobial use in plant production and protection. In parallel, FAO will launch the Reduce the need to use antimicrobials in farms initiative (RENOFARM) in 2023, and establish a list of antibiotics used in horticulture, ranking them according to their importance in plant health.

These new tools will support countries to make sound decisions to reduce AMU in crops and prevent or minimize the development of AMR.

K1-4

EMPOWERING AN INDIGENOUS PERSPECTIVE IN THE RESPONSE TO INVASIVE PATHOGENS

MARK-SHADBOLT Melanie. (1), LAMBERT Simon. (1), SHADBOLT Marcus-Rongowhitiao. (1), FORDYCE Phoebe. (1), HEIMLICK Micheal. (1)

(1) Te Tira Whakamtaki, Rangiora, NEW ZEALAND

Text
Many hundreds of pests and pathogens have been introduced, either deliberately or inadvertently, to Indigenous Peoples lands and waterways the world over. Unfortunately, the current biosecurity and eradication practices and processes implemented to address these incursions employ principles, values and knowledge that often undermine or even oppress local Indigenous Peoples and their extensive practices to sustainably manage biological heritage. So, whilst Indigenous Peoples have a millennia of information and experiences that are valuable in the often urgent response to invasive pests and pathogens, inclusive approaches are needed to ensure those experiences and information are able to inform and lead biosecurity events in a timely and effective manner.

At the same time, public attitudes to novel biosecurity management tactics such as toxins and gene editing need to be explored in advance of their deployment and ways of improving public engagement developed with social researchers and community leaders. We also need to consider ways to improve existing tools to increase the chances of successful eradication of new incursions. Greater use of advanced information technologies will move pest management towards “real time” control. Better understanding of the biology of pests, their interactions with ecosystems, and their impacts on assets that Indigenous Peoples value are required to ensure appropriate and just biosecurity strategies.

Food security in an unsecure future

K2-1

AGRIFOOD SYSTEMS FOR A FOOD AND NUTRITION SECURE WORLD: FROM EFFICIENCY TO RESILIENCE

GOVAERTS Bram. (1)

(1) CIMMYT, Mexico City, MEXICO

Text
The 4Cs – Climate Change, Conflict, COVID-19 and the Cost-of-living crisis – have brought about the worst food security crisis of the 21st Century. Dr. Bram Govaerts, Director General of CIMMYT will discuss strategies to overcome these challenges in his keynote presentation, “Agrifood Systems for a Food and Nutrition Secure World: From Efficiency to Resilience.” Govaerts will talk about early warning and surveillance systems as an important defense to help build resilience to external shocks in food insecure communities and regions. He will share success stories implementing systems that have effectively prevented the spread of prevalent and emerging pests and diseases in sub-Saharan Africa and South Asia. In addition, Govaerts will present CIMMYT’s work with national agricultural research systems and private sector partners on crop breeding programs that every year disseminate dozens

of disease resistant and climate resilient maize, wheat and dryland crop varieties where they are most needed to supplement surveillance efforts. He will describe ongoing efforts to promote the adoption of conservation agriculture-based sustainable intensification practices that transform food systems with a gender and social inclusion approach in the Global South. The training offered and the advisory systems supported by CIMMYT's work aim to empower women and offer more opportunities for fulfilling livelihoods to a new generation of farmers who will grow sustainably nutritious food for all.

K2-2

THE BEAUTY AND COMPLEXITY OF WHEAT DISEASE CONTROL

DOOHAN Fiona. (1)

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Text

Wheat has evolved distinct pathways, genes and systems to cope with particular pathogens. Pathogens such as *Fusarium* produce virulence factors, including the mycotoxin deoxynivalenol, to facilitate disease development. In turn, wheat has evolved genomic hotspots associated with disease responses and unique disease control strategies, including those involving taxonomically restricted genes and novel transcription factors. We now have the genomics tools to rapidly introgress these into commercial genotypes. Biocontrol also shows potential for the control of wheat diseases. Pathogens can have an intricate relationship with the host microbiome and endophytic microbes from wild crop relatives show potential for the control of diseases such as Fusarium head blight and Septoria tritici blotch. Now we are moving into an exciting era where we have the affordable tools to evaluate the relationships between the environment, crop biome, disease development and host genetic diversity across diverse ecosystems and this will give us new insights into plant-pathogen evolution and more refined solutions for disease control. Our work now moves to understanding the relationship between deoxynivalenol, wheat and the microbiome, with compelling indications that mycotoxins play an important role in regulating ecosystem interactions.

K2-3

CRISES ABOUND: HEALTH, CLIMATE, ENERGY, FOOD, PANDEMICS... HOW SUPERCOMPUTING, AI, AND LARGE-SCALE SYSTEMS BIOLOGY IN A ONEHEALTH FRAMEWORK CAN HELP ADDRESS THE MAJOR CHALLENGES WE ARE FACING.

JACOBSON Daniel. (1)

(1) Oak Ridge National Laboratory, Oak Ridge, UNITED STATES

Text

The recent flood of data generation has opened a new era of systems biology in which there are unprecedented opportunities to gain insights into complex biological systems. Integrated

biological models need to capture the higher order complexity of the interactions among cellular components. Solving such complex combinatorial problems will give us extraordinary levels of understanding of biological systems. The disease, traits or phenotypes of an organism, including its adaptation to its surrounding environment and the interactions with its microbiome, are the result of orchestrated, hierarchical, heterogeneous collections of expressed genomic variants regulated by and related to biotic and abiotic signals. However, the effects of these variants can be viewed as the result of historic selective pressure and current environmental as well as epigenetic interactions, and, as such, their co-occurrence can be seen as omics-wide associations in a number of different manners. We have developed supercomputing and explainable-AI approaches to find complex mechanisms responsible for all measurable phenotypes as well as an organism's ability to detect and modulate its microbiome. The result is progress towards a comprehensive systems biology model of an organism and how it has adapted to and responds to its abiotic and biotic environment which has applications in bioenergy, precision agriculture, ecosystem studies, precision medicine, and pandemic prevention among other disciplines.

Invasive and Emerging Plant Diseases

K3-1

EMERGING DISEASES IN THE VEGETABLE SECTOR: CHALLENGES AND PERSPECTIVES

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Text

The vegetable sector is vast and complex. The high volume of trade of products contributes to the vulnerability to contamination by pathogens of the goods in which production is often concentrated in relatively few locations and then marketed all over the world. The vegetable production sector is continuously interested by the emergence of new diseases. The main drivers of such epidemics are a) concentration of few crops and varieties; b) intensive cultivation systems; c) globalisation; d) just in time supply chains; e) climate change. In the vegetable sector new pathogens are often introduced into new areas throughout contaminated seeds. The leafy vegetables represent an interesting case study, also due to their dynamism. Fusarium wilts, Alternaria leaf spot and downy mildews are described as examples of how new pathogens or races can easily and quickly spread, causing severe losses. Reliable diagnostic tools help in controlling seed health, permitting seed treatment when needed. Such tools should be applied already at the industry level. The international trade of agricultural commodities makes the mediation efforts and cooperative research efforts across national barriers critical. The management of these diseases is challenging, since few fungicides are registered on these minor-use crops. However, they are generally grown under protection, often soilless, which is an environment helpful to implement innovative control strategies.

K3-3

RISK ASSESSMENT AND MANAGEMENT OF PESTS AND DISEASES IN THE EU: PAST AND PRESENT

POTTING Roel. (1)

(1) Netherlands Food and Consumer Safety Authority, Utrecht, NETHERLANDS

Text
The number of nonnative insects and pathogens is increasing and these may pose major threats to (agro)ecosystems. Pest risk analysis is the process of evaluating scientific evidence to determine whether a pathogenic agent is a pest, whether it should be regulated, and which official phytosanitary management measures should to be taken against it. Pest risk assessment is the evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences. In this presentation the evolution of plant health and pest risk assessment in the EU will be discussed.

A Global Plant Health Assessment (GPHA) of the state of Plant Health and its Impact on Ecosystem Services

K4-1

STATE AND EVOLUTION OF PLANT HEALTH GLOBALLY ACROSS PLANT SYSTEMS AND ECOREGIONS

BOVE Federica. (1)

(1) Università Cattolica del Sacro Cuore, Piacenza, ITALY

Text
This presentation is the first talk of the keynote session K4 "Global Plant Health Assessment (GPHA)". The GPHA is an initiative conducted under the aegis of the ISPP undertaken to contribute to the International Year of Plant Health. The general philosophy and the steps taken by the GPHA will be described. The Plant Systems assessed in the different Ecoregions of the world will be mapped. The overall results pertaining to the assessment of plant health status and evolution over the last 10 years will be outlined. Specific results for a limited number of Plant Systems (rice, potato, peri-urban horticulture and household gardens, softwood forests, and oak forests) will be described in more detail, allowing to highlight key findings. These include: plant health as affected by climate change, pathogen invasions, and the issue of pesticide misuse.

K4-2

SYNTHESIS AND IMPLICATIONS OF THE FINDINGS FROM THE GPHA

SAH Sonam. (1,2)

(1) G B Pant University of Agriculture and Teachnology, Pantnagar, INDIA; (2) ICAR-NIASM, Baramati, Pune, INDIA

Text

This presentation is the third talk of the keynote session K4 "Global Plant Health Assessment (GPHA)". This talk will first discuss the outcomes highlighted by the findings of the GPHA and its 26 reports for the [Plant System x Ecoregion] combinations that the project considered. This will be done with respect to the linkages between plant health and crop loss information, food security, biodiversity and conservation of species, climate change, pathogen invasions, and pesticide use and misuse. General insights gained from the GPHA initiative will then be highlighted. Links between the GPHA and the concept of One Health will be discussed. The notion of Ecosystem Services will be revisited and challenged. The talk will emphasise the fact that plant health is a common good, shared by all citizens across the world. The value of the GPHA specific design, as a collective action undertaken by a collective toward a common good, will be delineated. The GPHA recent as well as on-going activities, together with its outputs will be described. Perspectives will be offered, using the GPHA as an example of a collective action undertaken by a collective belonging to a Scientific Society toward common good.

K4-3

IMPACTS OF PLANT HEALTH ON THE SERVICES RENDERED BY PLANT SYSTEMS IN WORLD'S ECOREGIONS

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(1) Alliance of Bioversity International and CIAT Africa Hub – Senegal Office , Dakar, SENEGAL

Text

This presentation is the second talk of the keynote session K4 "Global Plant Health Assessment (GPHA)". The overall results pertaining to the assessment of the impact of disease on ecosystem services (provisioning, regulating, and cultural) and its evolution over the last 10 years will be outlined. Specific results for a limited number of Plant Systems (rice, potato, peri-urban horticulture and household gardens, softwood forests, and oak forests) will be described in more detail, allowing to highlight key findings of the results. These include: the paucity of hard data on crop losses caused by plant pathogens worldwide; the impact of plant diseases on food security; pathogen invasions threatening food security and biodiversity; climate change impacts on crops and crop losses; the challenges of pesticide misuse; and the conservation of plant species that are endangered by diseases, especially in the case of forest species.

Current Topics in Molecular Plant-Microbe Interactions

K5-1

REGULATION OF BACTERIAL GROWTH AND BEHAVIOR BY PLANT IMMUNITY

TSUDA Kenichi. (1)

(1) Huazhong Agricultural University, Wuhan, CHINA

Text

Plants are colonized by commensal, beneficial, and pathogenic bacteria that significantly affect plant health. Plants require bacterial colonization for their survival in nature, but ironically, some bacteria cause disease in plants, pointing to the dilemma that plants face. Therefore, the fundamental question is how plants regulate different bacteria for their benefit. However, we still lack fundamental knowledge of how plants actually regulate bacterial growth and behavior in plants. Through multi-omics and molecular genetics, we uncovered several mechanisms of how plant immunity regulates the growth of the bacterial pathogen Pseudomonas syringae. For instance, we found that plant immunity targets the bacterial iron acquisition system, the type III secretion system, and the bacterial protein MucD, all of which are required for virulence in plants but not their growth in vitro. More recently, we found that reactive oxygen species (ROS) generated by plants directly suppresses the type II secretion system (T2SS) of a potentially pathogenic commensal Xanthomonas isolated from healthy Arabidopsis thaliana plants. ROS-mediated inhibition of the T2SS converted the potentially harmful Xanthomonas into a commensal bacterium that protected plants against P. syringae. Thus, these results suggest that plant immunity targets the virulence mechanisms of bacteria to control their behavior in plants and turns them into beneficial bacteria in some cases.

K5-2

UNCOVERING THE FACTORS THAT SHAPE THE DISTRIBUTION OF VECTOR-BORNE PLANT PATHOGENS

HOGENHOUT Saskia. (1)

(1) John Innes Centre, Norwich, UNITED KINGDOM

Text

Insect vectors of the order Hemiptera, such as aphids, psyllids, leafhoppers and froghoppers, are critical for the transmission of bacterial plant pathogens such as *Phytoplasma* and *Liberibacter* species and *Xylella fastidiosa*. These pathogens, along with their vectors, are invasive and have caused the collapse of fruit and crop production industries and the decline of native flora. However, the drivers of the success of these pathogens are largely unknown.

We shed new light on how these pathogens and their effectors modulate plant processes and interactions with insect vectors. We created genomic and transcriptomic resources for ±40 small hemipteran insects and uncovered the geographic population structure and routes of global dispersal of several invasive insect vectors. Our findings indicate that the successes of the pathogens are due to their abilities to promote insect vector attraction and performance, as well as the migration of insect vectors over long distances.

However, the pathogens' dependence on insect vectors is also their Achilles' heel, and we found that this reliance can be exploited to generate plant resistance. Therefore, it is essential to gather information on sap-feeding insects to build resilience against vector-borne plant pathogens. By understanding the drivers of success of these pathogens and their vectors, we can develop strategies to combat their spread and reduce their impact on crop production and native flora

K5-3

IMPACT OF CLIMATE ON PLANT-PATHOGEN INTERACTIONS

HE Sheng-Yang. (1)

(1) Duke University, Durham, UNITED STATES

Text
The "Disease Triangle" concept states that plant disease outbreaks require not only a susceptible plant and a virulent pathogen, but also conducive environmental conditions. Molecular studies in the past four decades have made major strides in understanding the mechanistic bases of plant resistance and pathogen virulence. However, less effort has been devoted to addressing an increasingly important question - why climatic conditions, such as humidity and temperature, have a profound effect on host susceptibility and disease development. Moreover, current studies often ignore the potentially pervasive effect a plant's endogenous microbiome may have on host-pathogen interactions. In this talk, I will give an example of interplay between disease, environment and microbiota during *Pseudomonas syringae* infection of host plants. Results suggest that future studies should increasingly consider the multi-dimensional nature of "disease-environment-microbiome" interactions, which are likely more reflective of what occur in natural ecosystems.

New Developments in Plant Disease Management

K6-1

DISEASE EARLY WARNING AND ADVISORY SYSTEMS – THE CASE OF WHEAT RUSTS

HODSON David. (1)

(1) CIMMYT, Texcoco, MEXICO

Text
Rust pathogens are one of the main biotic threats to wheat production globally. Highly mobile, constantly changing, and capable of devasting epidemics; they are a priority for disease management and epidemic prevention. Throughout wheat growing areas an increasing number of virulent pathotypes are being detected with rapid evolution and long-distance movements observed. Through a coordinated, large-scale, operational disease surveillance network it has been possible to track emerging threats, notably for stem and stripe rust. This international, multi-disciplinary surveillance and monitoring network is primarily focused on hot spots in developing countries but has global reach and implications. The integration of new technologies is permitting faster accurate detection, improved early warning and widespread dissemination of timely, actionable advice for disease management and reduction of epidemic threats. Over the past 15 years, considerable progress has been made in developing advanced early warning and advisory systems for wheat rusts in East Africa and South Asia. These systems are resulting in the protection of vulnerable wheat crops through early decision-making and on-time deployment of disease management strategies. Progress and status of these early warning and advisory systems will be presented. Future directions, including the expansion to include other important wheat diseases, are also highlighted.

K6-2

ADVANCES IN PLANT VIRUS DISEASE MANAGEMENT IN SUB-SAHARAN AFRICA – THE CASE OF BUNCH TOP DISEASE

KUMAR Lava. (1), NGATAT Sergine. (2), FIABOE Komi. (2), ALABI Tunrayo. (1), MAHUKU George. (3), TRIPATHI Leena. (4)

(1) International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA; (2) IITA, Yaoundé, CAMEROON; (3) IITA, Kampala, UGANDA; (4) IITA, Dar es Salaam, UNITED REPUBLIC OF TANZANIA

Text
Plant viruses have caused several devastating epidemics to annual and perineal corps in sub-Saharan Africa (SSA). Virus disease management in SSA heavily reliant on host resistance. For the viruses lacking durable resistance management relied on integrated methods, including habitat management, clean seed systems and phytosanitary controls. However, these methods are less efficient in preventing the virus spread.

Production of banana in sub-Saharan Africa is hit by the banana bunchy top virus (BBTV, genus Babuvirus). The virus transmitted by an aphid, Pentalonia nigronervosa, and vegetative propagation is increasingly becoming a serious threat due to the expansion of the virus into new regions in West Africa and, most recently, into East Africa. In the absence of durable host resistance, BBTV management requires integrated approaches to prevent the spread and eradication of infected plants. Progress and status of the recent advances to control BBTV, including the search for host resistance for BBTV and its vector, surveillance using remote sensing and machine learning methods, development of rapid diagnostic tools, biocontrol for aphids, and transgenic approaches will be presented.

K6-3

NLRSEEK: HIGH-THROUGHPUT DISCOVERY PIPELINE FOR FUNCTIONAL RESISTANCE GENES

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Text

Breeding crop species that are safe from pests and diseases is vital to build sustainable food systems crucial for food security. An effective and environmentally friendly method of disease control is to enhance the plant immune system by introducing functional resistance genes. A major class of plant immune receptors are nucleotide-binding, leucine-rich repeat receptors (NLRs), however identifying NLRs for use in elite crop varieties is time-consuming and resource-intensive. Through analyses of copy number variation and expression data, we identify high expression as an overlooked molecular signature of functional NLRs. Combining this signature with high-throughput crop transformation, we developed an approach that enables rapid identification and in planta validation of NLRs from non-domesticated germplasm. Screening 995 NLRs from 18 grass species identified 19 new resistance genes against wheat stem rust, a critical threat to wheat production. This pipeline facilitates rapid resistance gene discovery from diverse plant species to generate disease-resistant crops.

CONCURRENT SESSIONS

A mechanistic approach of the varietal mixture effects on plant pathogens

C6.7-1

UTILIZING BIODIVERSITY SCIENCE TO GUIDE SUSTAINABLE CROP MANAGEMENT

LAINE Anna-Liisa. (1)

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Text

Pathogens are prevalent across all ecosystems, and an individual's reproductive success and survival will depend on its ability to resist infection. Natural populations have been shown to support considerable diversity in resistance, and theory predicts this diversity to offer an effective means of diluting disease risk at both population and community levels. Analysis of a large natural pathogen metapopulation shows host resistance to be shaped by spatial structure and to have direct consequences on disease dynamics. At the community level we find an association between host trait distribution and pathogen load across a steep environmental gradient. We test these predictions from natural ecosystems in a large field biodiversity trial to identify how undersown species contribute to disease dilution. Our results highlight variation among undersown species in their effectiveness to dilute disease on barley.

C6.7-2

HOW SPECIES MIXTURES SUPPRESS PLANT DISEASES: INSIGHTS FROM A META-ANALYSIS

HOMULLE Zohralyn. (1), LI Wenfeng. (1,3), **DOUMA Bob. (1)**, MAKOWSKI David. (3), VAN DER WERF Wopke. (1)

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Text
There is a strong policy push for the idea that integrating diversity into agricultural systems will reduce the impact of diseases and hence reduce the need for pesticides. It is, however, uncertain how strongly disease pressure is reduced by growing species in mixtures. Here, we present a global meta-analysis on the effect of species mixtures on disease incidence in agriculture. We compiled from the literature a large global dataset of 216 trials reporting disease incidence in intercrops and their corresponding pure stands. Intercropping reduced disease incidence from 35% in pure stands to 21% in mixtures, equivalent to a 52% reduction in the odds of disease, from approximately 1:2 to approximately 1:4. The intercropping effect was consistent across continents but varied for different pathogens and crop species combinations. Mechanisms underlying the suppressive effect were further explored. Among others, we show that: i) the degree of mixing was, surprisingly, not significantly associated with the degree of disease suppression; ii) taller companion crops tended to have a stronger disease suppressive effect compared to smaller companion crops, in particular for aboveground spreading diseases, and iii) intercropping both reduced the initial disease incidence, as well as the disease growth rate. This analysis gives further insight into the mechanisms that underly the disease-suppressive effect of species mixtures and may provide pointers to further optimize agronomic practices

C6.7-3

COMBINING SOURCES OF RESISTANCE IN VARIETAL MIXTURES TO MANAGE THE EVOLUTIONARY DYNAMIC OF PATHOGEN POPULATIONS OF CEREAL CROPS: A WAY TO SOLVE THE EFFICIENCY-SUSTAINABILITY TRADE-OFF?

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(1) INRAE, Palaiseau, FRANCE; (2) INRAE, Montpellier, FRANCE

Text
Varietal mixtures have been shown to limit plant pathogen expansion in numerous cases. This effect is related to the overall increase of functional diversity within fields, but the mechanisms involved, which are diverse and some of which are specific to the biology of the pathogen, are not all well characterised. Recent results obtained in the rice pathogen Magnaporthe oryzae and the wheat pathogen Zymoseptoria tritici suggested that the combination of resistances within field drives the epidemiological and evolutionary control of pathogen populations, through antagonistic interactions that limit the range of possible adaptations. The aim of this keynote presentation is to highlight most recent body of knowledge on the multi-scale mechanisms of plant-pathogen interactions that play a role on the control of plant diseases in varietal mixtures and drive the evolution of virulence and aggressiveness after the deployment of different resistance genes in neighbouring plants within a canopy. Applying this body of knowledge to control the evolution of pathogen populations would allow the design of “smarter mixtures” reconciling the objectives of efficiency and sustainability of this agroecological practice.

C6.7-4

PLANT-PLANT INTERACTIONS MODULATE WHEAT SUSCEPTIBILITY TO SEPTORIA

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Text

Because of their well-known impacts on yield and diseases, cultivar mixtures are a growing practice and also an increasingly studied research topic. Plant susceptibility to pathogens in mixtures is reduced in fields, notably through epidemiological mechanisms. However, a new mechanism has recently been identified in which plant susceptibility can be modulated by plant-plant interactions, in absence of epidemy. Our objective is to identify the physiological and genetical determinants responsible for such interactions in wheat. We investigated bread wheat susceptibility to Zymoseptoria tritici in 190 two-ways mixtures using a new high throughput image analysis. Overall, the wheat susceptibility to Septoria was identical between mixtures and pure crops, although specific positive and negative plant-plant interactions could be identified, which is consistent with field situations where effects of wheat cultivar mixtures on Septoria susceptibility are highly variable. These results thus allowed the identification of interesting pairs, representing 3% of all tested mixtures, in which neighbours have beneficial effects on focal plant susceptibility after one infection cycle. Using these pairs, we are trying to identify the below-ground signals triggering this modulation of wheat susceptibility by the neighbours and the physiological as well as molecular responses to such neighbours.

C6.7-5

SMARTER VARIETAL MIXTURES TO IMPROVE PLANT DISEASE MANAGEMENT

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(1) University of Reading, Reading, UNITED KINGDOM

Text

There is a large empirical and theoretical body of knowledge supporting varietal mixtures as an effective and sustainable approach to suppress fungal diseases in crops. Efficacy and durability of mixtures can be improved by adjusting their parameters [1,2]. We can choose the varieties comprising a mixture, thereby adjusting their degrees of disease resistance and tolerance. We can also adjust the number of component varieties and their proportions within the mixture. This optimization requires a detailed understanding of the epidemiology of diseases to be controlled and the population biology of the associated pathogens. I will review recent research on optimizing varietal mixtures drawing from both theoretical and empirical studies. I will discuss promising future directions: the role of new phenotyping technologies in optimizing varietal mixtures and the contribution of plant microbiomes to the efficacy of mixtures.

[1] Mikaberidze, A., McDonald, B. A. & Bonhoeffer, S., 2015 Developing smarter host mixtures to control plant disease. Plant Pathology 64, 996-1004, https://doi.org/10.1111/ppa.12321

[2] Ben M'Barek[a], S., Karisto[a], P., Abdedayem, W., Laribi, M., Fakhfakh, M., Kouki, H., Mikaberidze[b], A., & Yahyaoui[b], A., 2020 Improved control of Septoria tritici blotch in durum wheat using cultivar mixtures. Plant Pathology, 69, 1655-1665, https://doi.org/10.1111/ppa.13247 ([a]shared first authors; [b]shared senior authors)

C6.7-6

VARIABLE EFFECTS OF A WHEAT CULTIVAR MIXTURE ON SEPTORIA TRITICI BLOTCH: INVESTIGATING KEY FACTORS INVOLVED IN MIXTURE EFFECT VARIATION BETWEEN EUROPEAN SITES

VIDAL Tiphaine. (1), DUVIVIER Maxime. (2), GAUBRIE Sylvain. (3), HEICK Thies. (4), HELLIN Pierre. (2), KILDEA Steven. (5), SUFFERT Frédéric. (1)

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Text

Wheat cultivar mixtures can provide control against numerous diseases such as Septoria tritici blotch (STB) caused by *Zymoseptoria tritici*. One important criterion for mixture design is the contrast in resistance levels between the mixed cultivars, but these resistance levels can vary with local conditions (eg. composition of pathogen populations, climatic conditions). The objective of this work was to assess disease control provided by a cultivar mixture in different locations in Europe, and its relation to factors including the local resistance level of cultivars in pure stand and climatic conditions. A field experiment was carried out in seven sites in Europe (in France, Belgium, Denmark and Ireland) during three successive cropping seasons. In each site, disease severity was measured on each cultivar (Apache and Cellule) in pure stands and in a 50:50 mixture. The resistance level of each cultivar was variable between site x year. Mixture effect, computed as the difference of disease severity of the cultivar in mixture compared to pure stand, ranged from +3% (more disease in mixture) to -3% (less disease in mixture) on Apache and from +1% to -12% on Cellule. The largest disease reductions in mixtures were observed for each cultivar in sites x years where it appeared as susceptible in pure stand. These results show that the efficiency of a particular mixture can vary between sites and years and open perspective to better adapt mixture design to local conditions.

Advances in the use of exotic sentinel trees and novel monitoring programs

to detect incipient threats posed by forest pathogens

C4.5-1

LESSONS LEARNED FROM A RECIPROCAL INTERNATIONAL SENTINEL PLANTING PROJECT

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Text

Non-native, invasive tree pathogens and insect pests (PIPs) are among the greatest threats to forest ecosystems worldwide. Prevention and early detection are the most effective management strategies in mitigating PIP impacts. This includes risk analysis of the many possible PIPs in a source locale that can potentially become invasive if introduced into a susceptible sink environment. Ex-patria sentinel gardens can be a significant component of these efforts, as they involve the intentional planting and monitoring of host species of interest in potential source environments, thus exposing them to the local PIPs. In other words, target trees are used as the proverbial canary in the coalmine, i.e. an early warning system. Through an international collaborative study, we established plantations of Asian and European tree species in the U.S.A. and corresponding plantations in Europe, with Asian and North American species, and China, with European and North American species. Sentinel gardens were monitored for symptoms and signs of disease and insect attack over two seasons and fungal and insect species were collected and identified. Preliminary results show that several species of fungi and insects were described for the first time on several of the hosts used. Lessons learned from our experience with this approach so far will be discussed in the context of optimal strategies for invasion prevention.

C4.5-2

SATELLITE BASED MONITORING OF INVASIVE PESTS AND ALIEN PLANT BACTERIAS: THE XYLELLA FASTIDIOSA AND TOUMEYELLA PARVICORNIS CASE STUDIES

LASAPONARA Rosa. (1), TELESCA Luciano . (1), FARIDANI Farid. (2), ABATE Nicodemo. (3), LOVALLO Michele. (4)

(1) CNR, Potenza, ITALY; (2) Department of European and Mediterranean Cultures, Environment, and Cultural Heritage, University of Basilicata, Potenza, ITALY; (3) Institute of

Heritage Science, National Research Council, C.da S. Loja, 85050 Tito Scalo, Italy, , Potenza, ITALY; (4) ARPAB, 85100 , Potenza, ITALY

Text

The main goal of this study was to evaluate the potential of Fisher-Shannon statistical method applied to satellite time series to detect any anomalies happening in the vegetation behavior with particular reference to those related to Xylella fastidiosa and Toumeyella Parvicornis. For the purpose of this study, significant study areas have been selected in Italy focusing both urban forests, affected by Toumeyella Parvicornis, and agricultural areas, affected by Xylella fastidiosa, along with unaffected test areas. To account for the great variability exhibited by the seasonal variations while identifying small multi-year trends and changes, we devised a procedure made up of two steps: (i) firstly, the satellite-based time series has been analysed using the Singular Spectrum Analysis (SSA) to detect and remove the annual cycle including the seasonality and then (ii) the de-trended signals have been analysed using the Fisher-Shannon. The methodological approach has been applied soil-water-atmosphere-plant related satellite products available in the Google Earth Engine cloud database (LAI, NDVI, EVI, ET from MODIS).
In the Fisher-Shannon Information Plane (FSIP), the infected vegetated areas appear well characterized and discriminate from healthy areas. These preliminary results seem to envisage the usefulness of the Fisher-Shannon method as a reliable statistical tool to be included in an operational system for early diagnosis of status of deterioration of vegetation.

C4.5-3

FOUR YEARS OF THE EUROPEAN UNION REFERENCE LABORATORY (EURL) FOR FUNGI AND OOMYCETES

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(1) ANSES Plant Health Laboratory, EURL for fungi and oomycetes, Mycology Unit, Malzéville, FRANCE

Text

The European commission approved in 2016 a new Plant Health Act applicable for countries of the European Union (EU). This set of laws implemented in 2019 conferred authorities a greater competence to reduce the risks of introduction and further spread of plant pathogens within the EU. In response to this legislation, a network of EURLs in the main areas of plant health was created. Since 2019, the Mycology Unit of the Plant Health Laboratory of ANSES, has been appointed by the EU to carry this mandate as the EURL for fungi and oomycetes. The main objective of this mandate is to enhance the capacities for pathogens detection of the National Reference Laboratories (NRLs) of the EU member States. This goal is achieved through the organization of trainings, workshops and the validation and development of detection methods, as well as proficiency tests on quarantine or emerging plant pathogens. The latter allows to identify opportunities for improvement and implement corrective actions. Other activities comprise technical assistance and the release of literature reviews and newsletters. The EURL, also maintains a collection of over 100 fungal strains and distribute them as reference material to the NRLs for training purposes. Since its designation, the EURL for fungi and oomycetes has developed its work program on the above-mentioned activities in species such as *Fusarium circinatum*, *Geosmithia morbida*, *Phytophthora ramorum*, *Phyllosticta citricarpa*, *Tilletia indica*, among others.

C4.5-4

PRESCREENING AND MONITORING OF FOREST PLANT PATHOGENS USING SEQUENCING TECHNOLOGIES IN REGULATORY RESEARCH.

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(1) Canadian Food Inspection Agency, Ottawa Laboratory Fallowfield, Ottawa plant laboratory, Ottawa, On, CANADA; (2) Phytodata Inc. research company, Sherrington, Qc, CANADA; (3) Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, Qc, CANADA

Text

Early detection of fungi and oomycetes is the key to managing regulated and invasive alien species. These are difficult to study and even more in forest environments. Unlike insects, they can't be lured and requires a more significant sampling effort. Conventional qPCR-based monitoring approaches require a detailed knowledge of the target organisms. High throughput sequencing (HTS) technologies allow us to investigate different types of samples, process large numbers of samples and produce even greater volumes of genomic data. Metagenomics tools combining Ion Torrent sequencing and custom bioinformatic pipelines can be used to evaluate potential sampling methods for pathogens in forestry and agriculture and contribute to identify spreading pathways. Sampling methods exploiting eDNA isolated from insects, air, soil, tissues, and pollen have revealed sources of target pathogens. In addition, pest monitoring activities were evaluated to understand the limitations of the technology in biosurveillance. We aim to provide a framework combining sampling tools with HTS-based methods, appropriate bioinformatic pipelines and qPCR assays for early detection of emerging and invasive alien species. This was applied to *Bretziella fagacearum* responsible for the Oak wilt, *Phytophthora spp*. associated with root rot diseases and other forest pathogens in Canada. We believe that such a framework will help and improve early warning, promote public awareness, and support our regulatory activities.

C4.5-5

DORMANT WOODY PLANTS: A PATHWAY OF INTRODUCTION INTO EUROPE OF POTENTIALLY INVASIVE PESTS?

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(1) WSL, Birmensdorf, SWITZERLAND; (2) CABI, Delémont, SWITZERLAND

Text

Non-native pests (including pathogens) represent one of the major threats for trees worldwide. Most of the recently established non-native pests of trees were not known as harmful organisms or even to science prior to their establishment. Consequently, they were not targeted by practices and measures aiming at minimizing the risk of accidental introduction of harmful organisms.
Recently, new approaches to identify potential harmful invaders before they spread in a new environment have been proposed, including sentinel plantings and horizon scanning. Here

we assessed if the international trade of dormant woody plants may be a pathway of introduction of potentially invasive pests in Europe. For this, we characterized the communities of fungi and insects in and on (insects) asymptomatic dormant twigs of trees from 167 species in 14 genera grown at 49 sites in 33 countries around the globe. Our analyses showed that non-European plant samples hosted 10 fungal species and two specie of herbivorous insects not yet officially present in Europe. Most of these were found or are known to occur in association with tree species which are present in Europe, or which have congeneric species in Europe. Hence, when introduced into Europe such organisms would most likely find appropriate hosts on which, they could become established. To better assess the effective risk that such organisms pose to European tree species, however, specific risk analyses should be carried out in the future.

C4.5-6

OBSERVATREE: 10 YEARS OF EARLY WARNING CITIZEN SCIENCE

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(1) Woodland Trust, Grantham, UNITED KINGDOM; (2) Forest Research, Farnham, UNITED KINGDOM; (3) Fera Science Ltd, York, UNITED KINGDOM

Text
Observatree is a successful collaboration between Governmental agencies and NGOs in the UK. The project utilises citizen science to act as an early warning for tree pests and pathogens. Now entering its tenth year, what data has the project generated? Can volunteers submit accurate surveys for early warning and monitoring? By the end of 2022 over 18,000 reports had been submitted by a network of 200 highly trained volunteers. The volunteers reported on 9 priority pathogens identified by the Government, alongside reporting other worrying symptoms and, importantly, negative findings. The network has submitted 1400 new findings of the priority pathogens, contributing greatly to the known distribution of Hymenoscyphus fraxineus, and adding significant new findings for Cryphonectria parasitica and the acute oak decline bacterial complex. Analysis from 2019 reported 85% of the pest or pathogen findings were correct in identification, demonstrating the potential for volunteers in this space. Observatree also has a network of over 600 sentinel trees, consisting of over 30 species, distributed across the UK. The health of this sentinel network is showing decline. Over 50% of the monitored Fraxinus spp. and Aesculus spp. exhibit a damaging pest or pathogen. This data is vital for monitoring the state of the nations trees, aiding statutory action, research, and policy.

P4.5-001

SENTINEL PLANTS: A STRATEGY TO PREVENT NEW INVASIVE FOREST PEST AND PATHOGEN INTRODUCTIONS

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(1) National Research Council - Institute for Sustainable Plant Protection, Sesto Fiorentino, ITALY; (2) Department of Plant Pathology, CFAES, The Ohio State University, , Columbus, UNITED STATES

Text

The early detection of pests and diseases using sentinel plants has been exploited in some experimental work, to identificaty (unknown) plant pests and pathogens prior to introduction into new countries. Sentinel plantings can have different objectives: in-patria plantings estimate infestation rates of associations of native pathogens and native tree species that may be exported with that particular commodity, whereas ex-patria plantings are relevant to assessing new pest-host associations prior to the introduction of the pests, such as possible host shifts of pests native to the exporting country to hosts native to the importing country. In the frame of the EU project HOMED and an USDA funded project we have implemented this concept, widening the use of this tool. Here we report the results from the sentinel plantations established in Italy.
Eight European (in-patria), seven North American and five South African tree species (ex-patria) were planted in Florence, Italy according to a randomized block design. Over three years, symptomatic trees were sampled twice-a-year and the causal agent identified. The results show that the monitoring techniques developed and used in these trials are extremely effective: regularly monitoring over a period of three years has yielded a multitude of known and previously unknown pest-host associations. Our work presents the associations and discuss them in the context of risks associated with the global movement of live plants.

P4.5-002

REVEALING NOVEL INTERACTIONS BETWEEN OAK AND TUBAKIA SPECIES: EVIDENCE OF THE EFFICACY OF THE SENTINEL ARBORETA STRATEGY.

VANNINI Andrea. (1), OSKAY Funda. (2), DOGMUS-LEHTIJÄRVI Tugba. (3), MORALES-RODRIGUEZ Carmen. (1)

(1) Universita’ degli Studi della Tuscia, Viterbo, ITALY; (2) Çank?r? Karatekin University, Çank?r? , TURKEY; (3) T.C. Isparta University of Applied Science, Isparta, TURKEY

Text

In the present study, the sentinel arboreta strategy was applied, and its efficacy was evaluated at the Atatürk Arboretum (Istanbul, Turkey), having as a study case the interaction Tubakia spp. – Quercus spp. Thirty-four oak species native of America and Eurasia were sampled within the Fagaceae collection of the arboretum. Isolation trials were conducted from leaf necroses, and High Throughput Sequencing for fungal taxa was carried out from asymptomatic leaf blades. Four *Tubakia* species were identified, *T. dryina*, *T. suttoniana*, *T. hallii*, and *T. macnabbii*. Three out of four are of recent description and the present study contributed to updating their host-range. Thirty-two oak-Tubakia interactions new to science were described. Hypotheses were formulated on the possible movement across geographic areas of these species and on the risk posed in case of introduction in the distribution range of susceptible host species. As a conclusive remark, the present study confirmed the efficacy of the sentinel arboreta strategy to highlight new host-pathogen interactions and the risk of host-shift events.

P4.5-003

DEVELOPMENT OF ON-SITE QUICK DIAGNOSIS SYSTEM FOR DETECTING PHYTOPLASMA RELATED DISEASES IN SOUTH KOREA

LEE Sun Keun. (1), LEE Sang Hyun. (1), BAE Yoon Hee. (2), LEE Geon Woo. (3), HAN Sang Sub. (3), LEE Hyeong Woo. (4)

(1) Warm Temperature and Subtropical Forest Research Center, National Institute of Forest Science, Jeju, REPUBLIC OF KOREA; (2) BD Ferm Biotech Co., Ltd, Seongnam, REPUBLIC OF KOREA; (3) Department of Forest Environment Science, College of Agriculture and Life Sciences, Jeonbuk National University, Jeonju, REPUBLIC OF KOREA; (4) SpeegeneBio Co., Ltd, Seongnam, REPUBLIC OF KOREA

Text

The causes of broom disease prevalent in Korea are diverse, such as phytoplasma, fungi, and herbicides, and it is difficult to diagnose in the field because pure culture without host cells is impossible. So, we developed a simple quick diagnostic method for the efficient diagnosis of phytoplasmas infection in various hosts at outbreak sites. To do this, we designed a universal LAMP (Loop Mediated Isothermal Amplification) primer set for phytoplasma diagnosis by analyzing the nucleotide sequence of 16s rRNA from phytoplasma-infected *Ziziphus jujuba* var. *inemmis*, *Paulownia coreana*, *Rhus javanica* L., *Hovenia dulcis* Thunb., *Ulmus parvifolia* Jacq., *Elaeocarpus sylvestris* var. *ellipticus* (*Candidatus* phytoplasma malaysianum), *Elaeocarpus sylvestris* var. *ellipticus* (*Ca*. phytoplasma asteris). “Phytoplasma real time quick detection kit (PRTQ kit)” and “KN5 rapid DNA and RNA extraction kit (KN5 kit)” was developed (Speegenebio Co., Ltd). We evaluated both kits’ efficacy together with another portable nucleic acid amplifier (HARU-2000, SM electrical Co., Ltd.). When using PRTQ kit, All the phytoplasmas described above were detected within 14 minutes in “HARU-2000.” Phytoplasma DNA could be extracted by KN5 kit between 1 minute to 10 minutes. KN5 kit enhanced the availability of PRTQ kit in the field by allowing rapid DNA extraction from target samples. Therefore, “PRTQ kit” and “KN5 kit” in addition to “HARU-2000” can solve the difficulties of phytoplasma diagnosis in the field.

P4.5-004

DEVELOPMENT OF MARKERS FOR IDENTIFYING SPREAD ROUTES OF PINE WOOD NEMATODE IN KOREA

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(1) National Institute of Forest Science, Seoul, REPUBLIC OF KOREA; (2) Seoul National University, Seoul, REPUBLIC OF KOREA; (3) Kangwon National University, Chuncheon, REPUBLIC OF KOREA

Text

Pine wilt disease caused by the pine wood nematode, *Bursaphelenchus xylophilus*, is the most destructive forest disease in Korea. The pine wilt disease first occurred in Busan in 1988 and has spread to 135 regions over the past 30 years. This study was conducted to develop a molecular marker that can analyze the genetic relationship of the pine wood nematode populations by region because identifying the spread route is most important for

controlling pine wilt disease. The genome sequence of the Korean pine wood nematode was prepared, and genetic variations on 6 chromosomes and the mitochondrial genome were confirmed by analyzing the nucleotide sequences of 290 Korean pine wood nematode populations. As a result of phylogenetic analysis with this variation, it was possible to classify into three groups, and the number and location of SNPs by the group were analyzed and genetic markers were selected. Using the six selected SNP markers, hetero-type could be identified, more accurate tracking of the occurrence of pine wood nematode. Analyzing the genetic relationship of pine wood nematodes occurring in Korea and mapping a gene group, KL01, KL02, and KL01-03 groups are mainly distributed, and the KL01-03 hetero-type dominates in Korea. Our results can be useful for epidemiological investigations when pine wood nematodes spread to new areas and can be used as basic data for the development of genetic markers that can be subdivided by populations.

P4.5-005

FOUR YEARS OF THE EUROPEAN UNION REFERENCE LABORATORY (EURL) FOR FUNGI AND OOMYCETES

PARRA GIRALDO Pedro Pablo. (1), RENAULT Camille. (1), CERF Isabelle. (1), AGUAYO Jaime. (1), IOOS Renaud. (1)

(1) ANSES Plant Health Laboratory, EURL for fungi and oomycetes, Mycology Unit, Malzéville, FRANCE

Text

The European commission approved in 2016 a new Plant Health Act applicable for countries of the European Union (EU). This set of laws implemented in 2019 conferred authorities a greater competence to reduce the risks of introduction and further spread of plant pathogens within the EU. In response to this legislation, a network of EURLs in the main areas of plant health was created. Since 2019, the Mycology Unit of the Plant Health Laboratory of ANSES, has been appointed by the EU to carry this mandate as the EURL for fungi and oomycetes. The main objective of this mandate is to enhance the capacities for pathogens detection of the National Reference Laboratories (NRLs) of the EU member States. This goal is achieved through the organization of trainings, workshops and the validation and development of detection methods, as well as proficiency tests on quarantine or emerging plant pathogens. The latter allows to identify opportunities for improvement and implement corrective actions. Other activities comprise technical assistance and the release of literature reviews and newsletters. The EURL, also maintains a collection of over 100 fungal strains and distribute them as reference material to the NRLs for training purposes. Since its designation, the EURL for fungi and oomycetes has developed its work program on the above-mentioned activities in species such as *Fusarium circinatum*, *Geosmithia morbida*, *Phytophthora ramorum*, *Phyllosticta citricarpa*, *Tilletia indica*, among others.

P4.5-006

A RETROSPECTIVE OF INVASIVE FOREST PATHOGENS IN NORTH AMERICA: BIOGEOGRAPHIC PATTERNS AND SENTINEL TREES

WILLIAMS Geoffrey. (1), GOUGHERTY Andrew. (2), AUGUSTINUS Benno. (4), BROCKERHOFF Eckehard. (4), CHRISTEN Sarina. (5), SNIEZKO Richard A.. (3)

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Text

Risk uncertainty limits adoption and effectiveness of preventative forest biosecurity. To gather information on unknown threats, scientists have monitored trees outside of their range (i.e., “sentinel trees”) in new gardens and living botanical collections, where they may be exposed to novel pathogens lurking there. Although most invasive pathogens were known to science, the threat they posed to forest ecosystems in new environments remained unclear until a full-blown epidemic was already underway. In a wider context, Nearctic trees have been planted intercontinentally for > 500 years, resulting in thousands (> 2.7K) of recorded encounters with new pathogens. The analysis of retrospective data on prior knowledge of tree pathogens that became invasive could aid assessment of impending threats by quantifying pre-invasion risk uncertainty. To that end, we assembled chronological records of North American trees outside their range that could have functioned as sentinels for a comprehensive list of established invasive forest pathogens in North America. We then reviewed the biogeographic origins, dates of first observation, and prior records of host range for each pathogen. With this data we found support for the hypothesis that realized impacts of invasive pathogens could be partly accounted for retrospectively by observations from sentinel trees, phylogenetic host range, and human-aided dispersal of hosts to biogeographic regions where pests may be pre-adapted to colonize them.

APP-titude for social media in Plant Disease Research

C7.7-1

ENHANCING SOCIAL MEDIA DELIVERY TO PLANT PATHOLOGISTS

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Text

Time waster or community of trust? Views differ, but there is no denying the potential of social media to enhance plant disease research progress, record keeping and community engagement. This presentation introduces the use and engagement with social media by plant pathology societies and plant pathologists, and considers how to improve its relevance. The starting point was a 2023 survey of the plant pathology community to gauge the ethical use of social media to

1. improve access to the latest 'hot' findings in plant pathology and food security
2. improve extension and enhance contact with farmers and supply chain people
3. provide services and inspiration through plant pathology societies
4. support mental health and inspire the spirit
5. enhance the impact and access to journals and newsletters
6. promote meetings and report social news about plant pathologists

The survey findings will be summarized under

- topics most important to social media readership
- platforms respondents use to access plant pathology related topics and inspiration
- scientific societies and other sources of plant pathology information and
- the demographic profiles of users and non-users.

Finally, we will suggest opportunities for improving social media use to enhance science outreach, career prospects and well-being of plant pathologists.

C7.7-3

THE INTERNATIONAL YEAR OF PLANT HEALTH: BEING A JOURNALIST HELPS

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Text

The year 2020 offered a unique opportunity to plant pathologist worldwide, having been declared the International Year of Plant Health (IYPH) by the United Nations. Thanks to its activity in the International arena, as well as a very good relationship established with FAO, permitted AGROINNOVA to play a role, with the intent of letting people know the social and economic role of plant pathology. The IYPH indeed permitted to overturn the concept of plant disease into the one of plant health. Did help being a journalist? Of course yes. The celebration of the IYPH provided a very unique opportunity to invest resources in communicating our discipline to a much broader audience. Communicating the results of our research, nowadays considered into the broader field of public engagement, is an important task for researcher. Not all researchers are as good in communication as in research. This happens also because, at least in the past, the traditional *curricula* did not include courses on communication, so that most of us are self-taught. As a researcher active in public engagement, being also publicist journalist, taking courses and working closer to the journalism environment, help in selecting the topics to tell and a better understanding of the timing of journalism. A good relationship among researchers and journalists helps improving researcher's communication, sharing tools, ranking priorities, understanding what indeed matters to the public.

C7.7-4

FROM PHYTOPATHOLOGICAL STROLLS TO SOCIAL MEDIA SCROLLS: AN OPPORTUNITY TO RAISE AWARENESS OF PLANT PATHOGENS

SUFFERT Frédéric. (1), SUFFERT Muriel. (2)

(1) INRAE, Palaiseau, FRANCE; (2) EPPO, Paris, FRANCE

Text

The 2020 International Year of Plant Health was opportune for communication on plant diseases. During this year we illustrated the diversity and beauty of fungal plant pathogens found during “phytopathological strolls”, in which we observed and determined the origin of symptoms on diseased plants found in our garden, in the local streets of a city in the Paris (France) suburbs, and in nearby open spaces. We observed and described fungal pathogens through hundreds of photographs, shared our findings with a large audience on Twitter using the account @wheatpath, and received feedback. The lockdown imposed to control the COVID-19 pandemic created an additional motivation to take up the challenge and to involve our children. This experience was an opportunity to promote phytopathology as a part of our day-to-day life through a combination of classical approaches and digital tools in tune with the times, such as social media, by treating pathogen identification like a detective game and by making use of the addictive nature of collection approaches.

C7.7-5

THE INTERSECTION OF SOCIAL MEDIA AND INDIGENOUS RIGHTS IN PLANT PATHOLOGY

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Text

Communication and audience engagement is a key component of the modern science system with a direct benefit to the researcher, research team, organisations, and funding bodies. We now share and like posts of research progress and outputs through social media with the intent of building networks and getting our work out in the world; all with altruistic intentions of making an impact in the field of plant pathology. Yet, this thinking by researchers may not align with the values and practices of Indigenous/First Nations communities and/or could impact their rights. How can social media be used to uplift research projects with equitable benefit for Indigenous/First Nations peoples and rights when appropriate, and on the flip side, how can social media be problematic and unsafe? We will explore social media use and Indigenous/First Nations rights to provide insight and raise possible concerns about engaging in and promoting plant pathology research, and the

opportunities to be guided by strong and shared ethics. In addition to the presentation, we warmly invite you to attend ‘Getting rights right: A roundtable exploration of Indigenous rights and plant pathology’ at ICPP 2023.

At the heart of disease emergence: Determinants and consequences of host range contours of plant pathogens

C7.3-1

ON THE EMERGENCE OF NEW PATHOGENS: INSIGHTS FROM COMPARATIVE GENOME STUDIES OF THE SEPTORIA BLOTCH PATHOGEN

FAGUNDES Wagner. (1), ROJAS BARRERA Idalia. (1), ALIZADEH Alireza. (2), **STUKENBROCK Eva. (1)**

(1) Christian-Albrechts University of Kiel, Kiel, GERMANY; (2) Azarbaijan Shahid Madani University, Tabriz, IRAN (ISLAMIC REPUBLIC OF)

Text

The fungal pathogen *Zymoseptoria tritici* causes devastating disease in wheat. The history of this pathogen is tightly correlated with crop domestication and the spread of wheat cultivation with farmers since the Neolithic. We have traced back the origin of *Z. tritici* to the Middle East and identified a large diversity of sister species infecting wild grasses in natural grassland vegetations in Iran. This collection of isolates provides us with a unique resource to study speciation genetics and host specialization in natural and agricultural ecosystems. We have sequenced population genomic data of five *Zymoseptoria* species including populations of *Z. tritici* occurring on the wild wheat relatives, *Aegilops* spp. Plant inoculation experiments prove a strong degree of host specificity, even among closely related linages. In spite of the prominent signature of host specificity, comparative population genomic analyses reveal recurrent introgression including the exchange of functionally relevant traits and transposable elements across species boundaries. Interspecific hybridization may facilitate the rapid evolution of pathogens and new virulence traits in this group of important plant pathogens, and should be considered in the light of emergence of new pathogens in agricultural ecosystems.

C7.3-2

STEMPHYLIUM VESICARIUM CAUSES FOLIAR DISEASE ON CELERY IN MICHIGAN, USA

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Text

Celery (Apium graveolens L) is an important salad vegetable worldwide. Michigan is the second largest supplier of the crop in the United States; the state's crop was valued at $19.5 million USD in 2018. In 2022, foliar symptoms were observed on the lower leaves of celery in a Michigan field that had been previously cropped to onion. Brown spots and conidia were observed on the leaves' adaxial surface and border extending to the petiole. Fungal isolates (25) obtained from symptomatic tissue were identified as *Stemphylium vesicarium* using morphological characteristics. DNA was extracted from three isolates and two primer sets were used for sequencing the internal transcribed spacer (ITS) region (ITS1 and 4) and partial calmodulin (cmdA) gene (CALDF1 and CALDR1). According to a nucleotide BLAST search and multilocus phylogenetic analysis (neighbor-joining tree), the obtained sequences of the three isolates had 100% pairwise identity with *S. vesicarium* sequences MW798751 (ITS) and MK675706 (cmdA) and clustered into a single clade with *S. vesicarium* reference sequences. Selected isolates were tested and found to be pathogenic on celery seedlings. Three *S. vesicarium* isolates from celery incited leaf spot and tip necrosis on 'Bradley' onion seedlings. When two *S. vesicarium* isolates from volunteer onions growing in a celery field were used to inoculate celery seedlings, they caused leaf spots. While onion is a host of *S. vesicarium*, celery has not been considered a host.

C7.3-3

DEMOGRAPHIC AND GENOMIC CONSEQUENCES OF A RAPID ADAPTATION EVENT IN THE POPLAR RUST PATHOGEN

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Text

The intense and unidirectional selection pressure caused by the massive deployment of resistant plants can result in rapid adaptation of pathogen populations1. Such rapid evolution was documented for the poplar rust fungus. In 1994, poplar rust populations suddenly overcame the RMlp7 qualitative resistance carried by several poplar cultivars planted widely in Western Europe2,3. This recent event of adaptation from standing genetic variation caused a selective sweep on the rust genome4. We study a 25-year temporal sampling of poplar rust populations to decipher the demographic and evolutionary history of this pathogen while overcoming its host genetic resistance. We examine phenotypic and genetic variations throughout this adaptive event. Our analyses reveal that a unique and homogeneous genetic group overcame RMlp7 resistance and replaced the ancestral genetic group within five years. We then use forward simulations to 1) understand the interplay between demography and genetic evolution underpinning the rapid evolution of poplar rust

populations, and 2) disentangle the polymorphism signatures of selection from that of stochastic processes due to demographic changes. We show high stochasticity in evolutionary trajectories with the notable effect of evolutionary rescue scenarios on polymorphism signatures.Finally, we integrate our simulator in an Approximate Bayesian approach (ABC) to infer the demographic and selection parameters from temporal genetic data.Our statistical framework coupling modelling with temporal data is powerful to understand recent events of rapid adaptation. 1 Saubin et al. DOI:10.24072/pcjournal.10 2 Louet et al. DOI:10.1111/mec.16294 3 Persoons et al. DOI:10.1111/mec.13980 4 Persoons et al. DOI:10.1093/gbe/evab279

C7.3-4

HISTORY OF THE LAW OF BARBERRY ERADICATION: - WHY WAS THE LAW REMOVED AND SHOULD IT BE REINSTATED? A CASE STUDY FROM SWEDEN

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Text

Rust fungi a large group of plant pathogens and are one of the largest threats to plants in the world. Several of the rust fungi infecting grasses have *Berberis* spp. (barberry) as an aecial host, including two important diseases in cereals: stem rust (*Puccinia graminis*) and yellow rust (*Puccinia striiformis*), but also other species such as *Puccinia arrenatheri* and *Puccinia brachypodii*. Since the fungus *P. graminis* that causes stem rust cannot yet survive the winters in Nordic climates, the presence of the barberry bush is required in the landscape for the pathogen to survive. During large parts of the 20th century, the law on eradication of barberry was in force, with the aim of reducing attacks by stem rust. Since the removal of that law in 1994, the number of reported barberry bushes has increased in Sweden. Due to the law and resistant cultivars, Sweden was spared from the disease, but during the last decades stem rust has returned and is now causing disease in all cereal crops. The increased observations of stem rust correlate with an increased number of *Berberis* spp. in the landscape. With help of historical documents and knowledge about the biology of the fungus, we have analysed the importance of *Berberis* spp. in the landscape before, during and after of the law of barberry eradication from an epidemiological perspective. The result increases our knowledge about the management of cereal rust disease and about biological diversity in the agricultural landscape.

C7.3-5

Subpopulation differences in susceptibility to viral disease in North American switchgrass: Genetic and ecological considerations

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Text
A priority goal in pathosystem research is to probe differences in the genetic architecture of defense responses among diverse plant groups, including between crop and wild plants. For crop protection, numerous genome-wide association studies (GWAS) of plant viral disease expression have been conducted in common crops, such as maize and rice. To our knowledge, the only attempts at GWAS in wild plant species have focused on the biennial model plant Arabidopsis thaliana. Moreover, most such studies have been conducted under relatively artificial conditions. Here we report the first large-scale genetic analysis of wild virus interactions with a long-lived wild plant, conducted on Panicum virgatum (switchgrass), a native North American prairie under development as a bioenergy feedstock. We evaluated viral disease expression in a panel of 512 diverse tetraploid accessions originating from populations throughout the broad natural range of switchgrass. Plants were field-grown in Michigan, USA, in unencumbered soil with full exposure to natural environmental factors, including heat, cold, herbivory, and microbial interactions. We focused on naturally-occurring disease caused by switchgrass mosaic virus (SwMV), a wild virus (Genus Marafivirus, Family Tymoviridae) extant to our region that is transmitted by wild leafhoppers. We quantified variation among ecological subgroups in susceptibility to viral disease for three years, and we used GWAS to identify SNPs and candidate genes associated with disease phenotypes. Switchgrass susceptibility to wild viral disease varied among ecological subgroups from highly susceptible to very resistant. Notably, the range of the most susceptible genotypes overlapped the known distribution of switchgrass mosaic virus, suggesting that local interactions have not driven greater resistance in this region. GWAS results found that wild viral disease expression was associated with a broad network of small effect genes implicated in diverse pathways associated with cellular, metabolic, and immune response pathways, with only a small percentage of genes associated with identifiable characteristics of R genes. Among a conservative set of 143 candidate genes associated with 67 pruned SNPs, we found one in common with a candidate gene associated with virus resistance in rice, and 30 unannotated candidate switchgrass genes without known homology to model systems that may represent novel elements regulating plant virus interactions in natural ecosystems. These findings demonstrate the breadth of viral interactions with wild plants and offer opportunities for identifying new genetic mechanisms influencing viral infection dynamics.

C7.3-6

SURVIVAL NICHES OF CURTOBACTERIUM FLACCUMFACIENS PV. FLACCUMFACIENS

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Text
Knowledge of the ecological survival niches of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff), the causal agent of bean bacterial wilt, is essential for efficient disease management. We study the survival of Cff in the phyllosphere and rhizosphere of 21 weeds,

14 crops, and 4 types of soil. The aerial part of the plant was inoculated by spraying a bacterial suspension of Cff, while the soil of the growing pots and the pots containing only soil was infested with the same suspension. For the soil experiment, we evaluated the survival of one bacterium strain from a common bean and 3 strains from a soybean. The survival of Cff strains was evaluated every seven days until they were not detected and confirmed by PCR. High temperatures and rainfall reduced Cff survival in the phyllosphere, while high temperatures negatively affect survival in the rhizosphere. Our results demonstrated that weeds are potential hosts for Cff and their eradication in common bean fields is recommended. Barley, black oat, canola, forage turnip, maize, pearl millet, ryegrass, sorghum, soybean, sunflower, velvet bean, wheat, and white oat can be potential asymptomatic hosts. In the soil, the survival period ranged from 77 to 154 under laboratory conditions and for a maximum of 91 days under natural field conditions.

P7.3-002

THE PATHOGENS OF FUSARIUM SOLANI SPECIES COMPLEX (FSSC) CAUSING COLLAR ROT AND FRUIT ROT OF PASSION FRUITS IN TAIWAN

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Text

Two passion fruit varieties, Purple (*Passiflora edulis* Sims) and yellow (*P. edulis* f. *flavicarpa* Deg), are mainly grown worldwide. *Fusarium solani* (Fo) was important agent to cause collar rot of passion fruit in Brazil, Mainland China, and Japan etc. In Taiwan, Fo could cause collar rot and fruit rot in passion fruit, especially, symptom on fruit showing brown and water soak lesion. Recently, *F. solani* species complex (FSSC) have been classified more than 100 phylogenetic species based on phylogenetic analysis. The objective of this study is to clarify the species of FSSC pathogens causing collar rot and fruit rot of passion fruit in Taiwan and carry out their morphology and characteristics. The FSSC isolates from collar rot and fruit rot of purple or yellow passion fruit from different location in Taiwan were divided into four main molecular groups based on phylogenetic analysis. According to the results, *F. solani-melongenae* (FSSC 21) is dominant species to cause collar rot and fruit rot of passion fruit in Taiwan. Other species are including *F. solani* (FSSC 5), *F. liriodendri* (FSSC 24) and *F. noneumrtii* (FSSC 42). This result demonstrated that FSSC pathogens causing disease in passion fruit are diversities. In addition, *F. solani-melongenae* is homothallic type for perithecium production, and *F. liriodendra* could not produce macroconidia. Moreover, the pathogenicity of these FSSC species showed variation on different cultivars of passion fruit in Taiwan.

P7.3-003

DIVERSITY OF COLLETOTRICHUM SPECIES CAUSING APPLE BITTER ROT IN VIRGINIA

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Text

Apple is an economically lucrative fruit crop worldwide but can be severely impacted with bitter rot disease caused by multiple species in the *Colletotrichum acutatum* and *C. gloeosporioides* species complexes (CASC and CGSC). Identification of *Colletotrichum spp.* is important due to species-specific differences in life cycle, virulence, temperature requirements and fungicide sensitivity. We collected over 600 *Colletotrichum* isolates from symptomatic apple fruit from 35 locations in Virginia. After morphological examination, in Virginia the species in CGSC dominated with 65.5% of isolates in comparison to the 34.5% of CASC isolates. Using multi-locus phylogenetic analyses of *ITS, GAPDH* and *ACT* for CASC, and *ITS, GAPDH, CAL, ACT, APN2, ApMat* and *GS* genes for CGSC, 82 representative isolates were identified to the species level. In Virginia, we identified *C. fructicola, C. chrysophilum, C siamense* and *C. theobromicola* in CGSC and *C. fioriniae* and *C. nymphaeae* in CASC. The most dominant species in Virginia are *C. fructicola, C. chrysophilum* and *C. fioriniae*. So far, we sequenced 10 new genomes including two isolates of *C. fioriniae,* three isolates of *C. chrysophilum*, three isolates of *C. noveboracense* and three isolates of *C. nupharicola* collected from apple fruit, yellow water lily and *Juglans nigra*. Further work on genome sequencing of other isolates from Virginia is ongoing. Our work contributes to better understanding of *Colletotrichum spp.* diversity in the U.S.A.

P7.3-004

VIRULENCE AND PATHOTYPES OF PLASMODIOPHORA BRASSICAE IN CENTRAL EUROPE AND SWEDEN

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Text

Clubroot is a soil-borne plant disease caused by Plasmodiophora brassicae. The current study evaluates the distribution and prevalence of pathogen pathotypes in oilseed rape crops in the Czech Republic, Germany, Poland, and Sweden.
Field information revealed significant differences between disease incidences between

countries. Approximately 52% of infested fields had a low occurrence of the disease, 31% had moderate clubroot incidence, and 17% showed a high incidence of the disease. In total, 84 isolates of P. brassicae were collected in all countries. The pathotypes classification was determined using 17 Brassica hosts, including the European Clubroot Differentials (ECD), the Somé set, and the resistant oilseed rape cv. 'Mendel'.
The virulence analysis of the isolates using the ECD set revealed 42 designated pathotypes, the most prevalent being 16/31/31 in Germany, Poland, and Sweden and 16/06/12 in the Czech Republic, Germany, and Poland. The Somé set identified six pathotypes, with 1-4 per country, with P1 being the most widespread in Germany, Poland, and Sweden and P3 being common in the Czech Republic, Germany, and Poland. The study showed an increase in virulence in the P. brassicae population compared to previous studies, with several isolates overcoming the resistance of cv. 'Mendel' and Brassica rapa genotypes. A negative correlation was found between clubroot incidence and the frequency of oilseed rape in crop rotation and between soil pH and clubroot incidence.

P7.3-005

DIVERSITY AND CHARACTERIZATION OF FUSARIUM OXYSPORUM SPECIES COMPLEX (FOSC) AND FUSARIUM SOLANI SPECIES COMPLEX (FSSC) CAUSING ORCHID DISEASES IN TAIWAN.

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Text
The *Fusarium* causing diseases in Orchidaceae are more than eight species, especially, *F. oxysporum* and *F. solani* with wide hosts. The two *Fusarium* species are considered as species complex, FOSC and FSSC. In Taiwan, orchid plants are important flower for export; however, the population and characterization of *Fusarium* pathogens in orchids in Taiwan were obscured. The orchids showed *Fusarium*-like symptoms in garden were collected, including epiphytic orchid (8 species), semi-terrestrial orchid (1 species) and terrestrial orchid (2 species). Results indicated that 88 FOSC isolates and 80 FSSC isolates were obtained and confirmed their pathogenicity in original host. In addition, the terrestrial orchids are major host of FOSC and epiphytic orchids are major host of FSSC. Within phylogenetic analyses, FOSC isolates are divided into *F. nirenbergiae*, *F. curvatum*, *F. contaminatum*, *F. triseptatum* and *F. odoratissimum* based on *cal*, *rpb2*, *tef-1α* and *tub2* genes sequences (Lombard's classification system); meanwhile FSSC isolates could be separated into six molecular groups based on ITS rDNA and *rpb2* and *tef-1α* genes. Among the six groups, three groups were identified as *F. keratoplasticum*, *F. solani* and *F. solani-melongenae*; but another three groups distinguished from knew FSSC species. Moreover, the isolates in two unknow species groups belong homothallic type for perithecium production. Host rang test indicated that FOSC and FSSC isolates showed cross-infection in different orchids.

P7.3-006

BIOLOGY OF PENTASTIRIDIUS LEPORINUS AND APPROACHES TO MONITOR THE MAIN VECTOR OF THE SYNDROME ‘BASSES RICHESSES’ IN SUGAR BEET

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Text

Pentastiridius leporinus is the main vector of Syndrome ‘basses richesses’ (SBR), a fast-spreading sugar beet (Beta vulgaris) disease in Central European sugar beet growing areas. The disease is caused by two procaryotic phloem-limited bacterial pathogens, the γ-3 proteobacterium ‘Can. Arsenophonus phytopathogenicus’, and the stolbur phytoplasma (16SrXII-A subgroup) ‘Can. Phytoplasma solani’. SBR infections in sugar beet can lead up to 5 % sugar content loss and high yield reduction. P. leporinus has adapted from its natural host reed to sugar beet in crop rotation with cereals such as winter wheat or spring barley. Here we present a mass rearing protocol and vector life history data that will help to overcome an important bottleneck in SBR research and enhance efforts in developing integrated pest management tools. More, monitoring of this insect vector based on morphological identification is challenging as two other cixiid species Reptalus quinquecostatus and Hyalesthes obsoletus with similar external characters are known to additionally appear in sugar beet fields. A PCR-based method is provided for simple and reliable detection of P. leporinus collected via nets or traps. This method also detects eggs and all nymphal stages and differentiates this vector from the most common Auchenorrhyncha species occurring in sugar beet fields. Furthermore, the phylogenetic relationship of these morphologically close species was investigated based on cytochrome oxidase I gene.

P7.3-007

GENOMES AND PATHOTYPES OF PLASMODIOPHORA BRASSICAE IN POLAND

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Text

Highly pathogenic protist-like organism Plasmodiophora brassicae belonging to the infrakingdom Rhizaria is the cause of clubroot, a damaging soilborne disease of brassicas. Sixteen isolates collected in 2017-2021 originating from the roots of winter oilseed rape (Brassica napus) with clubroot symptoms were gathered from nine regions of Poland and

subjected to pathotype studies. using four identification systems (Buczacki, Som?, Strelkov, Williams) and two thresholds of disease index (25%, 50%). There were 10, 3, 6 and 4 pathotypes according to 25% threshold respectively. The systems by Buczacki, Som? and Williams evaluated using 50% threshold resulted in 13, 4 and 6 pathotype designations respectively. Differences in thresholds changed the pathogen designation in 31 out of 48 designations (65%).Twelve isolates were able to infect the cultivar Mendel, which is currently the main source of resistance of oilseed rape to clubroot in Poland. Genome sequencing of two isolates was performed using the Illumina MiSeq platform with 250 bp reading. Studies have shown differences from the reference isolate deposited in the NCBI database (approx. 5000 nucleotide changes). Differences between the tested isolates were also found. Bioinformatic analyses and genome sequencing of the other P. brassicae isolates/pathotypes from Poland are under way.

P7.3-008

FIRST REPORT OF RHIZOBIUM RHIZOGENES CAUSING CROWN GALL ON IN CHILE

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Text

Crown gall is an important disease worldwide caused by several species belonging to the Rhizobiaceae family. In Chile, only Agrobacterium tumefaciens has been reported causing this disease. In the 2018-2019 season a survey was conducted to detect other species causing galls in fruit trees in southern Chile (32°47'S to 49°50'S). Bacterial isolating was made by sowing dilutions of galls macerates from blueberry, raspberry and cherry plants onto Yeast Extract Mannitol with potassium tellurite (YEM+Kt). Twelve bacteria with typical Rhizobiaceae characteristic were isolated. Pathogenicity of the isolates were tested in tomato, kalanchoe, blueberry and cherry plants by injecting the bacterial suspensions (1 x 108 cells ml-1). Strain C-58 and sterile water were used as positive and negative control, respectively. After 30 to 90 d in a greenhouse depending on plant species was evaluated. Of the 12 isolates only the strain RGM (3430) isolated from blueberry and RGM (3422) isolated from cherry were able to induce tumors in four plant species inoculated. Genome sequencing revealed the presence of the tumor inducing (TI) plasmid in both strains. A multilocus phylogenetic analysis (atpD, gyrB, recA, rpoB) clustered both strains with Rhizobium rhizogenes reference strains (95% bootstrap). Average Nucleotide Identity (ANI) of complete genome confirmed the identification with ANI value of 98%. This is the first report of R. rhizogenes causing tumors in blueberry and cherry trees in Chile.

P7.3-009

A COMPREHENSIVE SURVEY OF PREVALENCE OF ROOT-KNOT NEMATODES AND CULTIVAR RESISTANCE ASSOCIATED WITH PEPPERS IN HAINAN, CHINA

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Text

Root-knot nematodes (RKNs) are considered as a huge threat to agricultural crops, including pepper in China. Hainan Island is the main producer of pepper, where the climate conditions and crop planting patterns are favorable for infection by RKNs. We first conducted a detailed investigation about the occurrence, severity and population distribution of RKNs infected pepper in 310 fields located in all 18 geographical areas of Hainan. Our results showed that RKNs belonging to *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* were found in Hainan. Notably, *M. enterolobii* is now the dominant population in this area, which were found in 87.5% of RKN infected pepper samples. *M. enterolobii* was first reported in Hainan from the roots of pacara earpod trees. It has caused more concerns worldwide due to its high aggressiveness, increasing geographical distribution, wide host range and pathogenicity in plants carrying resistance genes. We then evaluated the resistance level to common pepper cultivars targeting *M. incognita* and *M. enterolobii*. Among the tested cultivars, about 67% of cultivars showed resistant or highly resistant to *M. incognita*. However, *M. enterolobii* was highly pathogenic on all tested cultivars, which could explain the reason for its rapid expansion throughout Hainan. In conclusion, this study promotes the comprehensive understanding of RKN distribution and host resistance level in Hainan, which will guide the effective control of root-knot nematodes.

P7.3-010

DISEASE EMERGENCE SCENARIOS LINKED TO SPATIAL DISTRIBUTION OF A NOVEL CROP

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Text

Disease-ecology studies often address a specific disease scenario. Many crops have evolved natural resistance to diseases and pests in their native environments, but when a novel crop is introduced into a new environment, it can encounter new pathogens, possibly resulting in severe diseases.

The drivers of emerging diseases in macadamia, an evergreen tree nut crop that is native to Australia but now widely produced in several tropical and subtropical regions in the Americas, Asian, and African countries, may be categorised into three distinct disease patterns. These include (i) interplay of novel pathogens and lack natural host resistance resulting in the development of novel diseases, (ii) a spill-over effect from other plant hosts, and (iii) complex interactions of microbial communities with climatic factors that influence or drive disease outbreaks.

Conceptual framework for unravelling host susceptibility and exposure to multiple pathogens that result in various diseases is critical for prevention and management practices. Using fungal and oomycetes diseases in macadamia, this study demonstrated limits of ‘one microbe-one disease postulate”. It revealed a set of drivers of pathogen profiles, disease ecology and transmission dynamics supports each of the three disease emergence categories. It is

important to survey and monitor disease diversity and take necessary steps to prevent disease outbreaks to ensure the long-term sustainability of crop production.

Bacteriophages: ecological roles and potential applications against bacterial plant pathogens

C3.6-1

ECOLOGY AND EVOLUTION OF PHAGE-BACTERIA INTERACTIONS DRIVE BACTERIAL WILT DISEASE DYNAMICS

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Text
Bacterial viruses - phages - shape the structure of natural bacterial communities via the direct killing of cells and by imposing selection for the evolution of phage resistance. While phages are abundant in soils, their effects on plant-pathogenic bacteria, rhizosphere bacterial communities and associated plants are poorly understood. In this talk, I will present our current work on the role of soil phage communities in constraining the invasions and infections by soil-borne Ralstonia solanacearum plant-pathogenic bacterium. Specifically, I will discuss direct and indirect phage effects in complex rhizosphere microbiomes and how pathogen density regulation could be mediated by complex phage-bacteria interactions. Furthermore, I will highlight how phage selection can rapidly select phage-resistant mutants and how this feature could be used as an evolutionary tool to steer pathogen virulence through costly life-history trade-offs. Together, our findings highlight that soil suppressiveness, which is most often attributed to bacteria, could be determined by the rhizosphere phage communities, highlighting the potential for developing phage therapeutics to control plant pathogen invasions in agriculture.

C3.6-2

BIOGEOGRAPHIC DISTRIBUTION IMPACTS XANTHOMONAS ARBORICOLA PV. PRUNI SUSCEPTIBILITY TO BACTERIOPHAGE

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Text
Xanthomonas arboricola pv. *pruni* (Xap) is the causal agent of bacterial spot on *Prunus spp*. Lytic phages are being considered to manage bacterial disease due to their ability to infect and kill their bacterial hosts (i.e., Xap); however, bacteria can evolve resistance to phage. Despite the abundance of phage and frequency of phage-bacteria interactions in the environment, there is little knowledge on how biogeography impacts the genetic mechanisms underlying phage infectivity. To examine how geography impacts these mechanisms, 12 phages isolated from symptomatic peach leaves in North Carolina (United States [**US**]) were tested for their ability to lyse Xap strains from Brazil, Uruguay, and the US. The four strains from Brazil and one from the US showed varying levels of susceptibility to the 12 phages tested, while a strain from Uruguay and two from Brazil were moderately and fully resistant to phage lysis. Illumina and PacBio were used to generate closed bacterial genomes and construct a core genome. Phylogenetic analysis revealed the five susceptible strains clustered into one group while the strain from Uruguay and the two resistant strains from Brazil clustered into a second group. Genes unique to the five susceptible strains and the three resistant strains were identified. Predicted annotations include outer membrane and other hypothetical proteins. Understanding phage resistance mechanisms is important if phages are to be used to successfully manage bacterial pathogens.

C3.6-3

COEVOLUTIONARY ANALYSIS OF BACTERIA-PHAGE INTERACTIONS IDENTIFIES POTENTIAL RECEPTOR TARGETS FOR PHAGE INFECTION

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Text
Bacterial canker, caused by *Pseudomonas syringae* pathovars including pv. *syringae* (*Pss*) and pv. *morsprunorum* (*Psm*), is a major disease of *Prunus* species such as cherry (*Prunus avium*). There is currently no treatment for this disease. One method of control is use of naturally occurring bacteriophage (phage) infective to the bacterial pathogens. We have isolated and characterised phages (MR) with host specificity to *Pss* and *Psm*. Before field application as a biocontrol agent, it is important to assess their efficacy as well as changes occurring in the bacterial population to prevent phage infection. The growth of *Pss* populations co-inoculated with MR phage individually or in combination were measured and showed that either the phages are antagonising one another or that the bacteria evolve differently under multiple phage pressure. In either case, this would reduce the efficacy of phage control of bacterial populations. To understand this interaction further, *Pss* and phage were coevolved over 10 generations and the genomic and behavioural changes in bacterial populations were measured. *Pss* evolved mechanisms of resistance to phages through modifications to lipopolysaccharide (LPS) or mutations in a glycosyltransferase involved in LPS synthesis. Coevolved phages were more potent at reducing the bacterial population. Therefore, understanding the genetic mechanisms of coevolution in generating more infective phages for precise targeting of the bacterial population is essential.

C3.6-4

DO PHAGES HAVE AN IMPACT ON THE DIVERSITY OF PSEUDOMONAS SYRINGAE ON APRICOT TREES?

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Text

Pseudomonas syringae is diverse and ubiquitous complex of bacteria whose life history is linked to the water cycle. Some strains of *P. syringae* are pathogenic and cause up to 60% of yield losses in a large variety of crops. Recent research suggests that strains from apricot are phylogenetically more closely related to environmental strains than to other plant-associated strains. Neither the pathogenicity nor the phenotypic characteristics of *P. syringae* strains in bacterial canker of apricot are predictable by their phylogenetic position.
Phages play a major role in the evolution of their bacterial host populations by influencing their population size and structure, driving their genetic diversity and also their pathogenic potential.
This project aims to unravel the role of phages and their influence on the population structure of *P. syringae* on apricot. So first, *P. syringae*-specific phages were collected from soils of apricot orchard. Our work reveals a ubiquitous presence of phages and their extraordinary diversity with 10 new genera, 20 new species and 23 new phages have been identified. Their host range on a set of 51 *P. syringae* strains was also characterised. This set of strains was constructed by selecting pairs of *P. syringae* strains isolated from apricot and isolated from the non-agricultural environment from the same phylogroup. This allows the infectivity of phages to be tested on bacterial strains from the same phylogroup but from different isolation sites.

C3.6-5

KEYS AND COST OF THE TOXIC RELATIONSHIP BETWEEN NOVEL PHAGES AND XANTHOMONAS HORTORUM PV. VITIANS : MOLECULAR DETERMINANTS AND TRADE-OFF IN PLANTA

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Text

The use of bacteriophages as biocontrol agents of phytopathogenic bacteria at different stages of the agricultural supply chain has shown promising results in recent years, notably on *Xanthomonas* spp. Bacterial leaf spot of lettuce caused by *Xanthomonas hortorum* pv. *vitians* (*Xhv*) is a major threat for lettuce producers worldwide due to the lack of effective disease control strategies. In order to explore the potential of phages to reduce the severity and incidence of this plant disease, we isolated and characterized several novel lytic phages. Their genome sequences, morphologies, growth kinetic parameters and host ranges were characterized. A transposon insertion sequencing experiment revealed that 36 genes

predominantly involved in lipopolysaccharide biosynthesis were required for successful ΦXhv1 infection of our *Xhv* model strain LM16734. Phenotypic analyses of transposon insertion and deletion mutants resistant to ΦXhv1 suggested that this phage specifically binds lateral branches of the O-antigen to achieve its adsorption. Interestingly, some phage-resistant mutants defective in O-antigen biosynthesis showed a decreased fitness in planta and reduced motility on soft agar assays, resulting in a trade-off unlikely to occur in planta. Altogether, these results would pave the way to the design a phage cocktail combining various infection strategies and complementary host spectra, thus preventing the occurrence of resistances and ensuring the sustainability of the biocontrol.

C3.6-6

EVADING INFECTION: TEMPERATURE AFFECTS INTERACTION BETWEEN DICKEYA FANGZHONGDAI AND ITS BACTERIOPHAGE

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Text

Soft rot diseases caused by pathogens like *Dickeya* spp. significantly reduce the yield of agricultural crops used to meet the food needs of the world's growing population. No efficient chemical control strategies exist, and bacteriophage biocontrol has been proposed as a promising alternative. However, complex interactions among phages, bacteria, and their environment are still poorly understood yet critical for efficient therapy applications. In our study, we investigated the influence of temperature and pH on the interactions in the previously described *Dickeya fangzhongda*i and a BF25/12 *Podoviridae* bacteriophage system (Alic et al. 2017).
Different *D. fangzhongdai* strains exhibit different sensitivity phenotypes at 28 °C (standard growth temperature), however all susceptible strains showed reduced bacteriophage susceptibility at higher temperatures (37 °C) including reduction in phage adsorption and absence of bacterial lysis in liquid culture. Other factors, such as pH and bacteriophage concentration, did not affect the observed differences in phage susceptibility. The results of our study demonstrate the ability of *D. fangzhongdai* to evade bacteriophage infection at temperatures relevant to its occurrence in environment and greenhouse production. This is particularly important for bacteriophage biocontrol applications in agriculture and plant health.

P3.6-001

CHARACTERIZATION OF NOVEL BACTERIOPHAGES AGAINST ERWINIA AMYLOVORA, A CAUSAL PATHOGEN FOR FIRE BLIGHT DISEASE AND THEIR APPLICATION

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Text

Fire blight disease caused by *Erwinia amylovora* has been a major problem for cultivation of apple and pear. The development of effective and sustainable strategies for controlling this disease is necessary as alternatives of antibiotics and other conventional agricultural chemicals, which have been widely used. In this study, we focused on bacteriophages that only infect target bacteria as an alternative control agent. Lytic bacteriophages killing *Erwinia amylovora* and *Erwinia pyrifoliae* were isolated from soil and water samples and selected based on their morphology and stability under diverse environmental conditions. Four selelcted bacteriophages, φ13, φ14, φ22, and φ33, were found to be stable under a wide range of temperature (4 °C, 26 °C and 37 °C), pH (pH 3 to pH 11) and UV (λ= 306 and λ= 365). Transmission electron microscopy showed that φ13, φ14, and φ22 belong to the *Podoviridae* family and φ33 belongs to the *Mmyoviridae* family. Using apple seedlings and young apple fruits, we evaluated the control efficacy of these bacteriophages against fire blight. For this assay, *E amylovora* was infected by non-wound inoculation method on plants, and bacteriophages were treated 2 hours before or 6 hours after bacterial inoculation. The disease was significantly reduced by pretreatment of bacteriophages, indicating that selected bacteriophages can be potential candidates as biocontrol agents of *E amylovora*.

P3.6-002

DEVELOPMENT OF PHAGE COCKTAIL FOR PREVENTING SOFT ROT DISEASE CAUSED BY PECTOBACTERIUM SPECIES IN KIMCHI CABBAGE

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Text

Chemical bactericides such as antibiotics and copper-based agents have been extensively used to control plant pathogens. However, antimicrobial resistance has emerged that are recognized as serious threats in human health as well as agriculture. To overcome this problem, phages have been in the spotlight as alternative antimicrobial agents to replace chemical bactericides. Pectobacterium species causes soft-rot disease in various crops by producing plant cell wall-degrading enzymes (PCWDEs). The aim of this study is to develop phage cocktail for preventing soft rot disease caused by Pectobacterium. Two virulent Pectobacterium phages (phiPccP-2, and phiPccP-3) were isolated from rotten cabbage, and had 171,484 bp, and 107,777 bp genome, respectively. Lytic activities of phiPccP-2 and phiPccP-3 were maintained after incubation under various environmental conditions such as pH ranging from 4 to 11, -80°C to 50°C, UV-A, and UV-B. Phage cocktail consisting of phiPccP-2 and phiPccP-3 could suppress the emergence of phage-resistant Pectobacterium in killing curve assay and efficiently prevent soft rot disease in detached mature leaves of Kimchi cabbage. Phage phiPccP-1 was added to phage cocktail to enhance the antimicrobial effect, and phage cocktail treatment effectively protected Kimchi cabbage seedlings from soft rot disease, compared to single phage treatments. These results suggest high potential of phage cocktail as alternative antimicrobial agents to control Pectobacterium.

P3.6-003

DEVELOPING PHAGE THERAPY TO REDUCE PLANT PATHOGEN VIRULENCE IN RALSTONIA SOLANACEARUM

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Text

Ralstonia solanacearum is a devastating plant pathogen which causes bacterial wilt and brown rot disease, leading to huge economic losses worldwide. Several factors contribute to this: a large host range, persistence in soil and water, and broad geographical distribution. Moreover, there is no effective method of plant protection against this pathogen, making it imperative to develop novel control approaches. Bacteriophages have been proposed as biocontrol agents for *R. solanacearum*, as they are highly specific to the pathogen and propagate rapidly in soil. Phage efficacy at reducing bacterial densities has been reported, but over time bacteria evolve resistance to phages. Resistance is highly costly however, compromising both bacterial fitness and virulence. Therefore, phages could provide durable control of *R. solanacearum*, by reducing pathogen densities and leading to evolutionary trade-offs that make resistant bacteria less pathogenic. This study aims to understand how phage resistance reduces pathogen virulence. We will create a library of phage-resistant mutants using experimental evolution to identify genes linked with resistance. The effects of resistance mutations on pathogen fitness will be tested in plant rhizosphere microcosms, focusing on pathogen metabolic versatility, root colonization capability, and ability to evade plant immunity. Transcriptomic analyses will also be conducted to elucidate phage-bacteria interactions and the role of phage defence systems.

P3.6-004

CHARACTERISATION OF PHAGE THAT LYSE BRENNERIA GOODWINII AND GIBBSIELLA QUERCINECANS, THE CAUSATIVE AGENTS OF BLEEDING CANKERS ASSOCIATED WITH ACUTE OAK DECLINE

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Text

Acute oak decline (AOD) is an emerging disease of oak trees (*Quercus* spp.), that includes the production of dark bleeding cankers on bark and potential tree mortality within 3 to 5 years. AOD is caused by a polymicrobial complex of several bacterial species, the most important of which are the gram negative phytopathogens *Brenneria goodwinii* and *Gibbsiella quercinecans*. No treatments are yet available against AOD, and environmental impacts restrain the use of antimicrobial treatments . However, a potential avenue for biocontrol of

these bacteria species is the use of bacteriophage (phage), which have proven effective in treating several other bacterial tree diseases. Multiple phages from diseased lesions of oak trees have been isolated that can lyse *G. quercinecans* and *B. goodwinii* in England. A subset of unique phages has been characterised via several assays, including killing curve assays, one-step growth curves, and temperature and UV survival assays to determine their suitability as biocontrol agents. In addition, their genomes have sequenced in order to perform taxonomic characterisation and comparative genomic analysis. The results of this study will help identify phages or phage cocktails to treat bacterial cankers and reduce the impact of AOD. It will also form the basis of research on phage identification and dynamics within the AOD pathosystem.

P3.6-005

ISOLATION AND CHARACTERISATION OF VIRULENT PHAGES AGAINST EUROPEAN XYLELLA FASTIDIOSA SUBSPECIES

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Text
Xylella fastidiosa (Xf) is ranked in the world top 10 most important bacterial plant pathogens [1] and its host range is estimated at 638 plant species [2]. This bacterium colonizes the xylem vessels and forms biofilms that block water flow, resulting in plant wilting and death [3]. *Xf* is transmitted by biting-sucking insects but also by the trading of plants.
The most spread and damaging subspecies *(Xf fastidiosa, Xf pauca, and Xf multiplex)* are present in many countries and infect plants of agricultural interest [4]. Therefore its potential economic, environmental and social impact are considered the most serious in the Union [5]. Since 2013, *Xfp* has been identified in Europe on olive trees in Italy. In Spain, clusters of infection on olive and almond trees have been detected, and in France heavy infection have been reported on olive trees in the PACA region. Corsica is entirely infected by *Xfm*.
Phages are a promising mean of biocontrol and our objective is to develop a cocktail targeting Mediterranean strains. For this, Xf-associated and non-Xf-associated environments were tested to find phages. To bypass the difficulties of experimentation on *Xf*, a surrogate host *Xanthomonas albilineans* was used to isolate phages whose efficacy is then tested on *Xf* [6]. We have now about twenty characterized phages having efficiency on *Xfm*, some being also efficient on *Xff*.

P3.6-006

BACTERIOPHAGE AS A BIOCONTROL AGENT IN BACTERIAL BLOOD DISEASE OF BANANA IN INDONESIA

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Text
Blood disease of bananas caused by Ralstonia syzygii subsp. celebesensis (Rsc) has been one of the limiting factors for banana production in Indonesia. The current disease management has not yet provided effective results to supress the spread of the disease. This study was aimed to examine the potential of bacteriophage as a biocontrol agent in controlling Rsc in several banana cultivars in a Greenhouse and in the large field. Four isolates of bacteriophage (?BTF1, ?BTF 2, ?BTF 3, and ?BTF 4) were applied on four cultivars (Barangan, Cavendish, Raja Bulu, and Kepok Putih) infected by Rsc. The treatments of four bacteriophage isolates did not show any symptoms on all infected cultivars. Furthermore, the four bacteriophage isolates were able to supress the disease incidence and disease intensity on the fourth week of observation in the Greenhouse and on the tenth month of observation in the large field. No disease incidence and disease severity was appeared until the end of the observation, compared with 100% of disease incidence and disease severity in the control treatments. The weight of fresh fruits per bunch of the treated cultivars increased from the range of 19.26-30 kg, compared to no fresh fruits were harvested in the control treatments. This study suggests the application of bacteriophage has a great potential to reduce the infection of banana blood disease.

P3.6-007

PHAGE BIOCONTROL OF BACTERIAL LEAF BLIGHT ON RICE CAUSED BY XANTHOMONAS ORYZAE PV. ORYZAE IN VIETNAM

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Text
Rice bacterial leaf blight (BLB), caused by Xanthomonas oryzae pv. oryzae, is a serve disease in in many rice-growing countries. Bacteriocides such as antibiotics and copper compounds have mainly been used for managing this disease. However, this is not considered good methods in sustainable agriculture. In this study, we used phage therapy for control the disease. One hundred seven bacteriophages were isolated from six provinces in the Mekong Delta of Vietnam. Five promising phages, designated ΦVL12, ΦDT63c, ΦHG48b, ΦDT60b and ΦAG68a, were selected based on their ability to lyse more than 45 out of 62 tested Xoo strains and produced large plaques. ΦDT60b produced the largest plaques. Under greenhouse conditions, four phages (ΦVL12, Φ?T60b, Φ?T63c and ΦAG68a) at a titre of 108 pfu/ml were most effective in reducing BLB infection, with ΦDT60b being the most effective. The four phages belonged to the lytic Xiphoviridae, so they are safe for applying in open fields. In the field, four phage treatments, i.e. ΦDT60b (107 pfu/ml or 108 pfu/ml) or mixtures of four phages (107 pfu/ml or 108 pfu/ml) showed efficacy in disease

reduction. Two treatments, i.e. ?DT60b (108 pfu/ml) and the mixture of 4 phages (108 pfu/ml) provided excellent disease protection and increased yield compared to other phage treatments and were equally efficient as the control bactericide treatment, Starner 20WP.
Keyword: bacterial leaf blight, bacteriophage, rice, Xanthomonas oryzae pv. oryzae

Bioinvasion in the urban environment: pathways, early warning, mitigation measures, institutional frameworks and policy implementation

C1.5-1

URBAN TREE INVENTORIES - AN EFFECTIVE TOOL IN BIOSECURITY?

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Text
Tree inventories are thereby one of the most fundamental components of planning, managing and developing urban forestry. Inventories are therefore being conducted all over the world, often with huge costs involved. However, many of these inventories contain too much information, with little focus on keeping the content updated, and are often conducted without a clear purpose. Nevertheless, inventories can be a huge resource that, with correct application, can have a significant impact on how urban forestry is governed and how it is perceived by the public. This presentation will have an international focus, looking at examples of how tree inventories are being used to tackle one of the main problems that urban forestry is facing, pests and pathogen. The presentation include examples of how managers have used urban tree inventories for communication, monitoring and prognosis of how future pests and pathogen might affect the urban tree population, the ecosystem services they provide and the nature based solutions they provide.

C1.5-2

COMPLEX HEALTH ISSUES FACED BY URBAN TREES

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Text
Trees growing in cities survive in a harsh environment, often with limited access to light, soil, and water and exposure to strong winds and elevated temperatures. These factors negatively impact health and vigour, influencing the various ecosystem services they provide. Additional to abiotic factors, biotic factors can adversely affect urban tree health. The ease with which goods and people move in cities reflects in elevated introduction and spread of harmful pests and pathogens. For these reasons, resistance to pests and diseases is particularly important for trees planted in urban environments. Assessment of the threat level and accurate diagnosis, along with optimally selected management measures that consider legal restrictions and the safety of inhabitants, are absolute necessities. Diagnostic facilities are required, along with systems and standards for monitoring threats from biotic factors. All such activities should aim to preserve the existing structure of parks and city gardens in a healthy, vigorous condition, minimizing costs. In Europe, tree species richness is higher in urban environments compared to the surrounding forests, with a different species assemblges as a result. Therefore, the interface between urban and surrounding forest creates opportunities for invasive species and pathogens to establish and spread.
This work will review the most critical issues impacting urban tree health.

C1.5-3

THE VALUE OF BOTANICAL GARDENS FOR GLOBAL PLANT HEALTH RESEARCH: A SOUTH AFRICAN CASE STUDY

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Text
Worldwide there is a growing number of damaging invasive plant pests, introduced largely by international trade and the movement of plant material. Notably many of these pests are not problematic in their native range or were unknown to science prior to their arrival in novel environments. It is challenging to respond to such incursions but improving surveillance, and in particular global cooperation in surveillance, is likely to have significant benefits. Sentinel plantings are increasingly being used to identify future threats before widespread invasions occur. Botanical gardens contain diverse plant collections, providing unique opportunities for sentinel research. Moreover, as they are often close to likely points of entry, botanical gardens are often among the first sites of establishment of new invasive pests. In 2016, a sentinel project was initiated in South African botanical gardens to improve surveillance and identification of new and emerging pest risks. The project has led to multiple first reports for the country, including the detection of the polyphagous shot hole borer. It has also provided opportunities for training activities with botanical garden staff and supported the development of novel management options for existing pest issues. By raising plant health and biosecurity best practice awareness and capacity, the intention is to provide increased opportunities for the detection of invasive plant pests in a country with a limited surveillance budget.

C1.5-4

TREE INSECT PESTS AND PATHOGENS: SOCIO-ECONOMIC AND ENVIRONMENTAL IMPACTS IN URBAN AREAS

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Text

Trees contribute greatly to urban environments and human well-being, yet relatively little is known about the extent to which a rising incidence of tree insect pests and pathogens may be affecting these contributions. To address this issue, we undertook a systematic review and synthesis of the diverse global empirical evidence on the impacts of urban tree insect pests and pathogens, using bibliographic databases. Following screening and appraisal of over 3000 articles from a wide range of fields, 100 studies from 28 countries, spanning 1979–2021, were conceptually sorted into a three-part framework: (1) environmental impacts, representing 95 of the studies, including those reporting on tree damage, mortality, reduced growth, and changes in tree function; (2) social impacts were reported by 35 of studies, including on aesthetics, human health, and safety hazards; and (3) economic impacts, reported in 24 of studies, including on costs of pest management, and economic losses. There has been a considerable increase in urban impact studies since 2011. Evidence gaps exist on impacts on climate-regulating capacity, including temperature regulation, water retention, soil erosion, and wind protection, but also on specific hazards, nuisances, human well-being, property damages, and hazard liabilities. As a knowledge synthesis, the findings will enable us to better forecast how growing threats will affect the urban forest and plan for these eventualities.

C1.5-5

REPORTING SYSTEMS AND CITIZEN SCIENCE FOR THE DETECTION OF REGULATED TREE PESTS AND PATHOGENS IN BRITAIN

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Text

Early detection of pests and pathogens is critical for their management, allowing rapid response and helping to prevent their establishment and spread. The approach from the UK government is to respond to plant pest and disease threats by focussing on a biosecurity continuum to minimise their potential impact, that includes increasing vigilance and prevention measures; better enforcement and responses to threats, and more public awareness and engagement with biosecurity. Active surveillance for pests and pathogens consists of regular and ongoing monitoring by official bodies. However, additional data (passive surveillance) on tree health from citizen scientists (including the general public, landowners and tree professionals) can support national surveillance programmes, and the detection of new threats in the wider landscape. TreeAlert was developed to allow

submissions of tree health reports in Britain. This was complemented with citizen science and the Observatree project, launched in 2013. Since then, Observatree volunteers have been trained to identify priority tree pests and diseases of concern and have carried out thousands of tree health surveys, some of which have led to eradication or mitigation measures. Now more than ever, there is a need to be vigilant to ensure that the benefits that trees provide to us are maintained for future generations. This is a good example of how working together with active and passive surveillance can help to build tree resilience.

C1.5-6

THE EPIDEMIC SPREAD OF PHYTOPHTHORA NICOTIANAE IN A MEDITERRANEAN PARK IN ATHENS IS ASSOCIATED WITH HIGH SITE INVASIBILITY AND PATHOGEN INVASIVENESS.

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Text

In the summer of 2016, extensive decline, and mortality of multiple species of Mediterranean shrubs and trees were recorded in a park in Athens (Greece). The progressive crown decline of shrubs and trees was occasionally associated with collar and stem bleeding cankers, and root necrosis. *Phytophthora nicotianae* was found to the responsible for such decline in the park. The possible drivers of this extensive decline were investigated and discussed with a specific focus on pathogen invasiveness and site invasibility. The pathogen was probably unintentionally introduced from commercial nurseries. Its spread was likely favored by intrinsic traits such as polyphagy, adaptation to warm climate, potential of recombination and reproduction, long persistence of inoculum in the soil, which determine the high invasiveness in the Mediterranean climate. A multiple linear regression model was elaborated that evidenced a significant association of the level of inoculum in the plant beds with specific site traits such as total host richness and the quote of susceptible hosts. Furthermore, a multivariate model evidenced that specific host taxa were the drivers of inoculum build-up. Based on the results of the present study, the strategies to reduce the site invasibility by the polyphagous *P. nicotianae* without sensibly affecting the park's ornamental value must consider the choice of plant taxa and their combination in the plant beds.

P1.5-001

BIOSECURITY SURVEILLANCE FOR EARLY DETECTION OF INVASIVE SPECIES IN URBAN FORESTS IN AUSTRALIA

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Text

Protection of Australia's forests from invasive species has recently been enhanced with the establishment of a National Forest Biosecurity Surveillance Program, funded by a forest

industry and government partnership. Governance of the Program is managed through a committee of government and industry stakeholders, with technical experts developing operational aspects of the Program. Locations for surveillance are identified through a risk-categorisation process both across Australia and within each State, with resources allocated accordingly. Insect traps with semiochemicals are used to monitor for invading or recently established insect pests. Sentinel trees are assessed annually for signs and symptoms of pest or pathogen attack. Stakeholder engagement and awareness is used to increase general surveillance, with workshops and field days held for local councils, arborists, botanic gardens. An app (MyPestTrees) has been developed to assist in general surveillance and reporting. Research (funded by the forest industry and government) has been undertaken, and is ongoing, to improve risk and pathway analysis, biosecurity surveillance, diagnostics, and response processes. Transformative technologies are being utilised: remote sensing and machine learning to semi-automatically detect and map key hosts in high-risk areas; metabarcoding and high throughput sequencing for diagnosis of samples and eDNA. Examples of responses to recent exotic pathogen invasions are provided.

P1.5-003

MONITORING EMERGING PATHOGENS IN HORSE CHESTNUT TREES ACROSS EUROPE AND THEIR CORRELATION TO THE LEAF MINER INFESTATION

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Text

Horse Chestnut trees (*Aesculus* spp.) are a crucial component of urban ecology, but their health is facing numerous challenges from the effects of climate change and the ongoing emergence of non-native pests and pathogens. To investigate the fungal communities causing disease symptoms, Horse Chestnut leaves were collected from a European transect covering POR, ESP, GER, DEN, and SWE. The fungi were identified using molecular markers (ITSu1/4, LSU/LR6, ELF1-α). Some fungi were present in all locations (*Alternaria* sp. or *Cladosporium* sp.), while others were found in specific regions (*Epicoccum* sp., *Biscogniauxia* sp., and *Leptosphaerulina* sp.). A field trial was conducted to investigate the role of the Leaf Miner (*Cameraria ohridella*) as a vector of the pathogens. Symptomatic leaves with Leaf Miner pupae were stored over the winter and following arranged with healthy saplings in net cages to prevent external interactions. Results showed that the Leaf Miner could play a significant role in transmitting fungal pathogens to healthy hosts. The Horse Chestnut trees are critical for the urban environment and provide ecological benefits such as air purification, carbon sequestration, and biodiversity. However, their health is threatened by the combination of climate change and the impact of the Leaf Miner and fungal pathogens. This study highlights the need for ongoing monitoring and management efforts to protect Horse Chestnut trees and their ecosystem benefits.

P1.5-004

PREVALENCE OF ASAV-INFECTED FLOWERING ASH (F. ORNUS) TREES OF TWO GERMAN METROPOLES

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Text

Viral diseases play a particular role in tree health as predisposing factors (Büttner et al, 2013, 2023). Therefore, the virus infection could counteract trees resilience, especially in cities with anticipated extreme climate conditions. Flowering ash (Fraxinus ornus L.) infected by the ash shoestring-associated virus (ASaV) develop characteristic leaf symptoms (Gaskin et al., 2021). The tree species is considered as a potential “climate tree” in urban environment. The prevalence of ASaV in this tree species should be assessed in the cities of Hamburg and Berlin.

In the vegetation periods 2019 and 2020, a survey on the occurrence of ASaV-associated symptoms was carried out in selected street sites of Hamburg considering 50 % of the 466 flowering ash trees planted in the city state. The trees were visually inspected twice per year. A selection of symptomatic and symptomless leaves was sampled and tested for an ASaV-infection by virus-specific RT-PCR.

In 2021, we examined 65% of the 1150 flowering ash trees grown in Berlin for ASaV symptoms. Visual scoring of the trees was followed by sampling of 82 symptomtic leaves, 85 samples without virus suspected symptoms, and 32 with ASaV-atypical discoloration and deformation, and 14 samples with mild ASaV-symptoms were taken and tested by ASaV-specific RT-PCR. The occurrence and distribution of ASaV-infected trees in these two cities make aware that viruses have to be listed as a considerable member of potential pathogens in trees.

P1.5-005

A DIVERSE RANGE OF PHYTOPHTHORA SPECIES RECOVERED FROM TWO SOUTH AFRICAN BOTANICAL GARDENS

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Text

The genus *Phytophthora* contains many destructive and globally important plant pathogens. In the last decade, targeted sampling efforts have resulted in a dramatic increase in the number of known species, as well as a better understanding of the global distribution of these important pathogens. Routine activities undertaken in botanical gardens, combined with great numbers of local and international visitors, place botanical gardens at risk with regard to the accidental introduction and establishment of pathogens. In this study, the

occurrence of *Phytophthora* was investigated in two South African botanical gardens. Symptomatic collar and stem tissues were collected, and root and rhizosphere soil samples were taken from trees exhibiting symptoms of decline. Standard baiting techniques and direct plating of symptomatic tissues revealed the presence of seven species of *Phytophthora* residing in four phylogenetic clades. Five of these species were already known to be present in South Africa. However, *P. aquimorbida* was recorded for the first time, and an undescribed species residing in *Phytophthora* clade 5 was detected. A novel host-pathogen association where *P. citrophthora* is causing tar-spot on indigenous *Celtis africana*, was also identified. This study highlights the importance of monitoring botanical gardens for the detection and discovery of pathogens, and emphasises their value as sites for the discovery of novel host-pathogen associations

P1.5-006

DIVERSITY OF PHYTOPHTHORA COMMUNITIES IN A SENTINEL ARBORETUM IN SOUTHERN ITALY

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Text

Phytophthora comprises numerous invasive plant pathogens threatening natural and anthropized ecosystems. Botanical gardens hosting diverse plant species of various origin may be ideal sites to study the diversity of communities associated to the plants, intercept exotic species and investigate the potential host range of species of this oomycete genus. In this respect, botanical gardens can be regarded as sentinel plant collections in surveillance and monitoring schemes. From 2016 to 2019, it was investigated the diversity of *Phytophthora* species associated with the rhizosphere soil of exotic and enedemic plant species in the Botanical Garden of Catania, Sicily. Isolations were carried out from plants with symptoms suggestive of *Phytophthora* infections using standard techinques and the NARPH agar selective medium. Samples were taken from soil, roots and stem bark of *Araucaria cookii*, *Phytolacca dioica*, *Sterculia diversifolia*, *Zelkowa sicula*, *Quercus suber*, *Olea europea*, *Coffea arabica*, *Pistacia atlantica* and *Morus alba*. Isolates were identified based on both morphological characters and the analysis of the ITS regions of rDNA. *Phytophthora multivora*, the prevalent species, was recovered from all hosts, except *C. arabica*. *Phytophthora nicotianae*, the second most frequent species, was recovered from *A. cookii*, *P. dioica*, *Q. suber* and *O. europaea*. *Phytophthora parvispora* was recovered from *C. arabica*. Some of these pathogen/host associations are first records worldwide.

P1.5-007

SMART URBAN FOREST MONITORING: A PROJECT FOR REMOTE SENSING DETECTION AND EARLY WARNING IN FOREST TREES.

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Text

The current phase of climate and environmental changes demands actions that enhance available resources and preserve the forest heritage to reduce biodiversity loss and safeguard socio-economic systems. Digitization, especially remote sensing, has emerged as an innovative tool for sustainable management of forest ecosystems. Smart Urban Forest Monitoring (SUFM) program proposes a multidisciplinary approach to detect alterations in vegetation indices caused by pests on trees and forests in urban areas. Multi/hyperspectral RGB-NIR and SAR images from PRISMA satellite will be interpolated with ground data derived by the Greenery Scanner developed at the Massachusetts Institute of Technology; further data will derive from additional aerial images, Tree Talkers sensors and traditional visual inspections for assessing the state of infestation. A data lake will be created to collect data, and models of data analysis that will be developed to correlate images to the degree of infestation. The platform will be developed firstly on a remote sensing system measuring the degree of infestation of the pine tortoise scale Toumeyella parvicornis on Pinus pinea in urban parks and forests in Rome, with the aims at extending the remote sensing system to the spectral anomalies caused by other biotic or abiotic stresses of forest systems in both urban and non-urban contexts.

P1.5-008

THE NOVI SAD POPLAR TREE DIE-BACK AND DECAY LINKED TO FUSARIUM SOLANI, GRAPHIUM PENICILLIOIDES, AND CYCLOCYBE AEGERITA

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Text

Since the introduction of poplar (Populus spp.) hybrids in Serbia in the 1960s, Populus euramericana cl. I-214 and cv. "Robusta" have been widely planted in Novi Sad, which is the capital of the Vojvodina Province. Apart from sporadic infections of Plagiostoma populinum (syn. Dothichiza populea) on P. euramericana, the poplars planted in this city over the years have remained healthy. However, these trees have recently begun to show symptoms of die-back and decay, making them vulnerable to windthrows and windbreaks. Urban tree vulnerability is important for the risk management strategies used by city authorities, but it also attracts the attention of environmental NGOs, academic institutions, citizen groups and

other stakeholders that want to create safe, green, and habitable cities. Therefore, Novi Sad has begun an intensive health monitoring of poplars which has been carried out by the Institute of Lowland Forestry and Environment in accordance with a city directive for the maintenance of urban tree's health. Several fungi were isolated during the surveys and were preliminary identified as Fusarium solani species complex, Graphium penicillioides species complex, and Cyclocybe aegerita using the ITS rRNA. Moreover, it was common to find C. aegerita mushrooms at the base of symptomatic trees. The study calls for the restoration of greenery in Novi Sad after documenting for the first time F. solani and G. penicillioides species complexes in urban environments in Serbia.

P1.5-009

PESTALOTOPISIS TUJAE ASSOCIATED WITH ARBORVITAE TIP BLIGHT IN NURSERIES, URBAN AND RURAL ENVIRONMENTS IN SERBIA

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Text
Arborvitae (Thuja occidentalis L.) is native to eastern North America and is widely grown in Europe as an evergreen ornamental. In Serbia, T. occidentalis is typically planted as a screen or hedge in private gardens and public green spaces like city parks and cemeteries. Moreover, T. occidentalis cultivars (e.g. "Woodwadii", „Smaragd", "Globosa", "Aurea Nana") are the most commonly produced ornamentals in Serbian nurseries. During the last decade, T. occidentalis has been endangered due to a canker disease caused by Botryosphaeriaceae fungi. However, T. occidentalis plants in Serbian nurseries as well as in urban and rural areas have recently displayed new, unique symptoms of a foliar blight disease, according to the plant health inspection service of the Institute of Lowland Forestry and Environment. The leaves had symptoms of tip blight with numerous pycnidia on the brown necrotic areas at the edge of the leaves and these symptoms differed from those caused by Botryosphaeriaceae. The fungus consistently isolated from the infected tissues was preliminary identified as Pestalotiopsis thujae using morphology and DNA sequence data for the internal transcribed spacer (ITS) rRNA. P. thujae is known to be opportunistic and recent stressful conditions caused by drought and heat waves might have weakened T. occidentalis, making it vulnerable to pathogens. The study is the first report of P. thujae in Serbia and it raises concerns regarding the impact of P. thujae on these trees.

BIOLOGICAL CONTROL - Part 1: The importance of augmentative biocontrol and plant microbiome function for plant health

C1.1-1

BENEFICIAL BACTERIA-FUNGI INTERACTIONS TO INCREASE PLANT GROWTH AND HEALTH

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Text

Fungi and bacteria cohabit in the plant environment. Separately they have been intensively studied for their impact on plant health. Except some particular cases, little attention has however been paid to the intricacy of fungi-bacteria interactions. Using different models, we described that specific multipartite interactions between fungi and bacteria can lead to an increase or reduction of plant growth and/or health.
For instance, we used *Serendipita indica* (syn. *Piriformospora indica*), which is a root colonizing endophytic fungus that holds capabilities to enhance plant growth and to confer resistance to different stresses. This fungus is further known as hosting a bacterial endosymbiont, *Rhizobium radiobacter*, living inside its hyphae. However, there was still a gap of knowledge if other bacteria can also have positive effects on the fungus. We analyzed how co-inoculations of endophytic bacteria and *S. indica* influence plant growth and increase protection against different fungal pathogens. Possible mechanisms behind these interactions were described based on genome and advanced microscopic analyses, using fungal and bacterial strains tagged with fluorescent markers. Bacteria-fungus seem to cooperate in the process of fungal root colonization and establishment, e.g., by increasing fungal sporulation and hyphae expansion. Genome analysis of positive bacteria revealed many genes potentially involved in fungal and plant growth stimulation, biocontrol and root colonization.

C1.1-2

THE ROLE OF SMALL RNAS IN REGULATING MYCOPARASITIC INTERACTIONS

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Text

Small-RNAs (sRNAs) are emerging as key players in host-microbe interactions, although their role in mycoparasitic interactions remain understudied. We used the mycoparasitic

fungus *Clonostachys rosea* and plant pathogenic mycohosts *Botrytis cinerea* and *Fusarium graminearum* and investigated the role of sRNAs in mycoparasitism. Deletion of dicer genes in *C. rosea* resulted in mutants with reduced antagonism towards *B. cinerea*, reduced biocontrol of fusarium foot rot disease on wheat, and reduced production of sorbicillin secondary metabolites. Transcriptome and sRNA sequencing of *C. rosea* strains (wild type and *dcl1* and *dcl2* deletion strains), *B. cinerea* and *F. graminearum* during *in vitro* interactions identified 61 novel microRNA-like RNAs (milRNAs) in *C. rosea*. Eleven of those were downregulated in the Δ*dcl2* mutant. In addition to putative endogenous gene targets, these 11 milRNAs were predicted to target *B. cinerea* and *F. graminearum* virulence factor genes, as these showed an increased expression during interaction with the Δ*dcl2* mutant incapable of producing the targeting milRNAs (cross-species RNA silencing). In summary, our work constitutes the first step in elucidating the role of sRNA-mediated RNAi in regulating mycoparasitism and poses the base for future studies focusing on the role of cross-species RNAi in interspecific fungal interactions. This also indicate an important mechanistic role of RNAi in biological disease control using *C. rosea* as a biocontrol agent.

C1.1-3

TRICHODERMA IN THE BIOCONTROL OF NON-CONVENTIONAL TARGETS: NEMATODES AND INSECT PESTS

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Text

Trichoderma is a multipurpose, plant-beneficial fungus of importance in agriculture as a direct biological control agent (BCA) that, because of its interaction with plants, has become an indirect BCA (inducing resistance in plants against pathogens and their vectors) and a biostimulant, which can reduce the application of agrochemicals, thus promoting an eco-sustainable agriculture (Woo et al. 2023). The direct action of *Trichoderma* as a BCA against phytopathogenic fungi and oomycetes is well documented. However, there is much less information on the biocontrol and mechanisms of action of non-conventional targets, such as nematodes and insects. *Trichoderma* has long been noted to have suppressive effects on *Meloidogyne* root-knot nematodes (RKN) and other phytopathogenic nematodes via parasitism, egg lysis by proteases and chitinases, or suppression of egg hatching by secondary metabolites (Woo et al. 2023). Furthermore, *Trichoderma* exhibits direct biocontrol of insect pests, and vectors of viral and bacterial diseases, through enzymatic activity on the midgut peritrophic matrix, inhibition of cuticle formation or displaying antifeedant effects mitigating herbivore attack. In addition, extracts of secondary metabolites can have inhibitory effects on insect larvae and attract predators and parasitoids (Di Lelio et al. 2023; Monte, 2023).

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C1.1-4

PLANT MODIFIES FUNGAL NON-SELF RECOGNITION TO FACILITATE MYCOVIRUS TRANSMISSION

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Text

Horizontal transmission of mycovirus is strictly limited by hyphal anastomosis that is regulated by fungal non-self recognition system. However, mycovirus transmission between vegetative incompatible groups occurs in nature despite of the non-self recognition system. Understanding the mechanism of mycovirus transmission in nature would facilitate using hypovirulent-associated mycoviruses (HAV) to combat plant diseases. Here, we show that the horizontal transmission efficiency of mycoviruses between vegetatively incompatible individuals of Sclerotinia sclerotiorum is significantly higher in planta than in vitro. Furthermore, plant proline is increased upon S. sclerotiorum infection, and results in suppressing expression of genes responsible for the fungal non-self recognition reaction. Additional in vitro experiments and genetic evidence confirmed that proline facilitates mycovirus transmission between fungal isolates via inhibition of non-self recognition reaction. The Application of HAV-mediated hypovirulent strain together with proline substantially improved the virocontrol efficiency of fungal plant pathogens in multiple agricultural fields. This work provides mechanism by which plants defend themselves against pathogenic fungi by enhancing mycovirus transmission and opens up a future strategy to promote HAV horizontal transmission efficacy for the biocontrol of plant diseases.

C1.1-5

INTRASPECIFIC PHENOTYPIC AND GENETIC VARIATION IN BIOCONTROL INTERACTIONS: CHALLENGES AND OPPORTUNITIES

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Text

In augmentative biocontrol, individuals from a single species typically display high variation in biocontrol efficacy. In order to investigate the basis for this phenomenon, we evaluated 63 genome-sequenced strains of the biocontrol fungus Clonostachys rosea for their ability to control fusarium foot rot disease on wheat, in vitro antagonism, plant growth promotion and growth rate on a range of different fungicides. There were significant ($P < 0.05$) differences between C. rosea strains in all investigated traits, and typically low correlations ($R2 < 0.27$) between traits. This suggests a mechanistically different basis for these biocontrol-related traits, allowing for genotypic adaptation. A transcriptome analysis further identified induction of distinct gene sets in C. rosea following interaction with Fusarium graminearum or Botrytis cinerea, suggesting different mechanisms of antagonism towards different pathogens. In parallel, 200 genotyped wheat varieties were evaluated for their responsiveness towards C. rosea-mediated biocontrol of septoria tritici blotch disease. There was significant ($P < 0.05$) variation among wheat genotypes for biocontrol responsiveness, which allowed the

identification of two genomic regions associated with the trait. In summary, we show a high degree of genotype-by-genotype effects on the outcome of biocontrol interactions, which opens opportunities for designing optimal biocontrol agent – crop combinations as well as biocontrol breeding programs.

C1.1-6

AUREOBASIDIUM PULLULANS: UP-AND-COMING BIOCONTROL AGENT AGAINST CROWN ROT, ROOT ROT, AND GREY MOULD

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Text

Wild populations of woodland strawberries (*Fragaria vesca*) typically host the beneficial yeast fungus *Aureobasidium pullulans*, which protects the wild plants (incl. fruits) from pathogens. We isolated *A. pullulans* (AP-SLU6) from wild strawberries and tested its potential as a biocontrol agent against several diseases on cultivated garden strawberry (*Fragaria* × *ananassa*) in a greenhouse setting as well as in open field plantations. Both traditional spray applications and a bee vectoring system (Flying Doctors®) for high-precision application were tested. In controlled greenhouse tests, we found that spray applications of *A. pullulans* significantly reduced crown rot, root rot, and grey mould caused by *Phytophthora cactorum* and *Botrytis cinerea*, respectively. However, the high-precision application of *A. pullulans* using the bee-vectoring system was most efficient against grey mould, leading to significantly reduced grey mould development on the harvested fruits by 45 % and increased shelf life by 100 % in comparison to control treatments. The field experiment in a commercial strawberry plantation confirmed the significant effects of *A. pullulans* on grey mould infestation and shelf life and furthermore showed that *A. pullulans* was more efficient than other established biocontrol products. We conclude that *A. pullulans* shows high potential for successful biological control of several strawberry diseases and discuss opportunities for further optimization of this beneficial fungus.

BIOLOGICAL CONTROL - Part 2: The importance of augmentative biocontrol and plant microbiome function for plant health

C2.2-1

MICROBIOME CONCEPTS FOR BIOCONTROL OF PLANT AND HUMAN PATHOGENS

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Text

Understanding and managing microbiomes offers promising perspectives for all kind of health issues. The synergistic impact of anthropogenic factors on the inter-linked plant microbiome such as biodiversity loss, pollution, ozone depletion, climate change and changing biogeochemical cycles is less understood. Recent studies indicated a general shift of the plant microbiota characterized by a decrease of evenness and specificity, and an increase of r-strategist and hypermutator prevalence as well as antimicrobial resistance. This typical microbiome signature of the Anthropocene is often followed by a dysbiosis, which leads to missing symbionts on one hand and outbreaks of pathogens in the other. Managing the microbiome and controlling pathogeny can provide solutions for sustainable agriculture. Beyond, the plant microbiome is connected across systems and crucial for human and planetary health issues as well. Examples will be discussed. In conclusion, diversity within the microbiome and resistome are interconnected, and should be managed by microbiome management together.

C2.2-2

THIRTY YEARS OF RESEARCH ON BIOLOGICAL CONTROL OF POTATO COMMON SCAB USING PLANT-BENEFICIAL BACTERIA: WHAT WE HAVE LEARNED, WHAT'S NEXT…

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Text

Potato common scab in an important disease caused by several *Streptomyces* species that significantly reduces potato tuber quality and market value. Management of common scab is difficult, and no conventional method can reliably control this disease. An alternative approach is the use of plant-beneficial bacteria as biocontrol agents, whose application in the field can significantly reduce common scab incidence and severity. However, inconsistencies in efficacy have been reported and yet no durable control has been achieved. In this presentation, the potato common scab disease, the diversity and distribution of scab-causing *Streptomyces* species and their phytotoxins and pathogenicity determinants will first be reviewed. Then, we will describe the diversity of bacterial strains successfully used to date to suppress potato common scab under controlled and field conditions, their biocontrol mechanisms and the factors influencing the biocontrol success. Finally, we will discuss the use of phenazine-producing *Pseudomonas* spp. as promising biocontrol agents against

potato common scab, an attractive approach supported by ten years of continuous research in our laboratory.

C2.2-3

BACTERIAL BIOPROTECTANTS IN NATURAL AND AUGMENTATIVE BIOLOGICAL CONTROL

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Text

The three most commonly studied bacterial genera in the biocontrol of plant pathogens are Bacillus, Pseudomonas and Streptomyces. In Europe, most commercial bioprotectants used for augmentative biological control are based on Bacillus, with a predominance of closely related cyclic lipopeptide-producing Bacillus velezensis (amyloliquefaciens) strains. In contrast, only two active substances based on Streptomyces and two based on Pseudomonas (2) strains are listed in the EU pesticides database showing that their commercial potential is insufficiently exploited. Streptomyces and Pseudomonas, however, play key roles in natural biological control as observed in disease-suppressive soils and produce secondary metabolites that directly or indirectly impact plant pathogens. In our lab, we are interested in the drivers of taxonomic and metabolic diversity in Pseudomonas populations in disease-suppressive soils. Our data from tropical soils suggest that the main drivers for Pseudomonas diversity are soil quality, plant age and interbacterial competition. This results in a diverse Pseudomonas population that produce a range of bioactive compounds, including structurally diverse cyclic lipopeptides and type VI secretion systems, that suppress plant pathogens.

C2.2-4

FROM BCA DISCOVERY IN CEREALS TO BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT

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Text

Fusarium head blight disease in cereals is feared as much for mycotoxins as for direct yield loss. Neither disease resistance nor chemical control are effective or efficient in controlling disease or mycotoxin accumulation. Biological control based on beneficial filamentous fungi is receiving increasing attention for controlling this disease [1]. We have isolated plant-associated fungi from the rhizosphere and endophytes from healthy cereal tissues and demonstrated their potential as biological control agents to combat Fusarium head blight in oats (*Avena sativa*) and wheat (*Triticum aestivum*). Disease control works both in controlled environments and/or the field. Studies of mechanisms using RNAseq and microscopy

implicate induced resistance as the primary disease reducing mechanism [2,3,4]. Furthermore, disease control by the BCAs *Penicillium olsonii* and *Clonostachys rosea* is associated with reduced accumulation of mycotoxins [2,4], and we have seen substantial stimulation of detoxification of DON (deoxynivalenol) in oats by glycosylation by *C. rosea* and direct treatment with DON itself [2].

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C2.2-5

PSEUDOMONAS PROTEGENS: BACTERIAL SWISS ARMY KNIVES FOR FUTURE PROSPECTS IN PLANT PROTECTION AGAINST PEST INSECTS

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Text

Root-associated bacteria of the *Pseudomonas protegens* subgroup are well known for their plant-beneficial activities, which include the suppression of plant pathogenic fungi and protists and priming of plant immune defense. These bacteria can also exhibit potent insecticidal activities towards plant-feeding larvae of certain Lepidopteran and Dipteran pest insects. The disease and pest control capacities of these *Pseudomonas* are enabled by the production and release of various antimicrobial metabolites and insecticidal toxins. *P. protegens* are highly competitive and capable of efficiently colonizing different ecological niches and hosts, notably plants and insects that are densely populated by competing bacteria. We identified several molecular determinants important for the modus operandi of these insecticidal *Pseudomonas* once ingested by pest insects. Among them are contractile phage tail related-structures, specifically the type VI secretion system and tailocins, that are important weapons for the invasion of the insect gut microbiome, competition with dominant microbiome members and related *Pseudomonas*, and consequently for insect pathogenicity. The in-depth understanding of the molecular basis of the insect pathogenic activities of these fascinating bacteria and of their role in the microbial ecology of plants and insects are a prerequisite to generate knowledge for the potential future implementation of such bacteria in agricultural pest management strategies.

C2.2-6

MECHANISM BY WHICH SELECTED BACILLUS STRAINS THAT CONFER TOLERANCE TO VERTICILLIUM WILT IN POTATO AND STIMULATE GROWTH

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Text

Potato Verticillium wilt caused by *Verticillium dahliae* is a vascular disease, that seriously affects potato yield and quality worldwide. Once the pathogen enters the vascular bundle, the use of chemicals as control is not only ineffective but also the polluting environment. Therefore, it is of great importance to find environmentally friendly biological control methods. In this study, five strains from potato-growing fields, with good antagonistic activity were selected. These strains had an antagonistic activity against the other three pathogens, *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora infestans*. Among five strains, *Bacillus velezensis* XS142 showed the highest biocontrol effect on *V. dahliae*, even higher than that of the SynCom of the 5 strains. XS142 colonize intercellular spaces within roots and also stimulated the growth of the potato. To study the mechanism of XS142, by which tolerance to *V. dahliae* is created, the genome of XS142 was sequenced and showed that eight gene clusters were involved in the production of secondary metabolites with potential antimicrobial properties. Further, the transcriptomes of potatoes treated with XS142 were analyzed and the mechanisms underlying the increased resistance and growth promotion will be discussed. Conclusion: This study showed that *B. velezensis* XS142 has a high potential to be used as a biocontrol agent for potato Verticillium wilt and/or plant growth-promotion agriculture.

F1.1-1

INSIGHTS INTO THE GENERALIST LIFESTYLE AND BIOCONTROL ACTIVITY OF FUNGAL SPECIES OF CLONOSTACHYS THROUGH ANALYSIS OF THEIR PREDICTED SECRETOMES

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Text

The fungal secretome comprises proteins that are involved in many aspects of fungal lifestyles, including adaptation to ecological niches. The aim of this study was to investigate the composition and function of predicted fungal secretomes in mycoparasitic and beneficial fungal-plant interactions of *Clonostachys rosea*, *C. byssicola*, *C. chloroleuca*, *C. rhizophaga*, *C. solani* and one unidentified *Clonostachys* species. The predicted secretomes of the analyzed species comprised around 8% of their proteomes. Mining of transcriptome data collected during previous studies showed that 18% of the genes encoding predicted secreted proteins were upregulated during the interactions with the mycohosts *Fusarium graminearum* and *Helminthosporium solani*. Functional annotation of the predicted secretomes revealed that the most represented protease family was subclass S8A, often involved in the response

to nematodes and mycohosts, while the most numerous lipases and carbohydrate-active enzyme (CAZyme) groups appeared to be potentially involved in eliciting defense responses in the plants. Comparison with three *Trichoderma* spp., another genus known for its mycoparasitic activity, revealed profound differences in secretome composition, such as differences in the dominant CAZyme classes. This work sheds light on the role of the secretome in the interaction of *Clonostachys* spp. with plants and fungi, and highlights differences with *Trichoderma* spp. sharing similar lifestyles and ecological niches.

F1.1-2

ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS FROM APPLE FLOWERS IN TRENTINO AND THEIR EVALUATION AS BIOCONTROL AGENTS OF ERWINIA AMYLOVORA

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Text

Fire blight caused by *Erwinia amylovora* (*Ea*) represents a great threat to apple and pear production worldwide. For instance, the outbreak of fire blight occurred in Trentino caused a relevant reduction of crop yield in 2020. It is now widely accepted that apple flowers may harbor bacterial taxa that might hinder the ability of *Ea* to colonize apple flower. Based on this body of knowledge, we aimed at investigating the microbiota of apple flowers to select new potential biocontrol agents active against *Ea*. Flowers of *Malus domestica* cv. *Golden Delicious* from Trentino apple orchards were sampled at the 'Baloon stage' and surface sterilised to isolate only bacteria residing within the flowers. According to the 16S rRNA gene sequencing, the bacterial isolates mainly belonged to the *Enterobacteriaceae*, *Pseudomonadaceae*, and *Microbacteriaceae* families. One member of each bacterial family was selected and tested against *Ea* both on newly open apple flowers and on pear slices. *Pantoea agglomerans* AFF2001 and *Curtobacterium flaccumfaciens* AFF2009 effectively controlled *Ea* in both conditions. To characterize their mode of action, these bacterial strains were grown in a specific medium mimicking the apple stigma nutrient conditions and their cultural filtrates were tested to evaluate their impact on the growth and virulence of *Ea*. In the future, we will investigate the molecular mechanisms involved in the biocontrol activity of these bacterial strains.

F1.1-4

BIOLOGICAL CONTROL OF POTATO SCAB BY PSEUDOMONAS SP.

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Text

Potato scab, caused by *Streptomyces* spp., is a complex disease widespread in the main producing regions of the world. The control of this disease is very difficult and therefore a set

of techniques is required for effective management. Biological control represents an alternative measurement and the bacteria *Pseudomonas* can be the solution. In the present study two strains of *Pseudomonas* sp. (IBSBF 3420 and IBSBF 3423), which are resistant to Fluazinam (fungicide widely used by potato growers), were tested with this chemical *in vitro* and *in vivo* against S*treptomyces scabiei*. *In vitro* assays, halos of inhibition with average of Ø 4.4 cm were observed in treatment with the two control agents (biological+chemical) while that those treatments with only one agent showed averages of Ø 2.8 cm. In greenhouse assays, tubers treated with *Pseudomonas* sp. IBSBF 3420 and IBSBF 3423 resulted in the reduction of incidence (85 and 75%, respectively) and severity of disease (63.9 and 60.8%, respectively). Fluazinam treatment showed better performances when used together with *Pseudomonas* IBSBF 3420 (reduction of 79.6% incidence/64.1% severity) or *Pseudomonas* IBSBF 3423 (75.7% incidence/56.1% severity), while the treatment with only fungicide the reduction of incidence and severity were 68.5% and 50%, respectively. The results obtained herein showed that *Pseudomonas* strains can be used to reduce potato scab disease and the combination with Fluazinam does not affect the biological agent action.

P1.1-001

MICROBIAL BIOLOGICAL CONTROL AGENTS (MBCAS): CONSUMERS' FRIENDS OR FOES?

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Text

Microbial biological control agents (MBCAs) are used as an alternative to synthetic chemistries and the application of MBCAs implies that these confront microbial communities, including plant pathogenic fungi. The competitions for ecological niches are decided by the toxicity of secondary metabolites (SMs) produced by the microbes. The SM compounds produced and secreted by a microbe are taken up and are further modified by another, creating a SM cocktail, which might be highly toxic and putatively have an adverse effect on human health.

In order to test the hypothesis that confrontations between pathogenic fungi and MBCA transcriptionally de-regulate SM gene clusters (SMGCs) and SM synthesis, we established confrontation experiments with maize fungus Colletotrichum graminicola and the MBCA Bacillus amyloliquefaciens. Microscopy showed that large hyphal swellings are formed in the vicinity of B. amyloliquefaciens. To elucidate the response of SMGCs, transcriptome studies were performed, and we observed differentially regulated genes belonging to PKS and NRPS clusters. Of the 42 SM clusters in Colletotrichum, more than 30 clusters harbor genes that were differentially regulated in confrontations. The metabolites extracted from the fungus-bacterium interface were analyzed by LC-MS/MS to reveal the chemical interaction and these studies showed that confrontations between microbes induce the production of a large number of chemistries belonging to various classes.

P1.1-002

IN-VITRO EVALUATION OF BIOAGENTS AGAINST CLAVICEPS FUSIFORMIS CAUSING ERGOT OF PEARL MILLET

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Text

Aims- Among the bajra ailments, ergot presents a severe problem, but scant research has been done on it by using bioagents. **Methods**:For the objective of pathogen isolation With the help of a sterilized procedure these dried drops of honeydew were inoculated on the autoclaved solidified and cooled PDA media. After a week of incubation, the well-developed mycelial growth was purified using the hyphal tip techniques. By using a dual culture approach, eight bioagents were examined in vitro for their antagonistic vigour against pearl millet ergot. The inhibitory zone was ultimately quantified by measuring the distance between the two sides of the Petri dish under test. **Results**- The pathogen was isolated on Potato dextrose agar (PDA) media. Results revealed that all the bioagents that were evaluated, exhibited fungistatic/antifungal activity against C. fusiformis and significantly inhibited its growth over untreated control. Among bioagents tested T. asperellum was found to be the most effective highest mycelial growth inhibition (59.73%) followed by T. harzianum (57.51%).
Conclusion- Among the bioagents, Trichoderma asperellum showed promising results for the control of ergot under invitro conditions and has the potential zone of inhibition growth than other bioagents. Additionally, it reduces the cost of production by means of reducing chemical application in fields, so it derives maximum benefits to farmers in an eco-friendly manner.

P1.1-003

TRICHODERMA AFROHARZIANUM – A NEW PATHOGEN IN MAIZE

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Text

Trichoderma spp. are ubiquitous soil fungi occurring worldwide. Due to their mycoparasitic and endophytic properties, Trichoderma species are used in agriculture as biocontrol agents. However, in 2018, a massive occurrence of T. afroharzianum on maize cobs was observed for the first time in Germany. Since then, Trichoderma ear rot has been observed at several locations in Germany, France, and Italy, especially in dry and hot seasons. Symptoms of Trichoderma ear rot consist in massive production of green to gray-green conidia on infected cobs leading to significant reduction of cob weight and quality as well as reduced germination rate and malformed seedlings. In addition, several Trichoderma strains used in approved biological fungicides and soil additives were pathogenic and caused heavy cob infection. In inoculation trials in the greenhouse with barley, rye, sorghum and wheat, T. afroharzianum caused visual symptoms of infection, such as browning and discoloration on the ears of wheat and barley, leading to high colonization rates and reduction of grain weight. Climate chamber experiments confirmed that growth rate and disease severity of pathogenic T. afroharzianum isolates is increased above 25°C and show a broader temperature optimum,

especially in the high temperature range.

P1.1-004

BIOLOGICAL CONTROL OVER SOURCES OF POWDERY MILDEW INOCULUM (ERYSIPHE NECATOR), GRAY ROT (BOTRYTIS CINEREA) AND ARM DEATH (DIPLODIA SERIATA), IN PERIODS OF WINTER DORMANCY OF VITIS VINIFERA.

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Text

The use of biological control agents, allow a more ecological management for plant diseases, using population ecology. This study evaluates the effect of applications of biological agents, in dormancy of Vitis vinifera, aiming to evaluate the effect of formulations of Trichoderma spp, (Mamull ®) and Bacillus spps (Nacillus pro), during the pruning period, on the control of wood diseases (Diplodia spp) incidence and parasitism of pycnidia's, incidence of powdery mildew (Erysiphe necator), as well as incidence of gray rot (Botrytis cinerea) and parasitism in sclerotia. A field trial was established, with random blocks, with 5 replications by treatments, Control, pruning paste and chemical fungicides and winter biological, to foliage 24 hours post pruning and pruning debris. The results showed a significant effect (P< 0,05) of the treatments, both chemical (3.5%), and biological (0.5%) showed reduction damage to wood with respect to control (15.8%), only the biological showed a parasitism of pycnidia 73.4%, in Botrytis the control showed an 82.5% incidence, chemical 3.5% and the winter biological of 56.5% and 60% of sclerotia parasitism. In oidium, control 100% of incidence in clusters and 74% in leaves in veraison, while the chemical 5.2% in bunches, 1.2% in leaves, the biological reached 45% in clusters and 34% in leaves. This study shows the possibility to use biological control agents, in dormancy to reduce inoculum with significative effect in full season diseases.

P1.1-005

IN VITRO EFFICACY OF SOME PLANT EXTRACTS ON THE INHIBITION OF PECTOBACTERIUM CAROTOVORUM, THE CAUSATIVE AGENT OF BACTERIAL SOFT ROT

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Text

Pectobacterium carotovorum, is one of the most important and widespread bacterial pathogens of a variety of plant products in storage. The aim of the present study was to evaluate the efficacy of some plant extracts on Pectobacterium carotovorum in the laboratory conditions. For this purpose, flower, leaf and seed hydroethanolic extracts of cloves (Syzygium aromaticum), savory (Satureja hortensis) and fennel (Foeniculum vulgare) respectively were prepared. Overnight culture of Pectobacterium carotovorum (PTCC 1675) in nutrient agar was cultured into nutrient broth and standardized with 0.5 McFarlands. The minimum inhibitory concentrations (MIC) of the plant extracts were determined by a serial two-fold dilution method in 96- well plates. The plates were incubated at 25ºC for 48 h with the lid on. The wells were then examined for evidence of growth and MIC values were determined as the lowest antimicrobial concentration that inhibited visible growth of the test microorganism. Experiment was repeated two times. MIC value for the fennel and savory extracts was 1/32 while the value for clove extract was 1/16; hence, according to our results, fennel and savory showed stronger antimicrobial activity against Pectobacterium carotovorum as comparison with clove. These results in overall are promising to use some plant extracts as potential botanical tools to control microorganisms in plant and their products.
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P1.1-006

INSIGHT OF THE BACTERIA COMMUNITIES IN THE PHYLLOSPHERE OF COFFEA ARABICA CATIMOR7963 ASSOCIATING WITH GEOGRAPHICAL DIFFERENCES

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Text
Coffee is one of the most important economic crops. Bacteria have been considered as the most abundant inhabitants of the phyllosphere while it is unknow for Coffea arabica Catimor7963, one of the main coffee cultivars in China. The objectives of this study were to reveal the status of the bacterial communities in the phyllosphere of Catimor7963 through sequencing by the Illumina HiSeq2500 of the 16S rDNA V4-V5 regions across 6 representative sampling sites located in the main cropping areas of China, including YN_HGGL, YN_ZFXH, YN_PE, YN_WS of Yunnan and HN_WN, HN_FS of Hainan. To the best of our knowledge, the current study revealed the status of the bacteria communities in the phyllosphere of Catimor7963 for the first time. The dominant family was Chloroplast norank followed with Mitochondria. PCoA based on Bray-Curtis dissimilarities displayed the differences of the phyllosphere bacteria communities were positively correlated with the geographical distances. RDA analysis of the alpha diversity index with geographical parameters demonstrated that the diversity and abundance of the phyllosphere bacteria communities in high altitude and high latitude were relatively higher than those in low altitude and low latitude. We aim to gain a better understanding of the biocontrol resources suitable for realizing ecological cultivation under different agroecological systems. Moreover, the ecological cultivation management should be adapted to the local conditions.

P1.1-007

A POTENTIAL BIOCONTROL AGENT FOR MANAGING POTATO COMMON SCAB IN TAIWAN

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Text

Potato scab is widely distributed in major potato producing areas in the world, and can be transmitted through the infested soil and seed potatoes. Potato scab mainly harms the underground part of potatoes, and due to the properties of the soil are complicated, the effect of chemical pesticides on controlling potato scab is not good. This study developed a biocontrol agent (BCA) that can control potato scab. The naturally and artificially infested seed potatoes were used for preliminary field experiments. Both naturally and artificially infested seed potatoes were cut into pieces, coated them with wettable powder formulation of BCA (50-fold diluted with diatomaceous earth), planted them in the field, and then drenched them with suspension concentrate formulation of BCA (400-fold diluted with water). The results showed that drenching the naturally and artificially potatoes with BCA could reduce the diseased area of potato scab by 33.57 and 62.72% as compared with the control group, respectively. If the naturally and artificially potatoes coated with BCA alone, the diseased area of potato scab could be reduced by 7.99 and 47.3% as compared with the control group, respectively. In the future, if healthy seed potatoes can be used in conjunction with field sanitation to avoid discarding previously diseased potatoes in the field, the success rate of biological control of potato scab can be further increased.

P1.1-008

APPLICATION OF A BIOCONTROL AGENT FOR MANAGING ROOT-KNOT NEMATODE, MELOIDOGYNE GRAMINICOLA

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Text

Most plant parasitic nematodes attack the underground parts of plants, and their life cycle can be completed in the soil. Because of the complex properties of soil, chemical pesticides are not effective in controlling plant parasitic nematodes. This study developed a biocontrol agent (BCA) that can control root-knot nematode (*Meloidogyne graminicola*) of green onion. The result of first preliminary field trial showed that treatment of green onion seedlings with 200-fold diluted BCA can reduce the nodulation rate by 65.5% as compared to the control group. The result of the second preliminary field trial showed that treatment of green onion seedlings with 200-fold and 400-fold diluted BCA can reduce the nodulation rate by 48.36% and 45.95% as compared to the control group, respectively. In addition, the effect of BCA on controlling *Meloidogyne incognita* of cucumber was evaluated in the greenhouse. Cucumber

seedlings treated with 200 and 400-fold diluted BCA can reduce the nodulation rate by 65.2% and 59.4% as compared to the control group, respectively. At present, there is no BCA registered for managing root-knot nematode in Taiwan. This study develops a BCA that is easy to mass-produce, has a long shelf life, and has a good effect on the control of root-knot nematodes. It will provide an alternative method to control the nematode diseases for organic and conventional farming in Taiwan.

P1.1-009

BACILLUS MEGATERIUM STRAINS ISOLATED FROM RHIZOSPHERE AS PROMISING AGENT FOR BIOSTIMULANTS

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Text

Global climate change affects plant growth deficiency and yield loss with abiotic stresses such as drought, salinity, greenhouse gases and extreme temperature. In this regard, it is necessary to develop biostimulants to enhance tolerance to abiotic stress, uptake nutrients and promote plant growth. The aim of this study is utilizing of plant growth promoting rhizosphere microorganism as microbial biostimulants. *Bacillus megaterium* GEB3 and GEB13 were isolated from the rhizosphere of ginseng and identified by sequencing 16S rRNA. GEB3 and GEB13 has been showed plant growth related activity such as nitrogen fixation, siderophore secretion, and indole-3-acetic acid production. In addition, rice seeds were treated with GEB3 and GEB13 culture solutions, and seed germination rates were measured by culturing the seeds under drought conditions of 0 Mpa, -0.15 Mpa, and -0.49 Mpa. In drought conditions, the seed germination rate was higher in the GEB3 and GEB13 treatment groups than in the microbial-free control group. In greenhouse, GEB3 and GEB13 were treated to rice seedlings under drought conditions. As a result, the chlorophyll content was 34.1 SPAD for GEB3 treatment and 33.4 SPAD for GEB13 treatment after 4weeks, which was significantly increased compared to the control value of 21.6 SPAD without microbial treatment. These results indicate that *B. megaterium* GEB3 and GEB13 can be used as biostimulants that promote plant growth and abiotic stress resistance in crop cultivation.

P1.1-012

A LAB TO LAND EXPERIENCE ON TRICHODERMA BASED TECHNOLOGY FOR BETTER PLANT HEALTH MANAGEMENT IN TRIBAL REGIONS OF NORTH EAST INDIA

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Text

Indigenous Trichoderma strains were isolated and characterized. Potential strains of

Trichoderma spp. effective against six soil-borne fungal phytopathogens identified through a series of studies. The mode of action of Trichoderma spp. was studied against the targeted phytopathogens. PGP, enzyme release activity, the ability for siderophore production, P and Zn solubilization, tolerance to Al and Fe toxicity, and compatibility with soil microbiome studies showed a positive result for better plant health management. Through a continuous effort of 15 years in different agroecological conditions of NER of India, standardized bio-intensive strategies and popularized among the farming communities. More than 5000 farmers, FPOs, extension personnel, and tea garden managers were trained on technical aspects of the technology and its field use. Technology adopters gained more profit due to the result of higher yield with nutritious farm produce. The farmers could fetch more income compared to the non-adopters and bankability increases. The success opens a hope that the technology may create a revolution among the farming communities of the nearby areas. Moreover, the low-cost technology has opened up a new vista for plant disease management and is likely to be a boon for seed industries that would like to provide protection to seeds as well as plants against a large number of seed, soil-borne, and foliar diseases.

P1.1-013

EVALUATION OF MULTI-BACILLUS STRAINS ON CONTROL OF SOUTHERN BLIGHT IN PEPPER

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Text

Southern blight caused by Sclerotium rolfsii is a major fungal and soil-borne disease damaging the economic crops worldwide. The management of southern blight relies primarily on the strategic application of synthetic fungicides. However, chemical fungicides are responsible for environmental pollution, health hazards, pest resurgence, development of resistance in pathogens, destruction of non-target species, and deterioration of natural habitats. Here, endophytic Bacillus spp. were evaluated the efficacy on control of southern blight. Results revealed that R8-25, R8-43, and PS6-2 strains showed best ability against S. rolfsii isolates with 59.1% to 71.1% mycelia inhibition rate. Moreover, these three strains could produce volatile organic compounds (VOCs) to inhibit mycelia growth and sclerotia germination. Characteristics analyses indicated that the three strains have abilities secrete amylase, cellulase, gelatinase, protease, iturin A, bacillaene and IAA. For the seed germination and plant-growth-promoting tests, the strains R8-25 and R8-43 have the best efficacy to induce seed germination and promote bell/chili pepper growth in greenhouse. In the control test, single strains or mixture of R8-25 and R8-43 could reduce the severity of southern blight in bell pepper based on seed coating combined with drenching method in greenhouse, especially two strains mixture. According to these results, the two strains have the potential to be agents on control of southern blight.

P1.1-014

PRESENT STATUS, CHARACTERIZATION & BIOLOGICAL CONTROL USING NATIVE BACTERIAL STRAINS FOR DIEBACK & BLACK ROOT ROT DISEASES OF STRAWBERRY IN PAKISTAN

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Text

During 2017-18 and 2019-20 surveys were conducted in 12 strawberry-producing districts of Pakistan. Out of 12, 08 districts of Punjab province, 03 of Khyber Pukhtunkhwa province and Islamabad for disease assessment and samples collection. Mean % disease prevalence and incidence ranged from 0-100% and 5-8% for dieback whereas, 0-100% and 0-16% for black root rot (BRR) respectively. Dieback symptoms appeared as wilting and dieback with collapsing of the entire plant. The BRR appeared as black discolorations on entire root and plant look stunted and collapsed. Purified cultures were identified on morpho-molecular basis. Total of 30 Lasiodiplodia theobromae (dieback) and 47 of Rhizoctonia solani (21), Macrophomina phaseolina (11) & Fusarium solani (15) isolates of (BRR) were studied. Pathogens were by culturally & morphologically identified. For molecular studies, nucleotide sequencing of 20 highly pathogenic isolates (5 each of L. theobromae, R. solani, M. phaseolina & F. solani were done by ITS, TEF1-α & EndoPG primers & phylogenetically analyzed. This is 1st detailed study of these diseases in Pakistan. For biological control 30 native bacterial isolates were processed of which 3 isolates viz. Bacillus subtilis (1) and Pseudomonas fluorescens (2) showed the highest antagonistic effectiveness (>70%) against the dieback and BRR pathogens during bioassays. The selected bacterial isolates were further tested in the greenhouse and also showed promising disease control of 21-57%

P1.1-015

ISOLATION AND IDENTIFICATION OF PENICILLIUM CORYLOPHILUM AS ANTIBACTERIAL-PRODUCING FUNGI IN THE SOIL ENVIRONMENT OF KOYA PROVIN

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Text

The study was carried out to isolate and identify several fungi from Koya soil. (48) soil samples were collected from four different locations in Koya district. 6 fungal genera which include: *P. spp., Asp. spp., Rh. spp., Aur. spp., Cl. spp. and Dip. spp.* were isolated by used SDA, PDA, and MEA. Serial dilution plate method was used for isolation of soil fungi. the results, reveal that the most predominant genera and highest number of colonies were Pen. and Asp. sp.. the result showed that the of occurrence of Asp. and Pen. were very high among the fungal isolates . Different species of Pen. obtained from soil samples especially from Shewashok soil. The P. coryl had significant antagonistic activity against five out of six (G-) and (G+) pathogenic ATCC . P. coryl. from Shewashok location was the best isolate for antibacterial production and it had more antagonistic activity against pathogenic bacteria than the other Pen.spp. this isolate was used for antibacterial production. Ethanol solvent was used for the extraction of the antibacterial substances form

fermented P. corylm. The crude extract was dried in rotary evaporator , centrifuge at 35?C. The antibacterial obtained was highly effective against Y. enterocolitica and S. aureus. (GC-MS) was used for analysis of the fungal extract obtained from P. coryl. The result had determined thirteen compounds from crude extract . Some of these compounds have antibacterial activity against some pathogenic microbes .

P1.1-016

A BIOLOGICAL AGENT SERENADE FOR PROMOTING THE BANANA GROWTH AND MODIFYING RHIZOSPHERE SOIL MICROBIAL DIVERSITY AND COMMUNITY COMPOSITION

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Text

Fusarium wilt of banana is becoming a serious challenge to the banana industry globally. Biological control is one of the most effective measures for this disease. In order to explore the biocontrol effects of a biological agent Serenade on banana plants, two different cultivars ‘Brazilian’ and ‘Yunjiao No.1’ were used in greenhouse pot experiments. Results showed that the plant height and pseudostem diameter of banana susceptible cultivar Brazilian increased by 11.68% and 11.94% respectively after Serenade application, while the plant height and pseudostem diameter of resistant cultivar Yunjiao No.1 increased by 14.87% and 12.51% respectively. The fresh weight of two cultivars increased by 20.66% and 36.68% respectively, these indicating that Serenade has positive effects on plant growth promotion. TR4 infection and Serenade application changed the bacterial community composition of two banana cultivars, and the fungal community composition of Yunjiao No.1 also changed significantly. Correlation analysis showed that the relative abundance of Bacillus and Pseudomonas in the rhizosphere of both cultivars increased significantly after Serenade application, which had significant positive correlation with plant height, psedustem girth, above ground fresh weight, leaf length and leaf width. Therefore, the outcome of this study suggests that Serenade could be used in banana field application for promoting plant growth and modification of soil microbial communities.

P1.1-017

SIMPLICILLIUM AS THE DOMINANT MYCOPARASITES OF HEMILEIA VASTATRIX REVEALING REGIONAL GENETIC DIVERSIFICATION

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Text
?Coffee leaf rust (CLR) as one of the most devastated diseases of *Coffea arabica*, caused by the obligate fungal parasite *Hemileia vastatrix* (Hv). Mycoparasites frequently follow Hv in the field, interfering with the reproductive structures to stop further growth and dissemination of Hv, to offer a chance to potentially limit CLR. Here, we applied high throughput sequencing of the rDNA ITS1-ITS2 regions of coffee phyllosphere microbiota with 3 leaves symptoms (C, HV, PHV) across 6 sampling sites located in China coffee regions. Simplicillium was evaluated as the dominated mycoparasites of Hv in all the investigated populations through a series approach of metabarcoding analysis, revealing genetic diversity of associating with the geographic differences. At the 0.97 clustered threshold, 22 OTUs were 98-100% homologous to *Simplicillium* spp., of which, 11OTUs identified as *S. lanosoniveum*, 6 OTUs identified as *S. subtropicum*, others identified as *S. lamellicola, S. obclavatum* and *Simplicillium* sp. These sequences had 68 segregating sites and Pi of 0.05305. The MJ haplotype networks were built from a total of 17 haplotypes produced by 22 OTUs of *Simplicillium* spp. while divided into 2 groups, demonstrating great diversification with 0.996 Hd and 0.00023 VarHd and displaying genetic divergence with clear geographic patterns and leaf symptom selectivity. All in all, the current study is helpful for developing management biocontrol measures against CLR.

P1.1-018

POTENTIAL GROWTH SUPPRESSION AND BACTERIOSTATIC ACTIVITY OF PLANT-ASSOCIATED LACTIC ACID BACTERIA (LAB) AGAINST PANTOEA STEWARTII OF THE JACKFRUIT BRONZING DISEASE

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Text
Pantoea stewartii subsp. stewartii is the causative agent of "Jackfuit Bronzing," an emerging disease in jackfruit crops (Artocarpus heterophyllus L.). The disease was first discovered in Malaysia in 2017, affecting the J33 variety, Tekam Yellow. This disease degrades the quality of fresh jackfruit, leading to economic losses, and has remained a serious problem for the Malaysian jackfruit trade. In this study, 58 lactic acid bacteria (LAB) isolated from fruits and vegetables were screened and characterized for their antagonistic potential against P. stewartii subsp. stewartii. Fourteen cell-free supernatants (CFS) of the LAB isolates were found significantly inhibit the growth of P. stewartii subsp. stewartii in vitro ($P<0.05$). They were morphologically, biochemically, and genetically identified and based on the 16S rDNA sequencing analysis, the 6 LAB isolates showing the greatest antagonism are Lactiplantibacillus pentosus, Lactiplantibacillus argentoratensis, Leuconostoc holzapfelii, Weisella cibaria and Weisella paramesenteroides. CFS of the 6 potential LABs was extracted using ethyl acetate, diethyl ether, dichloromethane, and n-hexane solvents to examine their bioactive metabolites qualitatively and quantitatively. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) identification of the purified bioactive metabolites from the extract that possessed the greatest effect against P. stewartii subsp. stewartii will be presented.

P1.1-019

INDUCTION OF PEPPER RESISTANCE AGAINST PEPPER MILD MOTTLE VIRUS BY BACILLUS VELEZENSIS TREATMENT

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Text

Pepper mild mottle virus (PMMoV), a member of genus Tobamovirus, is the major viral pathogen of peppers (Capsicum annuum L.) in Taiwan. Symptoms caused by PMMoV include various degrees of mottling and curling of leaves, distortion of fruit and dwarfing. PMMoV is a rigid rod shaped virus that can be easily transmitted via mechanical inoculation when primary inoculum was present. The primary inoculation of PMMoV is difficult to prevent because it often comes from contaminated cultivation media or seeds. In our study, Bacillus velezensis was tested as a biocontrol agent for inducing systemic resistance of sweet pepper against PMMoV under nethouse conditions. Foliar application and soil irrigation of B. velezensis was performed once a week when the seedlings germinated. Treatment and control sweet pepper plants were inoculated with PMMoV after 2 treatments. For challenge inoculation, diseased sweet pepper leaves were ground into 50 times (W/V) phosphate buffer, and the ground juice was mechanically inoculated on sweet pepper leaves. The virus infection and virus concentration were detected by ELISA. All control plants were infected with PMMoV, and only 40% plants in the B. velezensis treatment were infected by the virus, and the virus concentration was lower than control plants. In our results on sweet pepper, B. velezensis can be used as a biocontrol agent for induction of resistance against PMMoV or reduction of symptoms caused by this virus.

P1.1-020

PROSPECTS FOR THE USE OF MICROBIAL PREPARATIONS TO PROTECT FRUIT CROPS FROM FIRE BLIGHT

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Text

Fire blight is a dangerous disease of fruit crops and causes enormous economic damage to fruit growing. The bacterium Erwinia amylovora, the causative agent of the disease, belongs to quarantine objects.

In order to reduce the spread of morbidity, screening of microorganisms isolated from garden cenosis of various regions of Kazakhstan was carried out against the causative agent of bacterial burn of fruit crops.

It was established that the MB-40 and 17M isolates showed the highest antagonistic activity against E. amylovora (the zones of pathogen growth inhibition were 48 mm and 30 mm,

respectively).
It was determined that acetoin and 2,3-butanedione produced by B. amyloliquefaciens MB-40, as well as lactic and acetic acids produced by L. plantarum 17, are the components responsible for the inhibitory activity against E. amylovora.
It has been shown that double application of the culture broth of strains B. amyloleguefaciens MB-40 and L. plantarum 17M, containing inhibitory components, protects fruit trees from bacterial burn by 70%.
The study of the virulence properties of the B. amyloliguefaciens MB-40 and L. plantarum 17 M strains showed that they are not pathogenic for warm-blooded organisms and can be used as the basis of biological preparations against bacterial burn of fruit crops.

P1.1-021

LECANICILLIUM AS THE PRINCIPAL NATURAL ENEMY OF HEMILEIA VASTATRIX DISPLAYING GENETIC DIVERGENCE WITH GEOGRAPHIC PATTERNS AND LEAF SYMPTOM PREFERENCES IN FIELD SETS

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Text

Hemileia vastatrix (Hv) Berk & Broome is one of the greatest threats to the global coffee industry causing coffee leaf rust (CLR). *Lecanicillium* can naturally parasitize rust pustules which is highlighted in the biological control of CLR. Here, we explore the genetic diversity of *Lecanicillium* spp. across the main coffee cropping areas of China through high-throughput sequencing of ITS1-ITS2 regions of rDNA in a series of complex leave field samples. At the 97% clustered threshold, 15 OTUs were 98–100% homologous to *Lecanicillium* spp. with Pi of 0.13356 and 187 segregating sites. These OTUs were classified as *L. lecanii, L. antillanum, L. muscarium, L. fusisporum* and *Lecanicillium* sp. They generated 15 haplotypes with Hd of 1.000 and VarHd of 0.00059 and constructed MJ haplotype networks. Populations from various sample sites showed distinct geographic trends in their haplotype diversity. The highest Pi?0.47886?and the highest VarHd?0.07407?presented in WN, while the genetic variants of FS and PE was not observed. MJ network based on the leave symptoms presented leave symptom preferences. Of which, 12 haplotypes presented in symptom HV with VarHd of 0.00116, and 9 haplotypes detected in symptom PHV with VarHd of 0.00274. Specific haplotypes were observed in HV and PHV. Our results demonstrated that it is essential to underline the need for strategies to forge alliances between various components to convert these biocontrol agents into workable commercial product.

P1.1-022

REDUCTION OF PATHOGENS CAUSING FUSARIUM HEAD BLIGHT IN WHEAT GRAIN BY AUREOBASIDIUM PULLULANS STRAINS PRODUCING AUREOBASIDIN A

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Text

Modern European agriculture increasingly relies on organic farming methods and ecological approaches, or conventional pesticides are partially replaced with biocontrol agents such Aureobasidium pullulans. Some strains of A. pullulans produce aureobasidin A, a cyclic antifungal peptide, catalyzed by an amino acid complex encoded by the aba1 gene. The aim of this field experiment, conducted in 2021 and 2022 in north-eastern Poland, was to select A. pullulans strains with the use of molecular markers linked to the aba1 gene, and to analyze the inhibitory effect of selected strains on trichothecenes concentrations in wheat grain. In 2021, all biological treatments reduced the severity of Fusarium head blight (FHB), and their effectiveness reached 62.6% (Ap CC2) and 57.1% (Ap 15). Wheat grain was contaminated with type-B trichothecenes: deoxynivalenol (DON), Fus-X, 3ADON, 15ADON, and nivalenol (NIV), and type-A trichothecenes: STO, HT-2 toxin, T-2 toxin tetraol, T-2 toxin triol, and diacetoxyscirpenol (DAS). Biological treatments involving a cell suspension of A. pullulans strain Ap 15 decreased the NIV content of grain from 11 mg/kg to 8 mg/kg in the first year of the study, and completely eliminated this mycotoxin from grain in the second year. When FHB severity was low, a A. pullulans treatments did not decrease DON concentrations in grain, although it reduced disease symptoms and the abundance of selected Fusarium species.

P1.1-023

MICRORNA EXPRESSION PROFILE REVEAL THE REGULATION OF E-POLY-L-LYSINE ON NICOTIANA TABACUM ANTI-TOBACCO MOSAIC VIRUS

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Text

ε-poly-L-lysine (ε-PL) produced by microorganisms has broad antimicrobial spectrum and good stability. miRNAs play an important role in various plant processes such as growth and development, defense stress and disease resistance. To investigate the antiviral mechanism of action of ε-PL in plants, we analyzed the expression profile of microRNA (miRNA) in tobacco mosaic virus (TMV)-infected *Nicotiana tabacum* after ε-PL treatment. The results showed that expression levels of 328 miRNAs were significantly altered by ε-PL. Some miRNAs were screened, and joint network analysis was performed on their target genes and gene-enriched GO/KEGG pathways. The results indicated that ε-PL regulates expression of miRNAs involved in pathways such as plant hormone signal transduction, host defense response and plant pathogen interaction, such as nta-miR6146, nta-miR1446 and nat-

miR319a. Subsequently, TRV-VIGS gene silencing method combined with the short tandem targets mimic technology were used to functionally analyze these miRNAs and their target genes. The results of northern blot and RT-qPCR showed that the accumulation of TMV in *N. benthamiana* were increased after silencing miR1446 and miR6146. Meanwhile, the silencing of miR172, miR164, and miR319 reduced the content of TMV, indicating that these miRNAs may play diverse roles during ε-PL- mediated antiviral responses. Collectively, these results provide theoretical basis for further elucidating antiviral mechanisms of ε-PL.

P1.1-024

BIOLOGICAL SOIL CRUST MICROALGAE AS A NOVEL SOURCE FOR THE DEVELOPMENT OF BIOCONTROL AGENT

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Text
Soil-borne pathogens pose a significant threat to crops globally, leading to significant economic losses. These pathogens can survive without a host by producing resting structures that are resistant to environmental stress and pesticides. Pesticides are toxic and their accumulation in soil and plants is dangerous to the environment and humans. Hence, researchers are focused on developing sustainable solutions such as biocontrol agents (BCA). However, the complex interactions between BCAs and the environment pose a challenge in developing effective solutions. Biological soil crusts (BSCs) are the natural cover of many arid and semi-arid lands. The microorganisms inhibiting BSCs developed unique mechanisms to survive harsh environmental conditions such as desiccation, heat, and radiation. Green algae isolated from desert crusts show a remarkable ability to withstand harsh environmental conditions. Surprisingly, it is also one of the fastest-growing phototrophs known. Preliminary results show that co-culturing this alga significantly inhibits the growth as well as the production and viability of resting structure in various soil-borne pathogenic fungi, including *Verticillium dahliae* and *Rhizoctonia solani*. The combination of fast-growing (i), resilience (ii), and the observed antifungal activity (iii) make it a promising candidate for the development of a BCA against soil-borne pathogenic fungi, specifically for arid and semi-arid regions and in the face of climate change.

P1.1-025

FIGHTING LETTUCE BACTERIAL PATHOGENS WITH BENEFICIAL PSEUDOMONAS STRAINS

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Text
Lettuce is a popular vegetable crop worldwide, accounting for a production value of more

than US$20 billion in 2020. It is threatened by several bacterial pathogens, including *Xanthomonas hortorum* pv. *vitians*, *Pseudomonas cichorii*, and *Pectobacterium carotovorum*. These pathogens cause bacterial leaf spot, varnish spot, and bacterial soft rot, respectively. Control methods are limited and often ineffective. It is therefore important to develop and implement novel, effective, durable, and low environmental impact control methods, such as biocontrol. With this in mind, we screened a collection of 1,200 *Pseudomonas* strains for their ability to inhibit the growth of the three pathogens under study. In total, 35 effective antagonistic *Pseudomonas* strains where identified. Their genomes were fully sequenced and annotated, revealing their phylogenetic affiliation and potential genetic determinants involved in their antagonistic activity. These *Pseudomonas* strains belong to 27 different species distributed among the *P*. *fluorescens* and the *P*. *putida* phylogenomic groups. Only 15 of these species have been described to date. The presence of genes involved in microbial competition and antibiosis, including the biosynthesis of pyochelin, type VI secretion systems, tailocins, and hydrogen cyanide, correlated with their inhibition abilities. Some of these strains show promise for the development of efficient biocontrol products against lettuce bacterial diseases.

P1.1-026

EFFECTS OF PSEUDOMONAS ALCALIPHILA EJ2 ON THE ENDOPHYTIC MICROBIOME AND PROTEOME OF RICE UNDER SALT STRESS

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Text

Soil salinity is a prevalent environmental stress in agricultural production. Microbial inoculants could effectively help plants to alleviate salt stress. However, there is little knowledge of the *Pseudomonas alcaliphila* Ej2 mechanisms aiding rice plants to reduce the adverse effects caused by salt stress. We performed integrated field and greenhouse experiments, microbial community profiling, and rice proteomic analysis to systematically investigate the Ej2 mechanism of action. We found an increase in shoot/root lengths and fresh/dry weight in inoculated plants under salt stress. In the meantime, the strain Ej2 has important roles in controlling diseases and promoting rice growth. Furthermore, the alpha diversity of Ej2-inoculated plants was higher than the control plants, expect the Shannon index of the bacterial microbiome. The Ej2 inoculated samples clustered and separated from the control samples based on beta diversity analysis. Importantly, the enriched and specific OTUs after Ej2 inoculation at the genus level were *Streptomyces*, *Pseudomonas*, *Flavobacterium*, and *Bacillus*. Moreover, we observed that Ej2 inoculation influenced the rice proteomic profile, including metabolism, plant-pathogen interactions, and biosynthesis of unsaturated fatty acids. These results provide comprehensive evidence that Ej2 inoculation affects the rice endophytic microbiome and proteomic profiles under salt stress.

P1.1-027

THE IMPACT OF MICROBIAL VOLATILE ORGANIC COMPOUNDS ON PLANT AND MICROBIOME INTERACTIONS

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Text

The rhizosphere microbiome is vital for maintaining plant growth/development and expressing resistance/tolerance responses to (a)biotic stresses. Plant growth-promoting rhizobacteria (PGPR) can confer resistance/tolerance on plants against stressed conditions, e.g., through liberating volatile organic compounds (VOCs). On the plant's side, plants employ root exudates as attractants to recruit root-associated beneficial microorganisms. Thus, artificial manipulation of plant-microbe interaction in the rhizosphere would be an efficient way to cope with various (a)biotic stresses. Yet, the remote crosstalk among VOCs emitted by a donor strain, microbiota, and plants and their root exudates have been challenging to unravel. The present study investigated the direct impact of VOCs emitted by the *Bacillus zanthoxyli* HS1 strain for boosting induced systemic resistance (ISR) and the indirect impact on plant nutrient uptake. Cabbage and cucumber seedlings exposed to bacterial VOCs showed ISR in infected leaves with phytopathogenic microbes. A split root approach revealed that bacterial VOCs change the root exudates composition, altered microbiome diversity, and increase nutrients uptake by plants. In conclusion, the study of the tripartite interaction among pathogen-infected plants, bacterial VOCs, and root exudates proposes a possibility that VOCs released by *B. zanthoxyli* HS1 modulate the microbiome and composition of root exudate to suppress pathogen attack and promote plant growth.

P1.1-028

APPLICATION OF ANTAGONISTIC AND ENTOMOPATHOGENIC FUNGAL CONSORTIUM AGAINST FUSARIUM WILT AND APHIDS OF CUMIN

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Text

Potential of consortial application of bioagents against fungal, bacterial and nematodes can be utilized to fullest extent by selecting most potential strains and not by arbitrary use of consortia. Cumin (Cuminum cyminum L.) is an important seed spices crop in India is majorly infected by Fusarium oxysoprum f. sp. cumini (Fusarium wilt) and aphid (Myzus persicae (Sulzer)).The multi-location field trials were conducted at Zone III A semi-arid eastern plain zone (Jaipur- Jobner) and zone IVB southern humid zone (Banswara)to evaluate the efficacy biocontrol agents and insecticide against major pests of cumin (Variety: RZ19) at two different agroclimatic conditions for the years of 2017, 2018 and 2019 rabi seasons. The combined application of biocontrol agents for the management of wilt of cumin by soil application with T. harzianum Th3 and M. anisopliae Ma1 enriched FYM (1:20). Seed

treatment with Th3 and Ma1 @ 8g/kg seeds also drenching at 30 and 60 days after sowing and three foliar spray with Flonicamid at 0.015 % clearly shows the maximum reduction of disease (62.16%) compared to control and also maximum yield of 570.94 kg/ha at Jaipur agroclimatic conditions. The maximum disease reduction of 60.22 per cent and increased yield up to 541.50 kg/ha was observed at Banswara agroclimatic conditions. Based on the bliss independence hypothesis, the synergy factor was >1 (1.01) which demonstrated the interaction was synergistic in both pests of cumin.

P1.1-029

CONTROL OF ANTHRACNOSE AND THRIPS IN CUCUMBER BY BACILLUS SUBTILIS WITH PRODUCTION OF VOLATILE COMPOUNDS

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Text

Cucumis sativus L. is one of the most important vegetables worldwide. Cucumber anthracnose caused by *Colletotrichum orbiculare* can incite anthracnose symptoms on leaves, stems and fruits causing serious yield loss. *Thrips palmi* not only causes damage by sucking, but also transmits Tospovirus virus diseases. Here, we aimed to evaluate the potential of *Bacillus subtilis* for controlling cucumber anthracnose and thrip's population, and to reveal potential mechanism in plant health management. *B. subtilis* WMA1 and *B. subtilis* 151B1 were isolated in native Taiwan. Both strains showed antagonistic activity to *C. orbiculare* COC3 by dual culture assay. Application of culture broth of either *B. subtilis* WMA1 or *B. subtilis* 151B1 exhibited preventive and curative efficacy against cucumber anthracnose. Culture filtrates of *B. subtilis* WMA1 and *B. subtilis* 151B1 inhibited conidial germination of *C. orbiculare* COC3 to 26.7 and 22%, respectively, compared to 70.7% of the SYB medium control. Additionally, both *Bacillus* strains promoted the growth of cucumber seedlings, inhibited the mycelium growth of *C. orbiculare* COC3, and reduced oviposition and modulated the development and reproduction of thrips, which may be due to the volatile compounds produced by *B. subtilis* WMA1 and *B. subtilis* 151B1. Our findings suggested that *B. subtilis* strains WMA1 and 151B1 exhibited potential for the control of cucumber anthracnose, and significantly reduced the fecundity and development rate of thrips.

P1.1-030

SCREENING AND APPLICATION OF INDUCED RESISTANCE MICROBE FOR VIRAL DISEASE CONTROL IN CUCUMBER

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Text

During 2006 to 2007, cucumber (*Cucumis sativus* L.) was infected by melon yellow spot virus (MYSV) with symptoms of mosaic, yellow and necrotic spots on the leaves, and transmited by melon thrips. So, the viral disease control mainly on preventing vector by insecticides. On this study, we develop a system to screen induced resistance microorganisms for cucumber virus disease control. Among 130 microorganisms, 23 exhibited good proteolytic activity with cleared more than >2.0 cm zone on milk agar plate. Bioassays of induce resistance to viral disease was conducted on indicator host quinoa (*Chenopodium quinoa*) and zucchini yellow mosaic virus (ZYMV). After spreading the lower five leaves with the culture filtrate of the above-mentioned microorganisms for 4 hours, the upper five leaves were inoculated with freshly prepared ZYMV inoculum. The local lesions were account at 7 days post inoculation (dpi). Results showed that *Bacillus velezensis* (B34) and *Bacillus* spp. (FYC14) is effective in inducing plant resistance. In vivo screening of endophytic, *B. velezensis* was selection for MYSV control in screenhouse cucumber. The results showed an approximately 47% reduction in disease incidence compared to the control after 4 weeks of planting and a 22% increase in total cucumber yield. In summary, *B. velezensis* is effective control with ability to cleave proteins, induce plant resistance, promote plant growth and endogenously. So far, the molecular mechanism is still under investigation.

P1.1-031

INTERACTION OF AUUB 209 (STREPTOMYCES ENISSOCAESILIS) AND AUDT 626- (STREPTOMYCES RACEMOCHROMOGENES) RHIZOBACTERIA AND SCLEROTIUM ROLFSII ROOT ROT PATHOGEN ON RELATIVE EXPRESSION OF DEFENCE RELATED GENES IN SOYBEAN THROUGH QRT-PCR

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Text

The present investigations were carried out to assess the interaction of rhizobacteria and target pathogen in soybean ecosystem at Molecular Genetics Lab,UAS,Dharwad to gain the insights into molecular basis of host-pathogen interaction during 2022. Defence related genes were analysed for their expression levels in response to pathogen (Sclerotium rolfsii Sacc.) and rhizobacterial isolates (AUUB209 Streptomyces enissocaesilis and AUDT626 (Streptomyces racemochromogenes) through quantitative real time polymerase chain reaction. A total of five defence related genes viz., Pathogenesis related protein 1 (PR 1), Pathogenesis related protein 2 (PR 2),Pathogenesis related protein 2 (PR 10), Polyphenol oxidase (PPO) and Chalcone synthase (CHS) were selected and analysed for

their expression levels under different treatments where rhizobacteria were applied as seed treatment (10g/kg seeds) and drenching at 35-40 days after sowing. The results revealed that the highest expression levels of PR 1 (2.75 fold),PR 2 (7.88 fold), PR 10 (4.16 fold) and PPO (8.50 fold) observed in the treatment (Host + Pathogen + AUUB 209 + AUDT 626) and the highest (3.27 fold) CHS gene expression was recorded in the treatment (Host + AUDT 626). The positive check (Host + Pathogen + Trichoderma harzianum) recorded 2.42, 5.74, 4.15, 6.33 and 1.34 fold change of PR 1, PR 2, PR 10, PPO and CHS genes respectively.This is the first report on rhizobacteria and root rot pathogen interaction in soybean in India.

P1.1-032

CHARACTERISING AND HARNESSING THE HAEMATOCOCCUS ALGAL MICROBIOME – TOWARDS BIOCONTROL OF THE FUNGAL PATHOGEN PARAPHYSODERMA SEDEBOKERENSE

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Text

Haematococcus pluvialis is a unicellular freshwater microalga cultivated industrially to produce astaxanthin, a sought-after antioxidant for nutraceutical and feed industries. Its mass culture is hindered by the fungal pathogen *Paraphysoderma sedebokerense* (Blastocladiomycota)[1,2]. The complex life cycle of this fungus and its resistance to most disinfection methods lead to a difficult control of the disease. Like for plants, there is increasing evidence that the algal microbiota plays a role for host health, including defense against pathogens[3]. To investigate the potential protection of *Haematococcus* by its bacterial microbiota against *P. sedebokerense* we combine metagenomics with a culture-based approach where we isolate the cultivable bacteria and prepare axenic algal strains. We show first results of our metagenomic analysis of the *Haematococcus* spp. microbiome in presence or absence of *P. sedebokerense*, revealing insights into the taxonomic diversity and potential functions of bacteria linked to fungal infection. These results guide us in choosing bacteria from the cultivable microbiota which are re-inoculated on axenic algae with or without the pathogen *P. sedebokerense*. First results of the impact of these synthetic bacterial communities on the alga-fungus interaction are shown. Altogether, this work allows to set the bases for development of biocontrol tools in industrial *Haematococcus* production.

[1]Allewaert *et al.,* 2018
[2]Hoffman et *al.*, 2007
[3]Dittami et *al.*, 2021

P1.1-033

CHARACTERIZATION OF THE MODE OF ACTION OF A BIOCONTROL PRODUCT THROUGH THE PATHOSYSTEM TOMATO MICRO-TOM AND PHYTOPHTHORA INFESTANS

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Text

The tomato is the most consumed fruit in France and in the world. Among the different diseases affecting tomatoes and potatoes, the late blight caused by the oomycete, *Phytophthora infestans*, is one of the most devastating pathogens. The oomycete causes significant yield losses, involving the repeated use of phytopharmaceutical products harmful to the environment and public health. In order to reduce the use of pesticides, the use of biocontrol agents is one of the possible alternatives for crop protection.
In this context, my research project aims to characterize the mode of action of a biocontrol product (GA342) developed by the company Gaïago in order to improve its effectiveness against the late blight, using the pathosystem *Phytophthora infestans* (A36_A2 strain) - Micro-Tom, model cultivar for tomato. Preliminary results showed that 1) GA342 had a direct effect on the oomycete, decelerating or inhibiting totally the mycelial growth, in a dose dependent manner; 2) in planta, the treatment with GA342, prior to the infection, showed to reduced significantly the symptoms. The results of further studies will be presented on the direct effect of GA342 and more particularly on sporangia, zoospores and mycelia. In addition, targeted transcriptomic and biochemistry approaches in planta will be shown.
The promising results related to the action mode of GA342 will allow us to optimize the efficiency of the product for field application.

P1.1-034

CHARACTERIZATION AND IDENTIFICATION OF FUSARIUM SPP. SPECIES THAT AFFECT ORANGE CROPS (CITRUS X SINENSIS L.) IN CHILE

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Text

In Chile, there are a few studies about the etiology and level of damage caused by *Fusarium* spp. species in citrus. Among the symptoms described in literature caused by these pathogens are foliar chlorosis, epinasty, dry root rot, wilting, branch dieback, and decreased growth. Most of these symptoms have been observed during the last years in commercial citrus orchards in the central region of Chile. For this reason, this study aims to identify morphologically and molecularly *Fusarium* species that affect orange trees in Chile. Therefore, from three orange commercial orchards of "Lane late" and "Fukumoto" cultivars, twelve *Fusarium* isolates were obtained, which were identified as *F. solani* and *F. oxysporum.* These isolates were characterized by yellow to purple colonies and the micro and macroconidia were organized in false mucilaginous heads. The pathogenicity tests carried out

on eight-month-old plants of the Robidoux rootstock confirm that the two identified *Fusarium* species colonize the root and generate necrosis after eight months of evaluation. However, no significant symptoms have yet been evidenced in the canopy. Only plants inoculated with *F. solani* grew 11% less than the control during a nine-week evaluation period. For this reason, plant growth measurements continue in order to demonstrate the pathogenicity level of these species on orange trees. The previous results are promising and will allow the evaluation of *Fusarium* biocontrol strategies in orange orchards.

P1.1-035

SAPROBIC CONIDIAL FUNGI FROM THE SOUTHERN AMAZON IN THE IN VITRO CONTROL OF PHYTOPATHOGENS

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Text

Fungi are the main decomposers of nature. They can live in different environments, especially in the soil, live in decaying organic matter. Also collaborate to renew and recycle materials, playing a very important role in sustainable development. Studies on conidial fungus saprobes in tropical areas are developed mainly in South America. Amazon region is considered to have the greater biodiversity of species with great relevance in the medical and economic areas, highlighting the possibility of new biocontrol agents and resistance inducers. The objective of this study was to determine the potential of the saprobic conidial fungi of the Amazon region in control of *Colletotrichum truncatum*, *C. musae*, *Fusarium udum*, *Fusarium* sp., *Aspergillus clavatus*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani.* For this, we used the conidial fungi *Beltrania rhombica*, *Brachysporiella* sp., *Dictyochaeta* sp. and *Gonytrichum* sp. In the pairing test and evaluation through the note scale proposed by Bell et al. (1982). It was verified that in the direct comparison, there was a significant interaction, and the conidial fungi studied showed different degrees of growth inhibition of *A. clavatus*, *C. truncatum*, *C. musae* and *F. udum*, with scores of 1 to 2, being very efficient and efficient, showing promising antagonists to phytopathogens. We concluded that the saprobic conidial fungi studied have potential in the control of phytopathogens, and these relationships should be better studied.

P1.1-037

EXPLORING THE FEASIBILITY OF BIOCONTROL USING STREPTOMYCES STRAINS AGAINST SOYBEAN PHYTOPATHOGENIC FUNGI

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Text

Soybean (*Glycine max*) is one of the main crops globally, but various diseases, such as Anthracnose (*Colletotrichum truncatum*) and root rot (*Fusarium* spp.), have a significant impact on grain yield and quality. Traditional control strategies rely mainly on chemical fungicides, but the emergence of fungicide-resistant pathogen strains requires a new approach. Biocontrol methods with a focus on sustainable agriculture have gained attention, particularly the use of the genus *Streptomyces*. Despite numerous studies on crops and biotic stress, research on the immune response to environmental and biological stress is limited. Our previous studies found that *Streptomyces bacillaris* S8 and *S. globisporus* SP6C4 exhibit excellent antifungal and antibacterial activities and suppress plant diseases. In this study, we aim to demonstrate the feasibility of using these strains as biological control agents. Genome sequencing revealed the presence of antibiotic-producing gene clusters in SP6C4 and S8. To verify the antifungal properties of these clusters, CRISPR/Cas9 was used to create biosynthesis knockout mutants. Results showed that the mutants lost antifungal activity against the Anthracnose and root rot pathogens. These findings demonstrate the potential of *S. bacillaris* S8 and *S. globisporus* SP6C4 as biocontrol agents, warranting further research in this field to promote sustainable disease management in soybean crops.

P1.1-038

METATRANSCRIPTOMIC ANALYSES REVEAL FUNGAL FUNCTIONAL GENES WITH POSSIBLE ROLES IN THE INTERACTIONS AMONG DOMINANT FUNGAL GENERA DURING NOBLE ROT DEVELOPMENT OF GRAPES

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Text

During the noble rot (NR) process not only *Botrytis cinerea* but the complex interaction dynamics of other filamentous fungi and yeasts play a role enabling the production of botrytized wines. Metatranscriptomic data was analyzed from healthy berries (H) and berries representing the four NR phases (I-IV) from the Tokaj wine region in Hungary over three months. Since a previous, DNA metabarcoding study has indicated that the most abundant fungal genera in NR grape berries are the filamentous fungi belonging to the genera *Alternaria*, *Botrytis*, *Epicoccum* and the yeasts *Aureobasidium* and *Rhodotorula*, RNAseq reads were aligned to the reference genomes *Alternaria alternata*, *Botrytis cinerea*, *Epicoccum nigrum* and *Aureobasidium pullulans* and *Rhodotorula graminis*. The main antagonistic strategy for B. cinerea is by means of reactive oxygen species (ROS) synthesis, but it also degrades complex carbohydrates, making nutrients available for the rest of the microbiome. For *E. nigrum*, *A. pullulans* and *R. graminis*, the main antagonistic and synergistic interaction strategy is by competing for and liberating nutritional resources respectively. *A. pullulans* and *R. graminis* express genes involved in alcohol biosynthesis, which has an antagonistic effect on filamentous fungi. *A. pullulans* also expresses genes involved in sulfate biosynthesis, but this

appears to be countered by *E. nigrum* which synthesizes enzymes which lower the sulfate concentration.

P1.1-039

DETERMINING THE MICROBIOTA CONTRIBUTION ON CROP PERFORMANCE BY COUPLING IN SITU AND IN VITRO APPROACHES

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Text
The negative impact of chemical inputs used in agriculture on the environment and human health calls for the development of more sustainable agricultural managements. Recent studies have highlighted that host-associated microbial diversity has an effect on plant resistance to biotic stresses. Biological processes related to this adaptation include microbiota-pest interactions (competition, antagonism, parasitism) and/or modulation of plant immunity. The objective of the presented work is to use microbiota-pest interactions to estimate Synthetic Microbial Communities (SynComs) composed by bacterial and fungal strains with protective effects on crops against a pathogen. The study focuses on two different pathosystems: Rhizoctonia solanii infecting Brassica napus and Fusarium graminearum infecting Triticum aestivum. During this congress, I propose to present the first results obtained during my PhD program. A microbial collection has been established and characterized to identify the microbiota of the two crops grew under contrasted agronomic and pedoclimatic conditions in France. The first SynComs could be formed quickly to be able to do in vitro tests on B. napus in the presence of R. solanii. These results could be presented at the congress in August.

P1.1-040

STUDY ON PHENOLIC SUBSTANCES IN POPLAR ANTHRACNOSE

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Text
Phenolic substances are one of the important secondary metabolites of plants, which are of great significance in the resistance to the infection of pathogens and metabolic regulation. The early symptoms of poplar anthracnose are the oxidative allergic reactions of phenolic compounds, indicating that phenolic compounds play an important role in the occurrence and development of poplar anthracnose. Therefore, we determined the concentrations of phenolic compounds and their antifungal activities in three poplar species. The results showed that: (1) Three poplar species showed different resistance to poplar anthracnose: P. canadensis was resistant, P. tomentosa was susceptible, and P. beijingensis showed intermediate resistance; (2)This study selected 11 phenolic secondary metabolites with large difference and high content from three poplar species before and after inoculation. The results showed that these 11 substances showed different degrees of effects. Most of the

substances showed the lowest and highest concentrations to inhibit the growth of pathogen, while the intermediate concentrations promoted the growth of pathogen; (3) The kinds and content of phenolic substances in P. tomentosa are low, but P. tomentosa show resistance in the field. Therefore, we believe that phenolic substances do not play a major role in the interaction between P. tomentosa and Colletotrichum gloeosporum, but other structures, such as waxy layers, are important in antifungal activities.

P1.1-041

HARNESSING THE INNER BEAST: DEVELOPMENT OF BIOCONTROL AGENTS FOR SWEET ACACIA (VACHELLIA FARNESIANA)

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Text

Sweet acacia (*Vachellia farnesiana*) is a woody weed in Australia requiring Integrated Pest Management. Prospective biocontrol agents were sought among diseased plants from north and central Queensland. Symptoms were documented, samples collected, and stems were prepared for isolation using ½ strength Potato Dextrose Agar. Internal Transcribed Spacer sequencing and phylogenetic analysis revealed 53 fungal strains, several of which are undergoing formal description. Several isolates were also pathogenic on sweet acacia seedlings and were selected for use in a field study aimed to investigate the interaction between the fungi and a stress enhancer, glyphosate. The fungal inoculum was encapsulated and implanted into stems, while several were encapsulated with a sub-lethal dose of glyphosate. Untreated control and encapsulated glyphosate applications were included for comparison. All fungus-only and fungus+glyphosate treatments displayed significant stem lesions compared to control and glyphosate-only treatments. However, the canopy recovered and it is concluded that glyphosate addition did not increase fungal infection of sweet acacia within the year-long trial period. Investigation of these treatments is still ongoing to evaluate their long-term effect on sweet acacia. Further study on incorporating these isolates and investigating their pathogenicity as a potential biocontrol agent is also ongoing, particularly for the long-term management of sweet acacia.

P1.1-042

ACTION OF PYTHIUM OLIGANDRUM ON GRAPEVINE TRUNK DISEASES AND ITS IMPACT ON MICROBIAL COMMUNITIES

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Text

Grapevine trunk diseases (GTD) have become a major concern in viticulture. Since the ban in 2001 of sodium arsenate, the use of alternative methods, such as biocontrol, has become

a major issue. Among the promising microorganisms, the oomycete *Pythium oligandrum* is known to improve the plants health by increasing their natural defenses and reducing diseases to up than 40%.
The BIOBESTicide project, funded by the European Commission, aims to industrialize the production of a biopesticide solution to fight GTD. Efficiency evaluation of the product formulated from *P. oligandrum* will be carried out, and the environmental impact of this solution will be assessed.
Thus, an experiment was carried out in greenhouse for assessing the impact of the biopesticide on microbial communities by a high-throughput sequencing approach. Vines were treated with a *P. oligandrum* formulation and were inoculated with a fungus involved in Esca disease: *Phaeomoniella chlamydospora*. A three-month follow-up was carried out with samples from wood and rhizosphere environment to allow the evaluation of potential changes on microbial communities, whether as part of grapevine trunk disease or after the action of *P. oligandrum*.
The results obtained revealed that the biopesticide acts efficacy against *P. chlamydospora* with a reduction in the size of induced necrosis. Moreover, it had few effect on the rhizosphere microbial communities, which may suggest that the biopesticide is environmentally safe.

P1.1-043

DYNAMICS OF MICROBIOTA AND FUSARIUM SPP. RESPONSIBLE FOR FUSARIUM HEAD BLIGHT AND IMPLICATIONS FOR BIOCONTROL STRATEGIES

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Text
Fusarium Head Blight is a devasting disease in cereals caused mainly by *Fusarium* spp. (Fspp). Despite being primary inoculum sources, soil and crop residues have been less studied than grains. Knowledge of the diversity and dynamics of microbiota and Fspp populations in these compartments is relevant to elaborate efficient biocontrol strategies. Six min-till wheat fields were thus monitored for two years, with soil, maize residues, and wheat grains collected at four stages, before metabarcoding using 16S, ITS2, EF1α markers, and qPCR using Fspp-specific primers. *F. graminearum* (Fg) and *F. avenaceum* were dominant in both grains and residues. Despite similar Fspp loads in residues in both years, grains of 2021 were more severely infected than in 2022, most probably because of less conducive conditions (drier and hotter) at flowering for Fspp. Following metabarcoding, co-occurrence network analyses revealed significant negative correlations between Fg and *Epicoccum nigrum* as well as *Sphingomonas* sp.. In parallel, a collection of 1670 bacterial and fungal isolates from collected samples was built using two methods (culture on classical media or after confrontation with Fg using the double layer method). High throughput screening of their anti-Fg activities on wheat grain-based medium, followed by a taxonomic identification of positive isolates, is in progress and shows a high prevalence of *Trichoderma* spp. in soil, while *Epicoccum* spp. have also been found but to a lesser extent.

P1.1-044

SELECTION OF NATIVE TRICHODERMA ISOLATES OBTAINED FROM BANANA RHIZOSPHERIC SOIL IN THE CANARY ISLANDS FOR THE CONTROL OF FUSARIUM OXYSPORUM F. SP. CUBENSE (STR4).

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Text

The subtropical race 4 of *Fusarium oxysporum* f. sp. *cubense* (*Foc*-STR4) causes Panama disease in bananas crops of the Canary Islands. Hitherto, there is no effective control methods, thus the selection of biological control agents from the native microbiota could help to find a sustainable solution. Our objectives were: to analyse the diversity of biocontrol genes in the native Trichoderma collection and to evaluate the in vitro biocontrol capacity on *Foc*-STR4. Rhizospheric soil of infected and healthy plants were collected in 14 farms of Tenerife. It was obtained 109 isolates of the genus *Trichoderma*, in which 12 species were identified by phylogenetic analysis of the tef1 gene. Specific primers were designed to detect 9 genes involved in the biocontrol process: proteases (*p6281, tvps1)*, glucanases (*bgn13.1, glyc, lam1.3, egl1*), chitinases (*tv-ech1, 42-kDa, chit36Y*). Showing that 55.5 % of the isolates have genes involved in protease synthesis and most of them belong to the *Harzianum-Virens* lineage. In relation to glucanases (39.2% positive isolates) and chitinases (21.4% positive isolates), most of them belonging to the *Longibrachiatum* (glucanases) and *Trichoderma* (glucanases and chitinases) lineages. In vitro biocontrol tests were carried out in a Petri dish with PDA, evaluating the inhibitory capacity of *Trichoderma* isolates against *Foc*-STR4. Obtaining a percentage of Foc-STR4 inhibition up to 35 %, demonstrating the biocontrol potential of the native strains.

P1.1-045

USING BACTERIOPHAGES AS EVOLUTIONARY TOOLS TO CONTROL BACTERIAL WILT DISEASE: PLANT TRANSCRIPTOMIC RESPONSE TO PHAGE-RESISTANT BACTERIA

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Text

Ralstonia solanacearum causes disease in more than 200 plant species including bacterial wilt of tomatoes and brown rot of potatoes. This bacterium is part of the EPPO A2 list of quarantine pathogens due to its soilborne and waterborne nature, worldwide distribution and lack of effective control measures. The use of bacteriophages as biocontrol agents is promising, as they exhibit high specificity to individual bacterial species, and do not affect eukaryotic cells. However, one aspect of the use of phage should not be underestimated: the

quick ability of the bacteria to become resistant to the phage. This often leads to a trade-off, whereby in becoming resistant, bacteria lose virulence. This may be exploited to reduce the overall pathogenicity of *Ralstonia* populations, and can also help us to better understand plant-pathogen interactions. We have successfully identified phages which are effective at controlling *R. solanacearum*, and are now focused on understanding the transcriptome response of a susceptible tomato cultivar over time when inoculated with either a virulent ancestral strain, in-vitro evolved strains (non-virulent), or an ancestral strain plus phage treatment, which models co-evolution in the soil. The transcriptomic analysis reveals that there are clear differences in the immune response of the plant in each of the treatments. This work sheds light on the key genes activated in susceptible plants when phage resistance mutations occur in the bacteria.

P1.1-046

EFFICACY OF BIOPESTICIDE LIFEGARD® WG FOR CONTROLLING PLASMOPARA VITICOLA AND ERISIPHE NECATOR IN EUROPEAN GRAPEVINE (VITIS VINIFERA)

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Text

Fungicides are critical for modern grapevine (Vitis vinifera) production, but overuse has undeniable financial, environmental, and pathogen resistance consequences. This has led to a growing interest in biopesticides; however, it is yet unclear how best to integrate them into management programs. We conducted a metastudy of 7 years (2016-2022) of trial reports from the Cornell Pathology Vineyards (Chardonnay & Chancellor) in Geneva, NY to evaluate LifeGard® WG (Bacillus mycoides isolate J; BmJ) efficacy against grapevine downy mildew (Plasmopara viticola) and powdery mildew (Erysiphe necator). Disease incidence and severity was measured on leaves and clusters using the Horsfall-Barratt scale at harvest. We found that treatments containing LifeGard (e.g. alone, tank mix, or rotation), provided significantly greater protection compared to untreated control for both diseases. Generally, LifeGard provided better cluster control than foliar control. As expected, conventional materials provided more control than LifeGard when used alone, however, LifeGard provided exceptional control, beyond the commercial standards, when used in rotation. Overall, we find that integrating LifeGard into vineyard disease management can reduce conventional chemistry use while maintaining effective control. From these results, we speculate that increased biopesticide adoption can lengthen the useful life of highly effective yet resistance-prone fungicides by reducing resistance development pressure.

P1.1-047

RESPONSE OF CUCUMBER PHYLLOSPHERE MICROBIOME TO THE APPLICATION OF SYNTHETIC AND ENVIRONMENTALLY FRIENDLY FUNGICIDES IN MANAGING POWDERY MILDEW

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Text

Reducing the use of synthetic fungicides and implementing environmentally friendly alternatives are pivotal to achieving sustainable agriculture. However, the impact of these environmentally friendly fungicides on plant microbiomes has received limited attention. This study compared the effectiveness of two environmentally friendly fungicides (neutralized phosphorous acid (NPA) and sulfur) and one synthetic fungicide (tebuconazole) in controlling powdery mildew in cucumber. The differences in the phyllosphere microbiome are analyzed using high-throughput amplicon sequencing methods. The results showed that all three fungicides significantly reduced disease severity and the incidence of powdery mildew. However, while the α-diversity showed no significant differences of the phyllosphere microbial communities among treatments, tebuconazole had a significantly impact on the fungal community, as revealed by β-diversity analysis. The differential abundance analysis showed that tebuconazole altered the phyllosphere fungal composition by reducing the abundance of fungal OTUs, primarily from the Dothideomycetes and Sordariomycetes groups, which include potentially beneficial endophytic fungi. In contrast, NPA and sulfur had minimal effects on the phyllosphere fungal microbiome compared to the control. These findings indicated that the use of NPA and sulfur can effectively control powdery mildew while having fewer impacts on the phyllosphere fungal microbiome compared to tebuconazole.

P1.1-049

GROEL PROTEIN FROM THE POTENTIAL BIOCONTROL AGENT RHODOPSEUDOMONAS PALUSTRIS ENHANCES RESISTANCE TO RICE BLAST DISEASE

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Text

GroEL, which is a chaperone, plays a key role in maintaining protein homeostasis and, among other functions, serves to prevent protein misfolding and aggregation. In addition, the GroEL protein also has a significant effect on enhancing plant resistance and inhibiting plant diseases. However, the function of the GroEL protein in the inhibition of rice blast remains unknown. Field experiment results show that photosynthetic bacteria PSB-06 have a good control effect on M. oryzae. PSB-06 also can promote rice growth and enhance the stress resistance. A GroEL protein which was separated and purified from photosynthetic bacteria had a significant antagonistic effect on appressorial formation and pathogenicity of M. oryzae, meanwhile transcriptional analysis demonstrated that the GroEL protein could improve the expression of defense gene of rice. Our results show that the photosynthetic bacteria Rhodopseudomonas palustris significantly controls rice blast disease. Its action involves an extracellular GroEL protein, which inhibits appressoria formation, antagonizes the pathogenicity of Magnaporthe oryzae and promotes a host defense response. The research results provide evidence of the potential of this photosynthetic bacterium as biocontrol agent at least for rice blast control.

P1.1-050

MANAGEMENT OF ALMOND CANKER DISEASES WITH THE BIOCONTROL AGENT TRICHODERMA ATROVIRIDE STRAIN SC1 IN CALIFORNIA.

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Text

Almond is the most extensive crop in California producing about 80% of the world production. Canker diseases caused by *Botryosphaeriaceae*, *Ceratocystis destructans* and *Eutypa lata* are the main limiting factor for almond production in California. Currently, management of canker diseases relies mainly on chemical treatments, which constitute a concern for environmental contamination in California. The efficacy of the biocontrol product Vintec (*Trichoderma atroviride* SC1) and benefits a spreader-sticker adjuvant to protect almond pruning wounds from infection by canker pathogens was evaluated using different spray application technologies [Solo 425 4-gallon backpack sprayer (Solo®); 25-gallon brushbuster spot sprayer Ag Spray Equipment and 100-gallon Pak-Blast air blast sprayer (Rears Manufacturing)]. Vintec treatments were compared to a water control and the chemical fungicide thiophanate-methyl. All treatments were applied on fresh pruning wounds 24 hours prior to inoculation with 100 µl of a spore suspension (1×104 spores/ml) of the fungi *Botryosphaeria dothidea*, *Neofusicoccum parvum*, *E. lata* and *C. destructans*. Results indicated that the efficacy of *T. atroviride* SC1 to protect pruning wounds was greater or equal to that of Thiophanate-methyl for *E. lata* and *Botryosphaeriaceae* fungi. Wound protection was improved when Vintec was amended with a spreader-sticker adjuvant and using the Solo 425 backpack sprayer and the brush spot sprayer.

P1.1-051

EFFECT OF ADDITIONAL ARBUSCULAR MYCORRHIZAL FUNGI (AMF) APPLICATION INTO PLANTING HOLE FOR GANODERMA DISEASE MANAGEMENT IN OIL PALM PLANTATION

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Text

Oil palm remains to be vulnerable to basal stem rot disease caused by *Ganoderma boninense*, the most severe oil palm disease in Malaysia. Arbuscular mycorrhiza fungi (AMF) are ubiquitous in the soil and well known to play an essential role in plant growth, plant protection and soil amendments. Although the success of research in oil palm has been variable, this paper reports the benefits of additional AMF application observed in two field trials in coastal

area estates in Malaysia; after 13 and 20 years of evaluation for *Ganoderma* management in oil palm plantations. Several AMF treatments were evaluated. Data on *Ganoderma* incidence in the same planting point was compared before replanting and then, after 13 and 20 years with AMF treatment. Overall results showed that 50g AMF inoculated during seed sowing is highly recommended for timely contact of the fungus to the root cells for vigorous colonization in oil palm roots. A single application of 50g AMF onto 3-month-old oil palm seedlings (T5) and 100g AMF into the planting hole only (T2) seems not sufficient to control *Ganoderma*. A combination of 50g AMF either during seed sowing or to 3-month-old oil palm seedlings with an additional of 100g or 500g of AMF applied into the planting hole demonstrates a positive significant result in *Ganoderma* control. Subsequent application of 500g AMF at the 6-monthly interval, for the first 3 years of planting also showed an effective control of *Ganoderma* disease in the oil palm field.

P1.1-052

SCREENING BIOCONTROL AGENTS FOR CASH CROP FUSARIUM WILT BASED ON FUSARIC ACID TOLERANCE AND ANTAGONISTIC ACTIVITY TO FUSARIUM OXYSPORUM

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Text

Fusarium wilt caused by Fusarium oxysporum is one of the most notorious diseases for cash crops. Using of microbial fungicides is one of the effective measures for controlling crop Fusarium wilt, and the genus of Bacillus is an important resource to develop microbial fungicides. Fusaric acid (FA) produced by F. oxysporum can inhibit the growth of Bacillus, thus affecting the control efficiency of microbial fungicides. Therefore, screening biocontrol Bacillus with FA-tolerant ability is hopeful to improve the biocontrol effect for Fusarium wilt. In this study, the method for screening biocontrol agents against crops Fusarium wilt was established based on the tolerant to FA and antagonism to F. oxysporum, three promising biocontrol bacteria, named as B31, F68 and 30833 were obtained to successfully control tomato, watermelon and cucumber Fusarium wilt. Strain B31, F68 and 30833 were identified as B. velezensis by phylogenetic analysis of 16S rDNA, gyrB, rpoB and rpoC genes sequences. Co-culture assays revealed that strain B31, F68 and 30833 enhanced the tolerance to F. oxysporum and its metabolites, when compared with B. velezensis strain FZB42. Further experiments confirmed that 10 μg/mL FA could completely inhibit the growth of FZB42, while strain B31, F68 and 30833 kept normal growth at 20 μg/mL of FA and partial growth at 40 μg/mL of FA. Compared with strain FZB42, strain B31, F68 and 30833 significantly improved the tolerance to FA.

P1.1-053

A NATURAL METABOLITE, REJUAGRO, TO CONTROL APPLE FIRE BLIGHT, CITRUS CANKER, AND CITRUS GREENING DISEASES

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Text

Since 2017, our research team has built an extensive collection of microbes from different orchards and natural environments in Wisconsin and other states of the U.S. in order to identify natural metabolites produced from microbes for plant disease control. A novel metabolite named "RejuAgro" produced from Pseudomonas soli strain T3-07 was discovered. RejuAgro has been commercially formulated with a two-year shelf-life when stored at room temperature. RejuAgro shows high potency in suppressing multiple bacterial and fungal crop pathogens with large economic impact, including pathogens causing apple fire blight, citrus greening, and citrus canker. External field trials of RejuAgro have been performed to assess its inhibition efficacy on apple fire blight and citrus canker. RejuAgro was benchmarked against streptomycin, a commercial antibiotic that is considered the gold standard for controlling crop diseases such as fire blight in the U.S. A treatment of RejuAgro at 10-20 ppm can effectively control the fire blight and citrus canker in field trials.
There is no cure once a tree is infected with citrus greening. USDA predicted that citrus greening could destroy the entire U.S. citrus industry during our lifetime. RejuAgro suppresses Candidatus Liberibacter asiaticus, the cause of citrus greening by foliar spray. In addition, we observed a higher expression of pathogenesis-related defense genes PR1 and PR2 when RejuAgro was applied to the citrus.

P1.1-054

FIELD EPIDEMIOLOGY OF AN OBLIGATE BIOTROPHIC PLANT PATHOGEN IN THE CONTEXT OF CLASSICAL BIOLOGICAL WEED CONTROL

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Text

Kordyana brasiliensis, an obligate biotrophic fungal pathogen native to South America, was deliberately introduced to Australia as a classical biological control agent for the environmental weed Tradescantia fluminensis. A field-based monitoring study was established in New South Wales at 14 sites where K. brasiliensis was released across a broad latitudinal (~ 1000 km) and climatological gradient. The study's objective was to evaluate the climatic conditions conducive to K. brasiliensis establishment and severe disease development as well as the ecological impacts of sustained disease on T. fluminensis. Sites were monitored 6, 18 and 24-months post release, assessing K. brasiliensis disease incidence (number of stems infected per plot) and severity (percentage of leaf area covered by lesions) and T. fluminensis abundance (cover and volume). Kordyana brasiliensis established at all release plots 6-months post release with an average disease incidence of ~ 80% at release plots. At 6, 18 and 24-months post release, disease severity was lower at southern, cooler, and drier sites compared to sites in the northern region with warmer, humid climates. At 18-months post release, K. brasiliensis high disease severity was strongly correlated with a significant negative decline in T. fluminensis abundance, with greatest reductions observed in the northern region.

P1.1-055

BIOCONTROL ACTIVITY AGAINST SOIL-BORNE PLANT PATHOGENS BY BACILLUS SPP.

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Text

Soil-borne diseases are plant pathogens worldwide that cause disease in many economically important crops. Soil-borne pathogens are notoriously difficult to control. However, people using insecticides to control soil-borne diseases have developed pathogens that are resistant to the fungicides. Here, Bacillus spp. is used to control strawflowers stem rot caused by Sclerotinia sclerotiorum and cucumber damping-off caused by Pythium aphanidermatum. Seven strains of Bacillus spp. isolated from mushroom compost, it has the ability to promote plant growth. Among them, B13 and B36 inhibited the mycelial growth of S. sclerotiorum and P. aphanidermatum on PDA (potato dextrose agar) plates. In the greenhouse experiments, B13 and B36 bacterial cultures were irrigated on 5-week-old strawflowers and 14-day-old cucumber seedlings. After 7 dpi, plants inoculated with mycelium blocks of S. sclerotiorum which grew on PDA for 7 days, or inoculated with zoospores solution (10^8 CFU/ml) of P. aphanidermatum. The disease incidence was recorded after 3 weeks and 10 days, respectively. Results showed that the incidence of plant irrigated of B36 were reduced 27% compared to control. The incidence of Pythium damping-off of cucumber seedlings was reduced from 92% of the control to 32% of Bacillus spp. B36 treatment. B36 was identified as B. velezensis and B13 was B. aryabhattai of 16S rDNA sequence. The strain B36 has the potential to control stem rot of strawflower and Pythium damping-off of cucumber.

P1.1-056

SELF-RESISTANCE MECHANISMS DURING THE BIOSYNTHESIS OF ANTIMICROBIAL N-OXIDE PHENAZINE IN LYSOBACTER ANTIBIOTICUS

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Text

Phenazines are redox-active compounds exhibiting broad antibiotic activity. *Lysobacter antibioticus* OH13, a soil bacterium emerging as a potential biocontrol agent, produced phenazine 5,10-dioxides myxin with outstanding antimicrobial activity. Antibiotic-producing microbes always employ self-resistance mechanisms, mainly including efflux, target modification, sequestration, and enzymatic inactivation to escape self-toxicity. A monooxygenase encoding gene *LaPhzX*, located in myxin biosynthetic gene cluster, deletion of which caused the mutants more sensitive to myxin and prolonged existence of myxin. Meanwhile, myxin decreased significantly in the LaPhzX protein reaction, and heterologous expression of LaPhzX in *Xanthomonas* increased its resistance to myxin. So LaPhzX is a

myxin detoxification enzyme for protecting *L. antibioticus* from suicide. In addition, we found a RND (resistance-nodulation-division) efflux pump encoding gene cluster *lexABC* in strain OH13, and their deficiency resulted in strains increased myxin susceptibility and reduction of myxin yield. Moreover, *lexABC* expression was induced by myxin and directly activated by a LysR type transcriptional regulator LexR. Myxin bound with LexR at valine (146) and lysine (195) residues. These results indicate a RND pump with regulation mediated self-protection strategy in *L. antibioticus*. The discovery of two self-resistance mechanisms against myxin in *L. antibioticus* is important for obtaining high-yield myxin strains.

P1.1-057

DOPING IN P. RADIATA: CAN PLANT GROWTH PROMOTING BACTERIA ENHANCE MORPHO-BIOCHEMICAL TRAITS

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Text

Pine pitch canker (PPC), caused by Fusarium circinatum, is an alarming forest disease that affects pine species differently. Pinus radiata is susceptible to PPC, while Pinus pinea is resistant. Previous work has shown that the Pinus pinea microbiome may be a source of plant growth promoting bacteria, which may also enhance resistance to PPC.
In this study, bacterial isolates were obtained from P. pinea and characterized. These were mixed into bacterial consortia (A1-A5 and B1-B5) presenting a progressively higher number of beneficial characteristics: IAA production, phosphate solubilization, siderophore production and ACC-deaminase. Pinus radiata seeds were soaked for 2 hours in solutions containing the bacterial consortia and sown in a peat:vermiculite (1:1, v/v) soil mixture. After 30 days of germination, seedlings' height, biomass and biochemical parameters (i.e. pigments, sugars and starch (STA), free amino acids (FAA), phenolics, flavonoids and malondialdehyde (MDA)) were assessed.
Concerning germination, B3 and A4 consortia increased germination up to 20%. The A4 group displayed significantly more adventitious roots, and higher STA and FAA content. B1 and A5 showed higher MDA content.
All in all, this study points to a high potential of the selected bacterial consortia to affect the germination and primary metabolism of P. radiata. Further experiments with larger numbers of plants and subsequent inoculations are needed to fully assess the impact of these bacteria.

P1.1-058

DIVERSITY OF NODULE-INHABITING BACTERIA ASSOCIATED WITH CULTIVARS OF PISUM SATIVUM AND THEIR BIOCONTROL POTENTIAL AGAINST APHANOMYCES EUTEICHES

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Text

Pea root rot, caused by the oomycete, *Aphanomyces euteiches*, is considered to be the most damaging pathology, as it can lead to significant losses in pea fields and there are currently no effective treatments for it. Studying plant microbiomes, such as bacterial endophytes present in pea nodules, can be a basis for valorizing them as potential biocontrol agents. For this purpose, nodules of three spring and three winter pea cultivars were investigated to isolate bacterial endophytes and test their potential biocontrol ability against *A. euteiches*. Moreover, a metabarcoding approach was performed to comprehensively assess the diversity of bacterial endophytes, with a particular emphasis on the biocontrol genera that are already described. Screening tests revealed 17 isolates, from five out of six cultivars, with an *in vitro* antagonist effect towards *A. euteiches*. High-throughput sequencing showed a predominance of *Rhizobium lusitanum* followed by *Rhizobium leguminosarum* in the nodules of all cultivars. High-throughput sequencing revealed a higher diversity of minor endophytes in the two winter cultivars, which are more resistant to frost, and known antagonist genera were more abundant in winter cultivars, making them better suited for biocontrol against *A. euteiches*. This research is the first to explore the microbiomes of nodule-inhabiting bacteria in multiple pea cultivars and provides a foundation for developing biocontrol strategies for managing pea root rot.

P1.1-059

METATRANSCRIPTOMIC ANALYSES OF GRAPES REVEAL DIFFERENCES IN EXPRESSED FUNCTIONAL GENES OF FILAMENTOUS AND YEAST FUNGI DURING NOBLE ROT AND GREY ROT

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Text

Botrytis cinerea is a necrotrophic fungus causing grey rot (GR) with crucial economic losses in fruit crops but can also cause the desired noble rot (NR) in grape berries used to produce botrytized wines. In both states, *B. cinerea* is associated with several other fungi, but the functional role of these is still poorly understood. Metatranscriptomic data was generated from healthy (H), noble rot (NR) and grey rot (GR) grape berries and RNAseq reads were aligned to the most prevalent filamentous fungi namely *Alternaria alternata*, *B. cinerea*, *Epicoccum nigrum* and yeasts, *Aureobasidium pullulans* and *Rhodotorula graminis* based on previous

culture-based studies. Differential enrichment analyses and pathway enrichment analyses revealed that all fungi and yeasts are most active in NR, followed by GR and H berries. Beside *B. cinerea*, several functional genes of other fungi and yeasts were linked to the well-known physico-chemical changes such as the increase of aromatic precursors, organic acids and favoured metabolites associated with NR berries. In addition, antagonistic microbial- and plant interaction genes were identified highlighting the complex population dynamics in a successful NR development and that *B. cinerea* is the main causal agent responsible for the necrotrophism associated with GR.

P1.1-060

IN VITRO CHARACTERIZATION OF PICHIA MEMBRANAEFACIENS FOR POSTHARVEST BIOCONTROL OF MONILINIA FRUCTICOLA.

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Text

Yeast-based biological control agents are sustainable and promising alternatives to control postharvest fungal diseases, such as brown rot, caused by *Monilinia fructicola*. *Pichia spp* is a yeast genus with interesting *in vitro* biocontrol attributes. The present study evaluated the biocontrol capacity of two native wine yeast strains of *Pichia membranefaciens* YCPUC66 and YCPUC144. Antagonistic action was observed in both strains, where YCPUC66 strain inhibited 78% of mycelium growth, while YCPUC144 strain inhibited 28%. Additionally, the antagonistic capacity based on the production of volatile organic compounds was evaluated by double plate assay. It was observed that YCPUC66 strain reduced mycelium growth by 89%, while YCPUC144 strain 61%. Postharvest conditions could reduce the cell viability of yeasts with biocontrol capacity due to stressors present in the medium, such as reactive oxygen species. Consequently, the tolerance to oxidative stress of both strains was evaluated by exposure to H_2O_2 concentrations. The results indicated that YCPUC66 strain showed greater tolerance to H_2O_2, close to 3mM. Until now, YCPUC66 strain has shown to have a greater inhibitory effect on the growth of *M. fructicola* and a greater tolerance to oxidative stress, becoming a promising biocontrol agent.

P1.1-061

ENHANCED BIOLOGICAL CONTROL AGAINST ACREMONIUM ACUATUM AND TRICHOTECIUM ROSEUM ON GRAPEFRUITS BY APPLICATION OF BACILLUS VELEZENSIS MWS28 WITH SODIUM ALGINATE

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Text

Grapes have many diseases caused by plant pathogens that reduce the quality of grapes, such as white stain symptoms, and anthracnose, and have been controlled mainly through

chemical control rather than biological control. In the case of beneficial microorganisms developed as biological control agents, due to their characteristics, much verification of grapefruit disease control has not been performed, and the control figures are insufficient. In this study, we selected the promising strain of Bacillus velezensis MWS28 which has induced systemic resistance and inhibitory effect on the plant pathogens such as White stain symptoms causing A. acutatum and T. roseum. Bacterial attachment increased as the concentration of alginate increased, and 0.3% alginate solution was most effective at gradient concentrations. As the attachment number increased, the biological control effect against grape white stain symptoms increased. At 3 weeks after MWS28 treatment, the grapefruits showed overweight, high sugar content, increased grapefruit size, and anthocyanin contents were significantly increased compared to the untreated control. These results showed that it can be used not only for grape disease control and yield increase during grape cultivation but also for eco-friendly pesticide-free cultivation.

P1.1-062

BIOLOGICAL CONTROL EFFICACY BY ANTAGONISTIC BACTERIA ON POSTHARVEST DISEASES CAUSED BY BOTRYOSPHAERIACEAE FUNGI FAMILY

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Text

Mango and avocado are the main subtropical crops in southern Spain. These fruits are grown and processed in the same geographical area, and are transported to the rest of Europe. However, postharvest diseases during storage and transportation could damage the export market. In this work, symptoms of rot in mango fruits were detected, and analyzed in search of the causal agent. Parallely, asymptomatic avocado fruits were also analyzed. These two fruits share, on many occasions, farms, processing and even transport, and could act as cross inoculation source. The main fungal genera found, both in mango and avocado, were Alternaria sp. and Neofusicoccum sp. Of these two genera, only Neofusicoccum sp. was able to reproduce the symptoms of rot in mango similar to previously detected. Likewise, Neofusicoccum sp. isolates, but not Alternaria sp., could produce rot symptoms in avocado inoculated fruits.

To study the control of this disease through sustainable strategies, two microbial biological control agents were tested. Both are isolated antagonists against phytopathogenic fungi, and correspond to the bacterium Pseudomonas chlororaphis PCL1606. and Bacillus velezensis UMAF6639. The applications of both microorganisms on the fruit showed significant levels of protection, although only UMAF6639 showed greater persistence in the fruit during the preventive applications in the field.

P1.1-063

THE RAPID DECLINE OF THE INVASIVE SPECIES AILANTHUS ALTISSIMA UNDER THE CHALLENGE OF VERTICILLIUM DAHLIAE: A PHYSIOCHEMICAL STUDY

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Text

The decay of *Ailanthus altissima* due to *Verticillium* spp. (Fungi, Ascomycota) represents a relevant opportunity for the biocontrol of this invasive tree, which is not properly counteracted by traditional physical and chemical approaches. Verticillium wilt symptoms remember those of drought as they are caused by vessel occlusions, even if phytotoxins produced by the fungus are also involved in the pathogenic mechanism. Here, outcomes from an open air pot experiment aimed to investigate the physiochemical responses of *Ailanthus* trees stem inoculated with *V. dahliae* (VdGL16 strain, isolated from the same host in Tuscany) are reported. Inoculated plants showed foliar injuries starting from 2 weeks post inoculation (wpi), and a final severe defoliation. Already at 4 wpi, the infection induced a reduction in leaf water content (-14% compared with uninoculated plants), stomatal opening and net photosynthesis (-46 and -38%, respectively). Moreover, the disease altered the translocation of mineral elements and carbohydrates, that reached minimum values at 8 and 6 wpi, respectively (-84 and -14%). An accumulation of abscisic acid, proline and phenylalanine was also observed at 8 wpi (3-, 10- and 6-fold higher, respectively), suggesting a potential response mechanism. Despite this weak attempt to counteract the fungal colonization, plants were prematurely compromised and death inevitably occurred, confirming the great potential of using *Verticillium* to control *Ailanthus* invasion.

P1.1-065

BIOLOGICAL CONTROL OF ZYMOSEPTORIA TRITICI IN WHEAT

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Text

The disease Septoria tritici blotch (STB), caused by *Zymoseptoria tritici*, is one of the most important and yield-reducing constraints in wheat production worldwide. Control relies heavily on fungicides, but there is an increasing desire to reduce fungicide use and the pathogen readily develops resistance against commonly used products. Biological control, using living microorganisms, is an upcoming trend within disease control, also for STB.
The mode of action of biocontrol agents is more complex than for traditional fungicides and therefore, the risk that they lose effect is considered much less than for chemical products. Potential biocontrol agents are often selected based on direct inhibitory effects *in vitro* and therefore, the mode of action is often not studied in detail. However, there is emerging evidence that one of the most important modes of action by biocontrol agents is induced resistance, which can only be discovered in assays using plants [1].
We use different fungi (e.g. endophytes and *Clonostachys rosea*) and bacteria to control STB and have found significant reductions of disease severity using spray applications of fungal

biocontrol agents under controlled and field conditions [e.g. 2]. Whereas *in vitro* studies showed limited inhibition of the pathogen, microscopy and transcriptomics implicated induced resistance as important mechanisms.

1. Latz et al. (2018). Plant Ecology & Diversity. 11: 555-567.
2. Latz et al. (2020). Biological Control. 141: 104128.

P1.1-066

CAN WILD BRASSICACEAE DEFENSE COMPOUNDS ENHANCE THE ANTAGONISTIC EFFECT OF SEED-BORNE FUNGI AGAINST ALTERNARIA BRASSICICOLA ?

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Text

Wild Brassicaceae plant species synthetize defense compounds that can have an impact on the interactions between seed-borne fungi, with contrasting effects depending on the identity of the compounds and of the fungal species. Some fungi can metabolize some glucosinolates while other fungi such as *Alternaria brassicicola*, the seed-borne causal agent of the black leaf spot disease on many Brassica crops, are inhibited by camalexin and isothiocyanates. To gain insights into the role of the host plant on the interactions between *A. brassicicola* and the potential seed-borne fungal antagonists, characterization of fungi associated with the seeds of wild Brassicaceae described as resistant to *A. brassicicola*, was conducted. Their antagonistic effect on *A. brassicicola* was tested in the presence of the main defense compounds encountered in their host plants. The impact of defense compounds identified in non-host Brassicaceae species was also measured. Through a series of confrontations conducted on solid media and on liquid media (by using nephelometry) enriched or not with glucosinolates and camalexin, the main goal of the study is to gain a better understanding of the mechanisms involved in the interactions between the host plant defenses and seed-borne fungal communities towards future design of biological control of *A. brassicicola*.

P1.1-067

THE MICROBIOME OF TUTA ABSOLUTA, IN SEARCH OF A BIO-CONTROL METHOD

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Text

ABSTRACT

Tomato is are regarded as one of the world's most economical crops that is only second to potato. They are important horticultural crop with significant contribution to world's food security as well as economic development from job creation. However, an invasive insect pest, Tuta absoluta, is a major threat to cultivation of tomato woldwide and is rapidly increasing its geographic presence. In a bid to develop an environmental-friendly control of this pest, next generation sequencing was used to unravel the microbiomes associated with the larvae of Tuta absoluta. DNA was extracted from larvae collected from different regions and sequenced using the illumina platform. Results of the sequencing showed that the dominant fungal phyla was Ascomycota, followed by Basidiomycota. Other fungal phyla present includes Mucoromycota, Glomeromycota, Mortiellomycota and Chrytridiomycota. Implications of these fungal occurrences in relation to biocontrol are discussed. There were also a large proportion of unidentified phyla, class, order, family, genus, and species.

P1.1-068

MULTIFUNCTIONAL BENEFICIAL BACTERIA FROM THE TOMATO ENDOPHYTOME AND THEIR BIOCONTROL ACTIVITY

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Text

A study of the tomato endorhizosphere microbiome was performed in commercial greenhouse conditions. Results showed that keystone taxa were represented not only by bacteria with high relative abundance, such as Bacillus and Pseudomonas, but also by numerous and lesser-known genera. The systemic selection of cultivable bacteria enabled us to obtain representative isolates which, in vitro, showed diverse PGPR abilities and marked antagonistic activity. We selected ten bacterial isolates both from genera commonly used as bioinoculants, such as Bacillus and Pseudomonas, and also from unconventional genera like Arthrobacter, Paenarthrobacter, Acinetobacter, Glutamicibacter, and Enterobacter. Bacterial isolates were evaluated individually or in different combinations as consortia for their PGPR activity, induction of plant resistance and biocontrol potential against the soil-borne pathogen Fusarium oxysporum f. sp. radicis-lycopersici (Forl), causal agent of crown and root rot and the leaf pathogen Xanthomonas euvesicatoria pv. perforans (Xep), causal agent of bacterial spot. Most of the treatments significantly reduced the symptoms of both diseases although to a different extent. The efficacy against pathogens that infect different plant organs suggests a multifunctional potential that combines different modes of action. The genomes of the bacterial isolates were sequenced using Oxford Nanopore long read, and Illumina short read sequencing. Genome analysis is underway.

P1.1-070

NEMATICIDAL ACTIVITY OF A BACTERIAL CONTROL AGENT AGAINST CLOVER CYST NEMATODE

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Text

Clover cyst nematode (CCN) is one of the important plant-parasitic nematodes worldwide, and mainly damage to Kimchi-cabbage production in Korea. Five chemical nematicides are available to control CCN in Kimchi-cabbage plant, but the nematicides are harmful to human and beneficial animals. Therefore, we need to develop the alternatives to control CCN. This study was performed to develop the bio-control agents (BCAs) using bacteria against CCN. Nematicidal activity of two bacteria (isolate BC1 and BC2) was assessed to CCN second-stage juveniles (J2s) using *in vitro* assay. As a result, culture solution of BC2 was highly toxicity to CCN J2s with 100% mortality. Culture filtrate of BC2 had also 74% of nematicidal activity to CCN J2s. To verify nematicidal activity of BC2 *in vivo*, pot experiment was conducted in a temperature-controlled room (25oC). The mean of fresh weight of Kimchi-cabbage in BC2 treatment were 1.4 and 2.2 times higher than that of the TSB (medium alone) and NemaO (nematode alone), respectively. The female reproduction on Kimchi-cabbage roots in BC2 treatment was inhibited by 77% (1st trial) and 73% (2nd trial) compared with control (NemaO), respectively. The BC2 treatments reduced the cyst size compared to NemaO treatment, but there was no significance. These results showed that the BC2 has nematicidal activity and potentials as BCAs against CCN.

P1.1-071

EPIGENETIC CHANGES IN TOMATO PLANTS MODULATED BY SOIL MICROBIOMES

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Text

The microbiomes in rhizosphere, phyllosphere, and endosphere lived in symbiotic relationships with plants can influence plants to be holobiont by the phenotypic plasticity and extended phenotype. Last several decades, soil microbiota has taken notice as a source to identify specific species able to contorl resistance and tolerance to biotic and abiotic stresses. However, the mechanism of change about phenotype of plants by soil microbiota has yet to be fully understood. In this study, we aimed to identify soil microbiomes that could promote the growth and development of the tomato (cultivar Micro-Tom). Our results showed that the fruit maturity of the Micro-Tom grown with microbiomes from Gijang B soil was 22.8% higher on average compared to those produced in the mock-treated soil. Additionally, the growth of Micro-Tom treated with microbiomes from Gyeongju soil increased by 138% compared with those grown under mock-treated plants. To understand if the phenotypic changes of the Micro-Tom were due to epigenetic modifications by microbiome, we checked the transcript level of the representative genes related epigenetics in different tissues and conditions. Furthermore, we analyzed the similarities and differences among microbiomes used in this study. Our study will give new insight into how soil microbiome can regulate plant phenotype and suggest that soil microbiome has a role in shaping the epigenome of plants from an evolutionary perspective

P1.1-072

RESISTANT KIWIFRUIT SPECIES OF HAYWARD CAN BENEFICIATION FLAVOBACTERIUM TO SUPPRESS BACTERIAL CANKER PATHOGENS

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Text

Bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) is the most destructive disease-causing great production loss to kiwifruit. Recent studies have shown that plant disease-resistant varieties can enrich beneficial microorganisms to inhibit the infection of pathogens. To investigate whether bacterial canker-resistant kiwifruit cultivars can enrich beneficial microorganisms, five common kiwifruit cultivars were identified for resistance by combining previous field surveys and laboratory resistance assays. Among them, the most resistant variety to Psa was *A. deliciosa* cv. Hayward, and the most susceptible was *A. chinensis* cv. HongYang. We researched the leaves, branches, root endospheric microbiome and rhizosphere soil microbiome of HongYang and Hayward by 16S rRNA gene amplicon sequencing in the field. The rhizosphere soil microbiome with the largest difference between the HongYang and Hayward, with more Flavobacterium in the resistant plants enriched. To identify whether Flavobacteria are involved in protecting plants against canker disease, we isolated and cultured 14 strains belonging to the high abundance of Flavobacteria OTU_1542, OTU_337, OTU_1542, OTU_6, OTU_193. It was proved that isolated Flavobacterium 55 and Flavobacterium B2 can reduce the occurrence of kiwifruit canker disease by inhibition zone methods and in the detached dormant experiments of leaves and branches.

P1.1-073

EFFECTS OF BENEFICIAL MICROBES ON DISEASE RESISTANCE IN TOMATO PLANTS

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Text

Sustainable agriculture is crucial to ensure food security for the expected 9 billion people in 2050. However, plant stress has led farmers to rely on chemical fertilizers and pesticides. This is also the case with managing the devastating tomato (*Solanum lycopersicum*) disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL). To address this issue, it is important to find an efficient, cost-effective, and eco-friendly solution. Beneficial microbes, which enhance nutrient availability and have anti-microbial properties, can serve as green alternatives to agrochemicals. This study aims to identify rhizobacteria with multiple plant

growth-promoting attributes and determine the key factors in stress mitigation. The bacterial strains were screened for various traits, such as solubilizing inorganic nutrients, tolerance to salt and drought, and production of indole acetic acid and siderophore. Strains were further assessed for *in vitro* inhibition on five economically significant phytopathogens, namely *F. oxysporum* f. sp. *niveum*, *Magnaporthe oryzae*, *Colletotrichum gloeosporioides*, and FOL. Thirteen potentially beneficial strains were obtained, and three of the best-performing strains were chosen for pot experiments in a greenhouse, which resulted in reducing disease severity of tomato infected by FOL. The use of these biocontrol agents is expected to regulate plant growth, defense-related genes, and chemical and physiological properties.

P1.1-074

EVALUATION OF SEVERAL ARBUSCULAR MYCORRHIZAL FUNGI PRODUCTS ON GROWTH AND TOLERANCE OF OIL PALM SEEDLINGS AGAINST BASAL STEM ROT DISEASE CAUSED BY GANODERMA BONINENSE

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Text

Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* has led to devastating yield losses in the oil palm industry and presently no effective solution to cure the disease spread has been discovered. Based on several product literatures, the use of Arbuscular Mycorrhizal Fungi (AMF) has been claimed to increase the oil palm tolerance against *Ganoderma* infection. AMF, being a beneficial microorganism, forms a symbiotic relationship with plant roots, which facilitates and increases the transfer of nutrients between the plant and the fungus. In Malaysia, several AMF products have been commercialised for use in oil palm. However, the effects of these products on oil palm growth and tolerance towards *Ganoderma* BSR disease have not been evaluated. Eight AMF products were selected in this study based on their contents. These products were applied during seed sowing, and their effects on oil palm growth were determined. Recorded growth parameters include oil palm seedling height and girth, as well as the number of fronds per seedling. After one year of inoculation with AMF, the oil palm seedlings were challenged with *Ganoderma boninense* PER 71 strain, using *Ganoderma* Rubberwood Block (RWB) to evaluate the AMF products' effectiveness against the BSR disease. Assessment based on the disease severity index was determined post-infection with the fungus.

P1.1-075

RECOVERY OF METAGENOME-ASSEMBLED GENOMES FROM THE PHYLLOSPHERE OF 110 RICE GENOTYPES

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Text
The plant microbiota plays crucial roles in sustaining plant health and productivity. Advancing plant microbiome research and designing sustainable practices for agriculture requires in-depth assessments of microorganisms associated with different host plants; however, there is little information on functional aspects of many microorganisms of interest. Therefore, we enriched microorganisms from the phyllosphere of 110 rice genotypes and subjected them to shotgun metagenomic sequencing to reconstruct bacterial genomes from the obtained datasets. The approach yielded a total of 1.34 terabases of shotgun-sequenced metagenomic data. By separately recovering bacterial genomes from each of the 110 rice genotypes, we recovered 569 non-redundant metagenome-assembled genomes (MAGs) with a completeness higher than 50% and contaminations less than 10%. The MAGs were primarily assigned to *Alphaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidia*. The presented data provides an extended basis for microbiome analyses of plant-associated microorganisms. It is complemented by detailed metadata to facilitate implementations in ecological studies, biotechnological mining approaches, and comparative assessments with genomes or MAGs from other studies.

P1.1-076

A BACTERIAL PROTEIN RHP-PSP MODULATES PLANT AUXIN PRODUCTION AND ALTERS LEAF METABOLITES COMPOSITION TO IMPLEMENT MUTUALISTIC INTERACTION WITH PLANT

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Text
Mutualism is a dominant feature in a variety of host-bacteria interactions. Mutualistic bacteria confer plants with growth promotion and pathogen resistance, reciprocally, plants preferentially supply carbohydrates and a stable habitat to mutualistic bacteria to support their efficient colonization. However, whether these bilateral activities act independently or are interlinked via the shared molecular mechanism remains largely unexplored. In this study, the reactive intermediate deaminase A family protein Rhp-PSP secreted by phyllosphere bacterium *Rhodopseudomonas palustris* JSC-3b was required for both JSC-3b-generated plant health and efficient JSC-3b colonization. The growth of *Nicotiana benthamiana* seedlings was promoted as a result of Rhp-PSP-mediated plant auxin production. Constitutively, the expression of Rhp-PSP gene in *N. benthamiana* caused superior seedling growth, concomitantly, the altered leaf metabolite composition repressed the in-vitro and in-vivo proliferation of phytopathogens *Xanthomonas oryzae* and *Pseudomonas solanacearum*, but promoted that of JSC-3b. In addition, protein-protein interaction analysis revealed that Rhp-PSP interacted with plasma membrane-localized protein, which was responsible for the Rhp-PSP-mediated plant health and efficient colonization on leaves. Collectively, our study reveals a mode of mutualistic interaction between plants and bacteria, in which both partners benefit from the Rhp-PSP-mediated plant physiological alternations.

P1.1-077

MICROBIOTA INTERACTIONS AND ASSEMBLY ON RICE LEAF

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Text

Plant-associated microbiomes confer fitness advantages to the plant host, including growth promotion, nutrient uptake, stress tolerance and resistance to pathogens. While many studies have illustrated those key roles of the root microbiota, less is known about the role of the leaf microbiota and how it is maintained and assembled. Here, we applied shotgun metagenomic sequenced leaf microbiomes of 110 rice genotypes to characterize leaf microbiota grown in the field. We identified that *Rhodopseudomonas palustris*, *Pseudomonas fluorescens* and *Ralstonia solanacearum* are microbial hubs in the co-occurrence network of rice leaf microbiota. Furthermore, we found that amino acids and derivatives were the most possible metabolic exchanges in the leaf bacterial community. Using Genome-wide association studies (GWAS), We identified rice genetic loci connected with the abundance of *Pseudomonadales*, *Burkholderiales*, *Xanthomonadales* and *Enterobacterales*. Notably, those genes of rice genetic loci were enriched in metabolic pathway and biosynthesis of secondary metabolites pathway. Finally, we demonstrate that our results can be used to improve future studies of the microbe-microbe and microbe-host interaction.

P1.1-078

ANTIFUNGAL CHEMICAL COMPOUNDS OF TRICHODERMA ISOLATES AGAINST THE INCITANTS OF CALONECTRIA LEAF BLIGHT OF EUCALYPTUS

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Text

Calonectria leaf blight is a serious disease of *Eucalyptus* in India. In this study, the efficacy of five *Trichoderma* isolates was tested against two prominent *Eucalyptus* pathogens, *Calonectria cerciana* and *Ca. pseudoreteaudii*. All the *Trichoderma* isolates exhibited >80% mycelial growth inhibition of both the pathogens in inverted plate assays. Solvent extraction of Trichoderma liquid cultures with ethyl acetate followed by gas chromatography-mass spectrometry (GC-MS), revealed naphthalene, 2-methyl-5-formylfuran, ethenone, and other volatile organic compounds (VOCs). Additionally, GC-MS analysis also detected n-alkanes (n-C11 to n-C21), 1-tetradecene, quinoline, α-phellandrene, 1-propyldodecyl phenylacetate, phenol, 2,4-bis(1,1-dimethyl)-phosphite (3:1), benzaldehyde, 4-propyl, tetradecanoic acid, myristic acid, n-hexadecanoic acid, and 9(11)-dehydroergosterol tosylate. *Calonectria* fungal hyphae treated with *Trichoderma* VOCs subjected to electron microscopy revealed ultrastructural and morphological damage. To our knowledge, this is the first report implicating the ability of *Trichoderma* VOCs to suppress the growth of *Calonectria* fungi

known to infect *Eucalyptus* in India. These results suggest that *Trichoderma* antifungals may be an eco-friendly alternative to chemical fungicides for the management of Calonectria leaf blight in *Eucalyptus*.
Keywords: VOCs, *Trichoderma*, *Calonectria*, biocontrol, GC-MS, electron microscopy, *Eucalyptus,* disease management

P1.1-079

INCREASED PRODUCTION AND USE OF INOSINE BY SPONTANEOUS VARIATION IN PAENIBACILLUS POLYMYXA E681

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Text
The variation in Paenibacillus polymyxa E681, a plant growth-promoting rhizobacterium (PGPR), occurs spontaneously, changing B-type (wild-typeical) to F-type (variant). To better understand the difference between the two types, a high-throughput analysis was performed using Biolog Phenotype MicroArray. Compared to the B-type, the F-type showed significantly different growth rates on 17 out of 960 substrates. There was no significant difference in substrates, such as osmolytes, pH, and sodium salicylate. The spectrum of substrates available for F-type was relatively wider than for B-type. In inosine, D-melezitose, and cytidine as single nutrients, the growth of F-type was significantly higher than that of B-type, and interestingly, a few endospores that F-type could not form were observed. To verify the phenotypic microarray result, each type was cultured in a flask containing a minimal medium, including inosine as a single carbon source, and similar results to those of the phenotypic microarray were obtained. A known inosine-related metabolism in E681 is de novo IMP biosynthesis, and all related genes including pur operon (purEKBCSQLFMNHD) were overexpressed in F-type as a result of RNA-Seq in our previous studies. In conclusion, F-type generated by a naturally occurring variation biosynthesized and utilized inosine better than B-type. The association between among inosine biosynthesis, endospore formation, and phenotypic variation needs to be further investigationed.

P1.1-081

SODIUM ALGINATE BIOENCAPSULATION FORMULATION ON THE EFFICACY OF BACILLUS SP. AS BIOLOGICAL CONTROL AGAINST PHYTOPATHOGEN

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Text
Plant disease control generally uses chemical pesticides. This method is not effective because it can cause environmental damage. To realize sustainable agriculture, effective control is needed and does not pollute the environment. One way that can be used is the use of antagonistic agents such as Bacillus sp. Encapsulation methods involve covering and protecting the microorganisms. The bioencapsulation formulation can protect Bacillus sp. from environmental stress such as chemical residues, unstable temperature , unsuitable pH, and sunlight. So that bioencapsulation can increase the effectiveness of Bacillus sp. as a biological control agent. The main topics discussed are bioencapsulation technology, bioencapsulation as a biopesticide formulation, materials, and manufacturing processes. This review presents a thorough analysis of the advantages and disadvantages of bioencapsulation technology. As well as closing with views on the prospects for bioencapsulation as a biopesticide formulation.

P1.1-082

IDENTIFICATION AND CHARACTERIZATION OF BREVIBACILLUS HALOTOLERANS B-4359: A POTENTIAL ANTAGONISTIC BACTERIUM AGAINST RED PEPPER ANTHRACNOSE IN KOREA

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Text
This study aims to screen and identify potential biocontrol agents (BCAs) from Freshwater Bioresources Culture Collection (FBCC), Korea, against major phytopathogens under in vitro conditions. Of 856 strains, only nine strains exhibited antagonistic activity, from which only one representative isolate *Brevibacillus halotolerans* B-4359 has been selected based on in vitro antagonistic activity and enzyme production. Cell-free culture filtrate (CF) and volatile organic compounds (VOCs) of B-4359 have been shown to be effective against the mycelial growth *C. acutatum*. B-4359 showed an excellent biological control effect of anthracnose on red pepper fruits. Further, B-4359 has been found to show a growth promotion effect in red pepper seedlings. Based on in vitro results, B-4359 played a role to control anthracnose disease effectively in field conditions when compared to other treatments and a non-treated control. The genetic mechanism underpinning the biocontrol traits of B-4359 was characterized using the whole-genome sequence of B-4359, which was closely compared to related strains. In a whole-genome sequence, B-4359 consisted of a 5,761,776 bp length with a GC content of 41.0%, including 5,118 CDS, 117 tRNA, and 36 rRNA genes. The genomic analysis showed 23 putative biosynthetic secondary metabolite gene clusters. Therefore, our results provide a better understanding of the B-4359 strain as an effective biocontrol against red pepper anthracnose for sustainable agriculture.

P1.1-083

ENDOPHYTES FROM HALOPHYTES: A SOURCE OF BENEFICIAL MICROBES FOR A SUSTAINABLE AGRICULTURE

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Text

The improvement of crop management strategies is needed due to the demands of adaptation to climate change, the emergence of new and more aggressive phytopathogens and the deterioration of agricultural soil quality. Beneficial endophytes are non-pathogenic microbes that live within plant tissues and can provide sufficient protection of their hosts against biotic and abiotic stress. For potential use in agriculture, we isolated and characterized over 600 endophytic microbes from olive trees and crop wild relative halophytes. We investigated thoroughly 26 beneficial Bacillus isolates using a multi-disciplinary approach and under biotic and abiotic stress conditions. We sequenced full chromosomes and plasmids of selected 26 Bacillus isolates. Comparative genomics reveal high genetic/genomic dissimilarity, novel secondary metabolism gene clusters and the discovery of new species. Six isolates grow in-vitro in high salinity (>15%). 15 isolates inhibited the growth of important phytopathogens (eg, Ralstonia, Clavibacter, Fusarium, Botrytis, etc). Several isolates retain these characteristics in-planta. The inhibitions were intensified when testing eluents, obtained from Bacillus cultures using flash column chromatography. Our studies provide strong evidence that specific beneficial Bacillus endophytes demonstrate high metabolic and genetic diversity and are excellent candidates as Bioinoculants for the enhancement of growth and tolerance of crops under biotic and abiotic stress.

P1.1-084

BIOCONTROL POTENTIAL OF BJERKANDERA ADUSTA AND SISTOTREMA BRINKMANNII AGAINST HETEROBASIDION SPP. PRIMARY INFECTIONS

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Text

One of the most efficient methods to control Heterobasidion root and butt rots is based on treatments of freshly cut coniferous stumps with biological or chemical products. Biological preparations based on the fungus Phlebiopsis gigantea are widely used in Europe as stump treatments against Heterobasidion spp., but these are more effective on pine stumps than on Norway spruce stumps. In the present study, we tested different Latvian isolates of

Bjerkandera adusta and Sistotrema brinkmanii for their antagonistic potential in vitro against both H. annosum sensu stricto and H. parviporum, using native isolates of P. gigantea and Finnish Rotstop® as controls. The best isolates were chosen using several features: growth rate on agar, antagonistic ability against Heterobasidion spp. and oidia production. Some of the B. adusta and S. brinkmanii isolates performed similarly to P. gigantea isolates. For those isolates the growth rate was measured in wood of Pinus sylvestris, Picea abies, Larix decidua and L. x eurolepis.
This study was funded by European Regional Development Fund project Nr. 1.1.1.1/20/A/095 “Biological control of Heterobasidion root rot using Latvian fungal strains”.

P1.1-085

IDENTIFICATION OF A STREPTOMYCES SPECIALIZED METABOLITE INVOLVED IN ANTIFUNGAL ACTIVITY, PLANT DEFENSE STIMULATION AND BACTERIA FITNESS IN THE RHIZOSPHERE

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Text

Rhizospheric microbiota harbors bacterial strains participating in plant immunity and resistance to root diseases. Recently, we identified a *Streptomyces* strain AgN23 isolated from grapevine rhizosphere, which produce a broad spectrum of antifungal metabolites and activates hypersensitive responses (HR) in *A. thaliana*. A metabolomic approach lead to the identification of a candidate compound produced by AgN23 which may impair sphingolipid metabolism in plants. Sphingolipid metabolism of plants is involved in HR, thus we characterized the role of this metabolite through a reverse genetic approach, based on the construction of AgN23 knock-outs strains. These mutants showed a reduced antifungal activity and are unable to inhibit Inositol Phosphorylceramide Synthase activity, a crucial enzyme in plant sphingolipid pathway. The induction by AgN23 of markers associated with HR or immune responses was compromised in AgN23 knock-out strains: nuclear calcium influxes, necrotic lesions, defense gene expression, and production of camalexin. Finally, we explored the role of the candidate metabolite in the soil and found that it is involved in the rhizosphere colonization by AgN23. Thus, we identified a specialized metabolite produced by a *Streptomyces* strain which is involved in antifungal activity, plant defense stimulation and strain fitness in the plant environment. Further work will aim to investigate how this strain and its cognate metabolite structure the rhizospheric microbiota.

P1.1-086

BIOLOGICAL CONTROL EFFICACY OF CHINESE CABBAGE CLUBROOT CAUSED BY PLASMODIOPHORA BRASSICAE

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Text

The club root of chinese cabbage can remain in the soi for a long period of 7 to 10 years, and after the dormant spores germinate, they break into the cabbage root hairs and multiply, forming root knots. Soil microbial community analysis and selection of useful microorganisms can be used as a basis for developing eco-friendly club root disease control technology from an agricultural point of view. Therefore, this study analyzed the microbial cluster difference between the outbreak and the undeveloped area of cabbage root knot disease for the development of the cabbage root knot disease eco-friendly control technology, and tested it to use as a basis for selecting eco-friendly biological control factors. The fungi soil microbiome examined the residual presence of club root pathogens in the soil to identify P. brassica in the site of the onset of club root disease, and not in the unendemic site. A comparison of the differences showed a 0.89-2.52% distribution difference in Trichoderma sp., Fusarium sp., and Purpureocillium sp.. The results of this study have provided a basis for the collection of root knot pathogen control microorganisms, especially, Trichoderma sp. is showed 47% club root disease inhibition effect in pot test. So it is believed that metagenomics data can be used as sufficient evidence when separating useful microorganisms for the development of eco-friendly club root disease control technology.

Key words : Chinese cabbage, clubroot, metagenomics, biocontrol

P1.1-087

PLANT GENOTYPE SPECIFIC MODULATION OF CLONOSTACHYS ROSEA-MEDIATED BIOCONTROL OF SEPTORIA TRITICI BLOTCH DISEASE ON WHEAT

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Text

Biocontrol agents are commonly used for disease management, however, biocontrol efficacy varies among plant genotypes, potentially because of genetic variation in plants for plant-biocontrol agent compatibility. This study aimed to explore the genetic variation in winter wheat for modulation of *Clonostachys rosea*-mediated biocontrol of septoria tritici blotch caused by *Zymoseptoria tritici*. In total, more than 200 wheat genotypes grown in the Scandinavian countries in the last 100 years were investigated under controlled greenhouse conditions. Foliar spray application of the pathogen and the biocontrol agent in two treatments, i.e. *Z. tritici* (Zt) alone and *Z. tritici* with *C. rosea* (ZtCr) was used to assess

disease progress over time and biocontrol efficacy. There was significant phenotypic variation among plant genotypes for disease progress in Zt and ZtCr treatments. Moreover, individual plant genotypes differed significantly between Zt and ZtCr treatments, indicating the plant genotype-dependent variation in biocontrol efficacy. Genome-wide association mapping using a 20K single-nucleotide polymorphism (SNP) marker array identified four SNP markers associated with *C. rosea* biocontrol efficacy and one distinct SNP marker associated with disease resistance. This work will serve as a foundation to further characterize the genetic basis of plant-biocontrol agent interactions, facilitating opportunities for concurrent breeding for disease resistance and biocontrol efficacy.

P1.1-088

MULTI-TRANSCRIPTOME ANALYSIS TO ELUCIDATE THE FLAVOBACTERIUM-MEDIATED SUPPRESSION OF BACTERIAL WILT AND THE CAUSATIVE BACTERIUM

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Text

The soil-borne pathogen *Ralstonia solanacearum* causes bacterial wilt and thereby crop losses in the Solanaceae plants including tomato, potato, pepper, and eggplant. Although the susceptibility to the wilt disease primarily depends on the plant genotype, the microbial community in the rhizosphere also contributes to the severity. A flavobacterium TRM1, isolated from the wilt-resistant tomato cultivar Hawaii 7996, suppresses *Ralstonia* wilt in a susceptible tomato cultivar. The antagonistic activity of TRM1 against *R. solanacearum* was also observed from co-cultivation of the two bacteria in mCPG medium. To infer the wilt-suppressing mechanism, a large-scale transcriptional characterization was conducted. The transcriptional changes of TRM1 and *R. solanacearum* under the co-cultivation condition were compared to those in mono-cultivation. The transcriptome data of TRM1, *R. solanacearum*, and tomato were also collected in the plant rhizosphere. Genes for several secretion systems in *R. solanacearum* were inferred to be associated with virulence, while genes encoding some membrane-bound proteins in TRM1 appeared to be associated with virulence suppression. Integrating the results of these transcriptional data helped us systematically understand the wilt-suppressing mechanisms between the plant pathogen, the disease-suppressing microbe, and the host plant.

P1.1-089

EVALUATING NEW ERWINIA PHAGES AS BIOCONTROL TOOLS AGAINST FIREBLIGHT DISEASE IN FRUIT TREES

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Text

Fireblight disease is caused by the bacterium *Erwinia amylovora* and has catastrophic consequences for apple and pear trees. The development of an innovative strategy against it is a priority challenge for fruit production worldwide. A potentially successful biocontrol tool could be the use of bacteriophages (or phages), the viruses of bacteria. To achieve this goal, a collection of 16 *Erwinia* phages newly isolated in the south of France were phenotypically and genotypically studied. The genomic analysis revealed the presence of 5 phage genera, including a new one, and 7 different phage species, including 4 new ones. Phage lifestyle analysis determined that all phages are virulent (only lytic cycle) and none can perform a lysogenic cycle, as advised for applied purposes. Their host range on a panel of 46 *E. amylovora* international strains and 4 closely related species was quantitatively assessed. Three phages have a broad host range (100% of strains), 12 phages have a medium host range (≥ 20 strains), and one has a narrow host range (13 strains). One phage can target two non-amylovora strains but the rest are restrained to the *E. amylovora* species, proving their specificity. The capacity of some candidate phages to inhibit bacterial growth *in vitro* was confirmed, and we are currently optimizing phage cocktails by adjusting phage types and ratios. Overall, this project seeks to prove the potential of phages as an efficient biocontrol tools against fireblight disease.

P1.1-090

SPORULATION POTENTIAL, DISPERSAL GRADIENT AND MYCELIUM GROWTH IN CONIFEROUS WOOD OF THE BIOCONTROL AGENT PHLEBIOPSIS GIGANTEA

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Text

During the vegetation season, natural stump colonization by Phlebiopsis gigantea plays a role in controlling Heterobasidion on coniferous stumps but this process may differ in cooler climate conditions. The aim of the study was i) to analyse P. gigantea spore deposition at low temperatures and gradients using Petri dishes and coniferous wood discs, ii) to compare P. gigantea and Heterobasidion spp. colonization in Pinus sylvestris discs and coniferous logs. Results showed that viable spores of P. gigantea are released in November and December. When air temperature exceeds 0°C, one cm2 of P. gigantea hymenophore can discharge on average 330100 basidiospores per day. The number of P. gigantea spores decreases with increasing distance from fruitbodies. However, at a distance of 10 m, the number of spores can reach up to 120 000 per m2 per hour. P. gigantea better colonize pine wood compared to spruce. When the area occupied by P. gigantea exceeded 7% of the P. sylvestris disc surface area, Heterobasidion spore infection was not observed.
This research was funded by JSC Latvian State Forests project No. 5-5.9.1_007q_101_21_79, “Investigation of the impact of root rot and reducing risks caused by root rot”.

P1.1-091

EFFECT OF BIOACTIVE METABOLITES OF NATIVE STRAIN OF TRICHODERMA HARZIANUM FOR MANAGEMENT OF MAJOR FOLIAR DISEASES OF MAIZE IN MEGHALAYA, INDIA

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Text

Trichoderma is a versatile ascomycetes fungus with lucrative biocontrol potential and plant growth promoting activity. To understand their biocontrol abilities, an isolate of *T. harzianum* (Th) native to Meghalaya, India was evaluated *in vitro* against three major pathogens of maize viz., *Rhizoctonia solani*, *Exserohilum turcicum* and *Sclerotium rolfsii* with significant inhibitory effect. The bioactive metabolite of Th was isolated, characterized and evaluated against the targeted pathogens, and found inhibitory effect. A field experiment was conducted for two seasons against Banded leaf and sheath blight, Exserohilum leaf blight and Sclerotium wilt of maize with eight treatment combinations of bioactive metabolite of Th. The results revealed a decrease in per cent disease incidence and severity with enhanced plant growth parameters and yield attributing parameters. A positive effect on total soil organic carbon percentage and total microbial populations as compared to the control was also observed during the experimentation. The results are indicative of the antifungal activities and PGP abilities of the bioactive metabolite of Th. The findings of the experiments may be useful as an effective way of managing the targeted diseases of maize.

P1.1-092

MICROALGA-ANTIFUNGAL BACTERIA SYNERGISTIC EFFECT ON PLANT PATHOGENIC FUNGI AND KING'S STRAWBERRY QUALITY

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Text

King's strawberry is one of large-fruited strawberries and is highly preferred by consumers due to their large fruits, but it is necessary to develop eco-friendly management technologies that can improve the disadvantages of soft fruits and weak powdery mildew. The purpose of this study was to evaluate the effects of phytopathogenic fungi on mycelial growth and quality of Strawberries when King's strawberry was treated with microalgae and antifungal bacteria. 0.4% microalga, Chlorella fusca (CF) and antagonist bacteria, AFB2-2 were mixed and replaced with Botrytis cinerea (BC), Colletotrichum gloeosporioides (CG), Phytophthora capsici (PC) and Sclerotinia sclerotiorum (SS). It was found to inhibit all mycelial growth of five plant pathogenic fungi. Hardness of King's strawberry with 0.2% and 0.4% CF and AFB2-2 increased by 22.7% and 9.1%, respectively, compared to untreated. In addition, the hardness of Seolhyang Strawberry mixed with 0.2% and 0.4% CF and AFB2-2 increased by 49.4% and 34.5%, respectively, compared to untreated. Through the above results, it is revealed that the combined treatment of chlorella (CF) and antagonist bacteria (AFB2-2) can be used as a biological management technology that can increase the hardness and sugar

content of King's and Seolhyang strawberry as well as inhibit the mycelial growth of plant pathogens.

P1.1-093

EXPERIMENTAL EVOLUTION TO STUDY THE ADAPTATION OF PLANT-BENEFICIAL PSEUDOMONADS TO INSECTS

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Text

Plant-beneficial pseudomonads are promising candidates for the biological control of plant diseases and insect pests. *Pseudomonas protegens* bacteria are efficient root and insect colonizer with antifungal as well as insecticidal activities. This versatility in lifestyles makes them highly interesting to study. Although many traits enabling root colonization and insect pathogenicity are already known, it is not clearly understood how these bacteria are adapted to a life in insects. We performed an experimental evolution with *P. protegens* CHA0 based on serial infection cycles of larvae of the crop pest *Plutella xylostella*. Although some evolved populations displayed altered insect killing speed compared to the original strain, bacterial virulence did not substantially change during the experimental evolution, indicating that *P. protegens* CHA0 is already well adapted to this insect species. *In vitro* screens of the evolved populations showed changes in growth rate and antimicrobial activities whereas genotyping revealed mutations in genes which are connected to the bacterial membrane structure. The adaptational phenotype of the identified genetic variations needs yet to be determined. Our experimental evolution provides new knowledge on the adaption of plant-beneficial pseudomonads to insects which is also important for their application in biological pest control.

P1.1-094

CHARACTERIZATION OF PEPPER-MICROBIOME FOR IDENTIFICATION OF PUTATIVE BIOCONTROL AGENTS AGAINST FUSARIUM SPECIES

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Text

Pepper (*Capsicum annuum* L.) is an economically and nutritionally important vegetable within the family Solanaceae. Biotic stresses impact the quality and productivity of the crop. Wilting caused by soil-borne *Fusarium* species are among the most challenging diseases of peppers to control. Integrated disease management (IDM) is a sustainable approach to control diseases. Biocontrol is an important component of IDM approaches to manage soil-

borne pathogens. Plant microbiome analysis has allowed opportunities for identifying associated microbes with plant beneficial functions, including biocontrol. Samples of bulk soil, rhizosphere, roots, and stem of diseased pepper plants showing wilting symptoms and healthy pepper were collected from the experimental farm of the WorldVeg, Shanhua, Taiwan. Metagenomic sequencing and analysis of the 16S (V3-V4) and ITS (3-4) regions were compared among samples. Species composition showed an abundance of *F. oxysporum*, *F. proliferatum*, and *F. solani* associated with diseased peppers. The unique taxa directly associated with healthy peppers and distinct from the pepper-conserved microbial community were identified as potential biocontrol agents. Thus, analyzing plant-microbial communities can provide insights into key microbes for biocontrol against diseases and their interaction with host plants and pathogens.

P1.1-095

DISSECTION OF THE ENDOPHYTIC AND RHIZOSPHERIC MICROBIOMES OF ATRACTYLODES LANCEA OF DIFFERENT ORIGINS AND VERIFICATION OF MICROBE FUNCTIONS ON A. LANCEA

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Text

Atractylodes lancea is a perennial herb and an important medicinal plant with a long history of clinical application in China. With the massive market demand and the subsequent development of *A. lancea* farming, severe issues including root rot disease outbreaks resulted from the continuous cropping of perennial *A. lancea*. We performed extensive studies on the microbiota associated with *A. lancea* and the soil to seek solutions.
Via next-generation sequencing of the 16S and ITS amplicons of microbe communities in the root endosphere and rhizosphere of *A. lancea* samples of diverse origins. We identified a group of steadily co-existing microbes in the rhizome consisting of the bacterial genera Rhodococcus, Ralstonia, Burkholderia-Caballeronia-Paraburkholderia, Sphingomona, and Pseudomonas, and a fungal genus Ascomycota, which we believe comprised the specific core microbiome of this particular plant species. We managed to isolate 33 endophytic bacterial strains and 13 endophytic fungal strains from the rhizome of *A. lancea* and performed inoculation experiments to study their resistance against root rot pathogens and their individual inducing effects on the medicinal compound accumulation and growth of *A. lancea*. We screened for endophytes with biocontrol potentials via dual-culture with two strains of Fusarium spp., the major pathogenic fungi of A. lancea root rot disease. The microbe strains we acquired showed promising application potential in future A. lancea farming.

P1.1-096

EFFICACY OF BCA'S AND PRI'S FOR THE CONTROL OF POTATO EARLY BLIGHT AND POTATO LATE BLIGHT

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Text
Early blight and late blight are both economically significant potato diseases worldwide. Control of potato early blight and late blight are currently heavily reliant on chemical fungicides. In the last decade, the use of biological control agents (BCA's) and plant resistant inducers (PRI's) for the suppression of plant pathogens has increased rapidly and has become a viable alternative for chemical pesticides. The aim of the present study was to assess the efficacy of different BCA's and PRI's for the control of both potato diseases in an artificially inoculated greenhouse studies. The influence of timing and dosage of BCA's and PRI's on disease development was evaluated on one moderately resistant and one susceptible potato cultivar. Efficacy of disease suppression was expressed through the area under the disease progress curve (AUDPC). Efficacies of biological products were compared with chemical fungicide Revus Top (mandipropamide, difenoconazole). There were significant differences in efficacies of used biological products. Results indicated that timing of application and product as well their interactions had marked effect on development of both diseases in both used varieties. The best products provided significant disease reduction, but none of them had the efficacy in the same level as used chemical fungicide. The experiment was carried out within the framework of the ECOSOL project.

P1.1-097

INVESTIGATION OF PTI ACTIVATION FOLLOWING THE PERCEPTION OF AN ELICITOR COMBINATION, IN ARABIDOPSIS THALIANA.

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Text
Plant elicitors (PE) have the ability to activate pattern triggered immunity (PTI), via there recognition by pattern recognition receptors (PRRs). PRRs contain extracellular domains and bind to PE in a receptor-ligand manner. For instance, PRRs containing leucine-rich repeats (LRRs) domains, are known to bind to peptides as bacterial flagellin, whereas PRRs containing lysine motifs (LysMs) are implicated in recognition of N-acetylglucosamines compounds as fungal chitin. PE perception results in the induction of a series of events such as reactive oxygen species (ROS) production, phytohormone signalling and defence molecules accumulation.

Here, we evaluated how the combination of two elicitors, perceived by different PRRs, affect A. thaliana PTI activation. Using an untargeted metabolomic approach, we found that

elicitors combination impacts a highest number of metabolites and deregulates specific metabolites pathway, when compared to the elicitors used alone. This highlights the interest to use a combination of elicitors in crop protection strategies. We then characterized a natural extract from Ulva algae from which we showed that plant eliciting activity was due to both ulvan polysaccharides and arabinogalactan-protein (AGP)-like compounds. We investigate defence response and metabolic changes induced by the Ulva extract treatment in A. thaliana and we studied how this type of complex extract is perceived by the plant.

P1.1-098

EFFECTS OF BIOINOCULANTS AND ORGANIC SOIL AMENDMENTS ON NEMATODE COMPOSITION OF APPLE ORCHARDS

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Text

Nematodes have versatile lifestyles and provide a suitable lens to decipher the ecosystem conditions. Here, 18S rDNA metabarcoding was employed to study the effect on the nematode composition of arbuscular mycorrhizal fungi, bioeffector, and organic amendments in apple orchards, which were sampled during spring and autumn. Sampling time more than treatment had a significant effect on the nematode diversity and composition, and higher alpha-diversity indices were observed during spring as compared to autumn. Although treatments were able to reduce nematode richness and diversity, their effects varied. The composition of bacterivorous and herbivorous nematodes showed seasonal variations, and a higher number of bacterivorous- as compared to herbivorous- nematodes were seen during spring. The composition of nematode trophic guilds was driven by dominant families like Rhabditidae and Tylenchidae. Nematode-based indices like structure and enrichment indices revealed maturing and moderately disturbed soils for the two apple orchards; and maturity- and plant parasite- indices were generally low. This indicates potential soil nutrient enrichment in the two different orchards resulting in high primary productivity for the herbivorous nematodes. Our study provides insights into the effect of soil treatment on nematode, with implications for the development and modification of bioinoculants, as well as the potential to improve the soil ecosystem services.

P1.1-099

IN VIVO AND IN VITRO ANTIFUNGAL ACTIVITY AND MOLECULAR MECHANISM OF DIMETHYL TRISULFIDE AGAINST COLLETOTRICHUM GLOEOSPORIOIDES FROM MANGO

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Text

Colletotrichum gloeosporioides can lead to huge economic losses during mango storage and transport, and dimethyl trisulfide (DMTS) was found in Streptomyces globisporus JK-1. The EC50 of DMTS to 66 representative strains of 13 mango Colletotrichum species in China was mainly from 0 to 20 μL/L, and the optimum treatment 80 μL/L for 6 h of DMTS to mango postharvest anthracnose could reach 66% control effect. A histological investigation demonstrated that DMTS exhibited strong inhibitory effects on the infection process of C. gloeosporioides in planta by inhibiting the germination of conidia and formation of appressoria, and contributing to deformation of appressoria prior to penetration. In vitro DMTS caused serious damage to the integrity of plasma membranes, which significantly reduced the survival rate of spores, and resulted in abnormal hyphal morphology. Moreover, DMTS caused deterioration of subcellular structures of conidia and mycelia, such as cell walls, plasma membranes, Golgi bodies, and mitochondria. In addition, to better understand the molecular antifungal mechanisms, the gene expression analysis showed DMTS significantly suppressed expression of ergosterol biosynthesis-related genes Cgerg6 and Cgerg11. The EC50 of ΔCgerg6 and ΔCgerg11 to DMTS was 3 folds and 1.9 folds of that of wild-type strain, respectively, and the wild-type phenotype was restored after the gene complements in situ, indicating that there was a close interaction between DMTS and the two genes.

P1.1-100

EXPLORING SOYBEAN AND SUNFLOWER MICROBIOMES FOR BENEFICIAL BACTERIAL MICROORGANISMS.

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Text

The control of Sclerotinia sclerotiorum in South Africa is limited to one registered fungicide for soybean and sunflower. There is an urgent need to identify biological control agents to combat white mold and promote plant health. In this study we isolate and identify beneficial microbes associated with soybean and sunflower to manage *S. sclerotiorum*. Soybean and sunflower phyllo- and rhizosphere samples from seedling to maturation were collected in Mpumalanga (South Africa). A total of forty bacterial strains were isolated and evaluated for plant growth-promoting properties, by determining phosphate solubilization, ammonium and indole acetic acid production. Sequencing of eleven bacterial isolates promoting plant growth and inhibiting four S. sclerotiorum isolates, varying in aggressiveness, was conducted using 16S-27F and 16S-1492R. Genera identified include *Bacillus*, *Lysinibacillus*, *Pantoea*, *Pseudomonas*, and *Sternophomonas spp*. In vivo root length, seedling vigor and biocontrol assays were conducted. *B. velezensis* significantly promoted root development and soybean vigor. Varying responses to *B. velezensis* was observed in *S. sclerotiorum* isolates with low and high oxalic acid production potential, ~50% and 67% inhibition, respectively. Aggressive pathogen strains must be considered when developing biological control strategies. Greenhouse and field experiments are underway to determine the reliability and efficiency of identified organisms against soybean stem rot.

P1.1-101

A CONSORTIUM OF BENEFICIAL MICROORGANISMS ALTERS THE RHIZOSPHERE MICROBIOME AND IMPACTS PLANT PERFORMANCE DIFFERENTLY IN CONTRASTING GROWING SEASONS

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Text

Intensification of agricultural management for yield maximization is associated with detrimental side effects on soil and plant health. We hypothesize that extensive management with integrated use of beneficial microorganisms (BM) promote plant stress resilience. The aim of this study was to examine whether the use of a consortium with BM affects the rhizosphere microbiome and plant health of maize depending on farming practices over two growing seasons. A long-term field experiment allowed the comparison of two tillage practices (mould-board plough vs. cultivator tillage) and two nitrogen (N) fertilization intensities (intensive vs. reduced extensive N-fertilization). In both years, successful root and soil colonization of the BM was detected in all treatments, associated with changes in the rhizosphere microbiome. A significant increase in biomass and nutrient content of the inoculated plants could only be detected in the growing season of 2020, which was characterized by severe spring drought. This was associated with increased expression of physiological stress indicators involved in drought stress defense, such as osmotic adjustment and detoxification of reactive oxygen species (ROS), and accordingly reduced leaf concentrations of ROS. This multidisciplinary study provides insights into the influence of BM applications on plant-microbe interactions and plant performance inclusive the relevance of abiotic stress factors under field conditions.

P1.1-102

EVALUATION OF TRICHODERMA SPP. OIL PALM ENDOPHYTE ON THE IN VITRO GROWTH OF PHYTOPHTHORA PALMIVORA

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Text

The Oomycete *Phytophthora palmivora* is the causal agent of Bud rot. This primary disease affects oil palm plantations in the Colombian North zone, which has reached high incidence and severity levels during the last five years. Endophytic microorganisms are of particular interest as biological control agents since they can colonize the internal tissue of plants

without causing apparent damage, compete for nutrients and space within the vascular system, and act as inhibitors of pathogenic microorganisms. Fungi of the genus *Trichoderma* comprise a group of filamentous fungi widely used in the biocontrol of plant pathogens. This study was carried out in six plantations in northern Colombia, where root samples were taken from 18 oil palms (*Elaeis guineensis*), which were processed in selective media for *Trichoderma*. Subsequently, 30 endophytic isolates of *Trichoderma* spp. were morphologically identified, and their antagonistic capacity against *P. palmivora* was evaluated with dual culture and mycoparasitism assays. The potential of 15 *Trichoderma* isolates for the control of this pathogen with PICR values >70% in the dual culture test and 100% parasitism, where mycelium coiling, sporangia and chlamydospore parasitism were observed. This study highlights the potential of using endophytic *Trichoderma* as biological control agents in the integrated management program of Bud rot in oil palm plantations in Colombia.

P1.1-103

MECHANISMS OF ACTION OF AKANTHOMYCES LECANII ON PEANUT RUST: ULTRASTRUCTURAL INVESTIGATIONS

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Text

Peanut rust caused by *Puccinia arachidis* is one of the most important peanut foliar diseases in the world. For sustainable peanut production, ecological disease management strategies are needed to limit the overuse of synthetic fungicides in the control of rust. Thus, the use of *Akanthomyces lecanii*, a hyperparasite of rust fungi, may be an alternative to control peanut rust. However, investigations of the mechanisms of action of *A. lecanii* are essential before its development as a biocontrol agent. To do so, peanut leaves bearing rust sori were detached, and each leaf was sprayed with 500 µl of a suspension of *A. lecanii* conidia (10^6 conidia/ml) at lower surface. Then, each leaf was incubated in a Petri dish on moist blotting paper at 20°C, under a 12:12 light/dark photoperiod. After 15 days' incubation, the inoculated and uninoculated rust sori were observed under photonic and electron microscopes. From our findings, pictures revealed that *A. lecanii* colonized the urediniospores of inoculated sori. Our results showed that the mechanisms of action of *A. lecanii* on *P. arachidis* could involve the following events: (i) attachment of the antagonist to urediniospores mediated by a mucilaginous extracellular matrix; (ii) penetration due to mechanical pressure and enzymes action on cell-wall; (iii) active growth of the antagonist inside of urediniospores and digestion of cell contents. From our study, the use of *A. lecanii* spores is promising tools for biocontrol of peanut rust.

P1.1-104

EFFECT OF CO-INOCULATION OF PINE SEEDLINGS WITH TRICHOLOMA SP AND/OR STREPTOMYCES ON HETEROBASIDION PATHOGENESIS AND HOST GROWTH

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Text

Forest trees frequently interact with a diverse range of microorganisms including ectomycorrhiza, bacteria, and fungal pathogens. Plant defense responses to individual pathogen have been widely studied, but very little is known on the effect of co-inoculation on host defenses. To study the impact of co-inoculation or tripartite interaction on plant growth and host defenses, Scots Pine (Pinus sylvestris) seedlings were inoculated with either Tricholoma sp or Streptomyces sp or both together with a root pathogen Heterobasidion annosum for three months. The inoculation with Streptomyces or Heterobasidion alone had negative effect on plant growth whereas co-inoculation of Tricholoma and Streptomyces sp in presence of the pathogen seems to promote plant growth (root length, number of lateral roots, seedling weight) of Scots pine over time. Based on the phenotypic examination, it was concluded that the ectomychorrhizal Tricholoma sp and Actinobacterial Streptomyces sp counteracts negative effect of H. annosum on plant growth. RNA-seq analysis of seedlings inoculated with Tricholoma sp, Streptomyces sp, infected with H. annosum will be analyzed for identification of differentially expressed genes (DEGs). The potential of pre-inoculation of seedlings to protect seedling roots before out-planting deserves to be further explored.

P1.1-105

IN VITRO EVALUATION OF FUNGAL ENDOPHYTES OF ROSEMARY (ROSMARINUS OFFICINALIS) AGAINST DIPLODIA BULGARICA

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Text

Endophytic fungi are a biodiversity-rich group of microorganisms that are widely present in internal plant tissues and often provide beneficial effects to their hosts. Rosemary (*Rosmarinus officinalis*) is an important medical and aromatic plant and has antimicrobial, antioxidant, antiviral, and immune system stimulatory properties. *Diplodia bulgarica* is one of the causal agents of canker and fruit rot in apples (*Malus domestica*). In this study, we isolated and identified endophytic fungi from healthy rosemary leaf, flower, and branch tissues collected in Aydin and Mugla provinces, Turkiye. An experiment on the in vitro screening of 452 endophytic fungi of rosemary against *D. bulgarica* CEE-273 indicated that four isolates showed more than 50% inhibition. Using the internal transcribed spacer (ITS) regions of rDNA, the isolates with biocontrol potential were identified as *Pyronema omphalodes* (Ro-321 and Ro-621), *Nigrospora gorlenkoana* (Ro-615), and *Sordaria fimicola* (Ro-611). This is the first report on the endophytic association of the above fungi with rosemary and the exploitation of their biocontrol potential against *D. bulgarica*.

This research was funded by Aydin Adnan Menderes University Scientific Research Projects Coordinator, grant number ZRF-21039.

P1.1-106

INVESTIGATING THE INVOLVEMENT OF TOMATO RHIZOBACTERIA IN RESISTANCE TO BACTERIAL WILT

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Text

The plant microbiome can modulate plants' physiology and phenotype, such as development, growth, and responses to biotic and abiotic stresses. Previously, we demonstrated that a flavobacterial sp., isolated from the rhizosphere of a wilt-resistant tomato, suppresses the disease caused by *Ralstonia solanacearum*. Here, we defined bacterial species that are enriched in either resistant or susceptible tomatoes using 16S rRNA gene sequences and whole metagenome sequences. We established a collection of rhizosphere microbes, to constitute a synthetic microbial community for bacterial wilt resistance. We selected strains that belong to *Flavobacteriaceae*, *Rhizobiaeceae*, *Xanthomonadaceae*, *Rhodobacteriaceae,* and *Cyclobacteriaceae* as members of the synthetic community. An uncultured *Sphigomonadaceae* strain, which was abundant in the resistant tomato, was also successfully isolated by utilizing the metagenome-assembled genome information. The synthetic community and individual strains were subject to assays that assess their effects on bacterial wilt resistance and plant growth promotion. Our study on the rhizosphere microbiome for its involvement in disease resistance and plant health will shed light on understanding intricate relationships between plant, pathogen, and microbiome. Further, bacteria recovered in this work may serve as a useful resource to study microbe-host interactions and can be utilized as plant probiotics.

P1.1-107

BIOFORMULATION OF PHYLLOPLANE PSEUDOMONAS SPP. FOR MANAGEMENT OF SHEATH BLIGHT DISEASE OF RICE (ORYZA SATIVA)

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Text

This study was conducted to formulate phylloplane Pseudomonas bacterial strain P. fluorescens and P. asplenii and to determine their efficacy on sheath blight suppression as well as on yield components of rice. Both strains were preserved in peat and talc as single

strains or in mixtures at 4°C and 28±°C. The peat formulation was found to be more suitable (at 4°C) than talc to retain longer shelf life of individuals and strain mixtures with sufficient viable cells. Evaluation of formulated strains for suppression of sheath blight revealed that the consortium and P. fluorescens alone significantly reduced the area under disease progress curve (AUDPC) compare to untreated control. Percent reduction of AUDPC was 32.79, 32.58 and 21.19 for consortium P. fluorescens and P. asplenii, respectively. Strain mixture also significantly reduced disease progress rate (0.011unit/day). In addition to disease suppression, strain mixture enhanced plant height, percent effective tiller and filled grain per plant. Effects of all the treatments on flag leaf area, total number of tiller and number of effective tillers were insignificant. However, the weight of 100-grain was significantly (1.65g) highest in strain mixture applied plants. This study suggested that indigenous Pseudomonas bacteria isolated from rice plant can be preserved in peat at 4°C until 6 months of storage and used as efficient biocontrol agents for management of sheath blight disease and increase grain yield as well.

P1.1-108

SCREENING OF VARIOUS MICROORGANISMS THAT INHIBIT THE GROWTH OF ERWINIA AMYLOVORA, THE CAUSATIVE AGENT OF FIRE BLIGHT OF FRUIT CROPS

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Text

The search for new approaches and solutions in the field of bacterial burn control is highly relevant. Microbial preparations based on microorganisms with antagonistic activity play an essential role. In this regard, the genera Bacillus, Pseudomonas, Lactobacillus and Saccharomyces, which are in the collection of the Scientific Production Center of Microbiology and Virology, have been screened and have inhibitory activity against Erwinia amylovora the causative agent of fire blight of fruit crops. It was established that in bacteria of the genus Bacillus only two strains suppressed the growth of the pathogen E. amylovora. B. amyloleguefaciens and Bacillus N2, the zone diameters of suppression were 30.0±0.6 mm and 14.0±1.0 mm, respectively. Among the bacteria of the genus Pseudomonas, no strains with inhibitory activity were found. Most strains of the genus Lactobacillus suppressed the growth of the pathogen. The maximum inhibition zone of E. amylovora was in strains L. paracasei 33-4 (39.6 ± 6.65 mm), L. plantarum M17 (35.6 ± 0.57 mm).
Among strains of the genus Saccharomyces, the largest growth inhibition zone (25.6 ± 2.08 mm) was found in S. cerevisiae (vini). It was found that the component composition of the culture fluid of B. amyloliquefaciens mainly contains acetoin and 2,3-butanedione. Strains of the genus Lactobacillus produce acetic acid and lactic acid to a greater extent. Strain S. cerevisiae forms phenylethyl alcohol, 1-butanol, 3-methyl.

P1.1-109

MYCOVIROIDS HAVE POTENTIAL TO CONTROL CROP FUNGAL DISEASES

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Text
Mycoviruses are the only acellular agents that have been extensively investigated and utilized to combat crop fungal diseases. Recently, a novel class of viroid-like RNAs naturally infecting a filamentous fungus Botryosphaeria dothidea were isolated from apple, and tentatively named Botryosphaeria dothidea circular RNAs (BdcRNAs) 1 to 3, which have been characterized and termed as mycoviroids referring to viroid-like RNAs naturally infecting fungi besides its original definition. BdcRNAs 1 to 3 in size of 450 to 221 nt display no detectable nucleotide identity to known RNA sequences, but share different identity levels with each other, replicate autonomously in the nucleus via a rolling-circle mechanism following a symmetric pathway. BdcRNAs significantly affect the biological traits of B. dothidea by regulating the gene expression and metabolic pathways related to important cellular processes of the fungal host. More importantly, BdcRNAs 1 and 2 can significantly attenuate or even erase the fungal virulence, while enhance the growth (for BdcRNA1) and increase the tolerance to some osmotic stress of the host fungus. These features provide an important alternative candidate to serve as a biocontrol tool for attenuation of fungal diseases similar to some mycoviruses that cause hypovirulence.

P1.1-110

WHEN COMPETITORS JOIN FORCES: USING CONSORTIA OF ENTOMOPATHOGENIC PSEUDOMONAS BACTERIA, NEMATODES AND FUNGI FOR PEST CONTROL

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Text
Below-ground pests are difficult to control because either no effective control methods exist or suitable insecticides are or will soon be banned due to their negative effects on the environment. We evaluated the potential of disease-suppressing *Pseudomonas chlororaphis* bacteria with entomopathogenic activity for insect control. Moreover, we combined the pseudomonads with entomopathogenic nematodes (*Steinernema feltiae*) and fungi (*Metarhizium brunneum*) with the aim to increase reliability and efficacy of biocontrol measures.
In a series of experiments ranging from the greenhouse to the field, *P. chlororaphis* emerged to be highly efficient in controlling the cabbage maggot *Delia radicum*, an important pest of Brassicacean crops. Furthermore, the triple consortium of *P. chlororaphis* with *S. feltiae* and *M. brunneum* increased the number of marketable radishes by 50% in a field trial. In several experiments, we observed increased pest control when combining the pseudomonads with the nematodes or the fungi. These synergistic effects were verified when applying the combinations against two further pests. The triple consortium was the most lethal and fastest killing treatment against *Pieris brassicae* and *Diabrotica balteata* larvae. In the early stages

of the infection, all three agents established inside the larvae.
Our results show that entomopathogenic pseudomonads, nematodes and fungi are compatible and could potentially be used to control a variety of below-ground insect pests.

P1.1-111

INHIBITION OF ACRAB-TOLC ENHANCES ANTIMICROBIAL ACTIVITY OF PHYTOCHEMICALS IN PECTOBACTERIUM BRASILIENSE

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Text
In nature, bacterial pathogenicity is counteracted mainly by plant-derived antimicrobial defense molecules. In return, efflux pumps (EP) are part of the resistance mechanism employed by bacterial pathogens to promote their survival in a chemical hostile environment. We studied the effect of combinations of efflux pump inhibitors (EPIs) and plant-derived antimicrobial phenolic compounds on bacterial activity, using Pectobacteriun brasiliense 1692 (Pb 1692) as a model system. Specifically, we measured the minimal inhibitory concentration (MIC) of two phytochemicals, phloretin (Pht) and naringenin (Nar), and of one common antibiotic ciprofloxacin (Cip), either alone or in combinations with two known inhibitors of the AcrB EP of Escherichia coli, a close homolog of the AcrAB-TolC EP of Pb 1692. In addition, we also measured the expression of genes encoding for the EP, under similar conditions. Using the FICI equation, we observed synergism between the EPIs and the phytochemicals, but not between the EPIs and the antibiotic, suggesting that EP inhibition potentiated the antimicrobial activity of the plant-derived compounds, but not of Cip. Docking simulations were successfully used to rationalize these experimental results. Our findings suggest that AcrAB-TolC plays an important role in the survival and fitness of Pb1692 in the plant environment and that its inhibition is a viable strategy for controlling bacterial pathogenicity.

P1.1-113

EXPLORING THE EFFICACY OF PLANT EXTRACTS IN VITRO AGAINST MANGO ANTHRACNOSE PATHOGEN: COLLETOTRICHUM GLOEOSPORIOIDES

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Text
Mango (Mangifera indica L.) is a delectable fruit grown in less than 90 tropical and sub-tropical countries in the world. Mango anthracnose, caused by Colletotrichum gloeosporioides, is a highly destructive disease. Chemical control is most frequently

practiced by the mango growers, which poses bad impacts on the environment and human health. Hence, alternate control strategies should be used for the management of disease and for increasing the potential yield of mango. The in vitro potential of neem, mint, and garlic for control of Colletotrichum gloeosporioides was investigated in the current study. The pathogen was isolated from diseased mango plant portions. Aqueous and methanolic extracts of neem, mint, and garlic were prepared and tested at different concentrations for their efficacy against fungal growth. All plant extracts significantly reduced the fungal growth in the poison food technique as compared to the control. The methanolic extract of neem was most effective in fungal growth suppression. Results of this study indicate that plant extracts can be used for the control of anthracnose disease in mango. It will be less expensive and safer, and it may be a viable alternative to synthetic fungicides.

P1.1-114

TOWARDS A MONITORING OF BIOCONTROL AGENTS : NEW TOOLS FOR A BETTER UNDERSTANDING OF THEIR ESTABLISHMENT IN THE ENVIRONMENT

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Text

Biological control agents (BCA) play an important role in crop protection and can be used to reduce the usage of chemical plant protection products as desired by public policy. Differents *Bacillus* and *Trichoderma* are used in commercial BCA products. However, the BCA efficacy depends on a lot of environmental parameters such as pH, temperature, hygrometry or cultural practices. It is important to quantify the BCA dynamics in the environment to have a better understanding of their efficacy and the differences observed between controlled and fields conditions. This study aims to (i) develop specific qPCR and dPCR methods for *Trichoderma atroviride*, *Bacillus velezensis* species and *Bacillus amyloliquefaciens* operationnal group and (ii) monitor BCA populations dynamics in plants and soils. The different tools were validated for each organism under laboratory conditions (specificity, sensitivity, …) and have been tested on samples from field trials. We were able to monitor the dynamics of the BCA in the rhizosphere and in wheat according to the time after application. In our field conditions, the implantation of the BCA either in soils or in plants was variable, often weak and not durable over time which could explain the weak efficacy of the BCA observed against the targeted diseases. Thus, the generic tools developed will be useful to monitor different biocontrol agents that use *B. amyloliquefaciens* or *T. atroviride* whatever the crop and the strains.

P1.1-115

ANTAGONISTIC EFFECT OF TRICHODERMA SPECIES AGAINST PATHOGENIC FUNGI ASSOCIATED WITH QUERCUS SUBER DECLINE IN TUNISIA

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Text

Cork oak decline is becoming a serious problem in Tunisian forests. This phenomenon is caused by a combination of biotic and abiotic factors mainly drought and several pathogenic fungi. Following a survey on the oak decline in Northwestern Tunisia, Biscogniauxia mediterranea, Diplodia corticola and Diplodia gallae were identified as the main causal agents of oak decline. Faced with the extent of this decline and the aggressiveness of these pathogens, control methods should be applied. For this purpose, the biocontrol potential of Trichoderma isolates towards these pathogens was investigated. Trichoderma strains, isolated from cork oak trees, were identified as T. harzianum, T. citrinoviride and T. saturnisporum through the sequencing of four DNA regions. Dual culture inhibition experiments were used to evaluate the antagonistic effect of Trichoderma isolates against B. mediterranea, D. corticola and D. gallae. The results showed the efficacy of T. harzianum in comparison with T. citrinoviride and T. saturnisporum by inhibiting totally the mycelial growth of pathogenic fungi. Microscopic observations showed a mycoparasitic relationship between T. harzianum and pathogens. These findings might prove the possibility of applying Trichoderma isolates in an eco- friendly way as biological agents against cork oak pathogens.

P1.1-116

INVESTIGATING CHANGES IN THE ROOT MICROBIOTA IN RESPONSE TO RICE'S CRY FOR HELP UNDER DIFFERENT FOLIAR PATHOGEN ATTACKS

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Text

In response to biotic and abiotic stresses, plants have evolved various defence strategies, including the recruitment of health-promoting root-associated microorganisms via a “cry for help” mechanism. This selected community of microorganisms is known to help minimize the damages caused by the stress, for example by modulating plant nutrition or immunity. However, the rules of microbiome assembly following foliar pathogen infection and the mechanisms that govern its assembly and function in the diseased host are still poorly understood. In particular, it is not known whether different pathogens can induce different root microbiome changes. Using *Oryza sativa* subsp. *japonica* cv. Nipponbare, we analysed the modifications of the root-associated microbiome after host exposure to five different foliar pathogens, including two bacteria (*Xanthomonas oryzae* and *Xanthomonas oryzicola*), two fungi (*Pyricularia oryzae* and *Bipolaris oryzae*) and one virus (Rice Yellow Mottle Virus). Rice was grown in a greenhouse on rice field-sampled soil, and inoculated with the respective pathogens. One week after inoculation, we collected the rhizosphere and analysed the diversity of the microbiome using 16S/18S/gyrB amplicon sequencing approaches. Whether the pathogen has an effect on the root microbiome and whether each pathogen affects it differently, with common signatures specific to each group of pathogens, will be presented in this poster.

P1.1-117

SECONDARY METABOLITES OF INSECT SYMBIONTS AND THEIR ANTIMICROBIAL ACTIVITY

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Text

The resistance of plant pathogenic fungi to fungicides is becoming more and more serious. It is of great significance to develop new agricultural fungicides. Insects are widely distributed in a variety of ecological niches, and their ability to live in unique habitats is often to promote symbiosis with their microbes, which were sources of new antibiotic metabolites. For example, the new metabolite isochromophilone XV from the symbiont of *Ectropis oblique* significantly inhibited *Colletotrichum graminicola* with IC_{50} value of 29.9 μg/mL. Insects are a group of organisms with the largest number of known species in the earth biosphere, and the special microorganisms symbiotic with insects are rich in diversity. However, compared with insect species, there is less research on insect symbionts and less research on their metabolites, so it is urgent to strengthen research.

P1.1-118

ASSESSING THE ANTAGONISTIC POTENTIAL AND BIOCONTROL EFFICACY OF RICE-ASSOCIATED BACTERIA AGAINST MAGNAPORTHE ORYZAE

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Text

The fungal pathogen Magnaporthe oryzae causes one of the most important rice diseases: blast. It is found in rice-growing regions around the world and causes significant yield losses. Resistant cultivars and fungicides are the most widely used methods of control. However, the recent concept of the holobiont opens the way to promising sustainable alternatives for plant protection, based on plant-microbe interactions. In this context, we initiated a study of beneficial bacteria using two approaches: i) in vitro, to search for bacteria antagonistic to M. oryzae, through a bacterial/fungal confrontation test on two culture media, and ii) in planta, to search for bacteria with biocontrol activity, through the observation of blast symptoms on rice plants primed with the beneficial bacteria. The results allowed the selection of seven bacterial strains from the genera Azorhizobium, Bacillus, Burkholderia and Cupriavidus with the ability to reduce mycelial development by up to 57%, as well as four bacterial strains from the genera Azorhizobium, Bacillus and Burkholderia with the ability to reduce blast symptoms by up to 45%. Phylogenetic analysis based on 16S rDNA showed that the bacterial strains probably correspond to new species distinct from those already described. Two strains from

the genera Bacillus and Burkholderia had both abilities. The bacterial genomes were sequenced, which should help us to further investigate the mechanisms involved.

P1.1-119

WHAT MAKES A COMPOST SUPPRESSIVE TO SOILBORNE PATHOGENS?

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Text

Composts have been shown to suppress soilborne pathogens in numerous greenhouse and field experiments. However, the effectiveness of disease suppression is highly variable between composts, and we currently lack reliable indicators to select composts for plant protection. We hypothesize that disease suppression is a complex interplay between abiotic and biotic compost properties. Investigating the microbial communities may help to develop tools for predicting suppressive properties and producing composts with strong biocontrol activity.

In the first part of the project, 17 composts were assessed for disease suppression in a cress–*Globisporangium ultimum* (syn. *Pythium ultimum*) system and assessed for their physico-chemical properties. Their microbial communities were analyzed using an Illumina metabarcoding approach, which identified bacterial taxa that are indicative for disease suppression. This data set has now been extended by 30 additional composts and a cucumber–*G. ultimum* and a cucumber–*Rhizoctonia solani* test system, which revealed differences in disease suppression between pathogens and plant species. The microbial communities are currently assessed by SMRT cell long-read sequencing with the goal to get a high taxonomic resolution to accurately relate the sequencing data with isolates obtained from the composts. Our comprehensive data set provides new insights into the contribution of different abiotic and biotic factors to disease-suppressive activity of composts.

P1.1-120

ENVIRONMENTAL CONDITIONS AFFECT PUCCINIA PUNCTIFORMIS TELIOSPORE LONGEVITY

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Text

The autoaecious rust-fungus *Puccinia punctiformis* is an obligate biotroph pathogen of *Cirsium arvense*; a cosmopolitan weed and one of the most harmful noxious weeds in

agricultural and forestry landscapes in North America and Europe. The pathogen completes its whole life cycle on *C. arvense*, occasionally causing a systemic infection characterized by the production of spore-bearing shoots that die before flowering or producing seeds, and a large reduction in above- and below-ground biomass. This high level of specificity and the severe damage caused by the systemic form of the rust disease makes *P. punctiformis* a promising biocontrol agent against *C. arvense*.
Although the pathogen has been found wherever its host is distributed and can persist in infected batches for years, it rarely reaches epidemic population levels. Since the viability of overwintering teliospores is crucial to forming basidiospores to establish systemic infection every new season, teliospore viability could be affected by field conditions of temperature and humidity through time. To test this hypothesis, teliospores collected in July-August 2022 were stored at -19°C, 6°C, or 23°C; and 5%, 22%, 62%, or 90% relative humidities (RH), to finally analyze their viability by measuring teliospore germination rate once each month later over 250 µl/L dodecyl-NSC in 1% agar. Germination results declined at 23°C under 90% and 62% RH suggesting that teliospore longevity decreases at high humidities and temperatures.

P1.1-121

DISEASE-INDUCED CHANGES IN SOYBEAN MYCOBIOME DETERMINE PLANT HEALTH

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Text
The plant microbiome is an essential part of the host and is gradually recognized as playing a critical role in plant growth and health. But still, the formation and functions of plant microbiomes during pathogen invasion are not fully understood. We investigated how the soybean plant attracts helpful microbes to suppress soil-borne diseases. We found that the soil mycobiome determined whether the plants survived or succumbed to infection. Surviving plant microbiomes were linked to unique taxa, pathogen-suppressing fungi, and fungi that induce plant immunity. Our findings imply that soil mycobiome composition and function might be recruited under pathogen attack, influencing the consequences of plant-pathogen interactions.

P1.1-122

BIOCONTROL EFFECTS OF RHIZOBACTERIA PRODUCING VOLATILE-ORGANIC COMPOUNDS AND CYLCOLIPOPEPTIDES AGAINST THE MAJOR PATHOGEN FUNGI OF WHEAT

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Text
The use of pesticides for managing crop pests and plant diseases had shown several

bottlenecks, stimulating research for developing alternative plant protection solutions. One of them relies on the use of biocontrol products to reduce pathogen populations and limit disease incidence. Plant Growth-Promoting Rhizobacteria (PGPR) could be considered as very good candidates.
In this work, we explore biocontrol potentials of a panel of PGPRs isolated from the wheat rhizosphere and belonging to several bacterial genera, against 2 major fungal wheat pathogens Fusarium graminearum and Zymoseptoria tritici. Characterization of bacterial volatiles and secreted secondary metabolites for their inhibition properties against mycelium and spore growth was made using in vitro antagonist confrontation tests on the two pathogens. It was followed by plant protection assays on wheat crown rot fusariosis under greenhouse conditions. This 2-step screening allowed us to identify strains with direct antagonist effects and others with indirect mechanisms related to plant defense stimulation. Combining genome mining and metabolomics approaches, we were able to identify some key molecular determinants for the inhibition of fungal pathogens in our PGPR library. Metabolic profiling and consecutive bio-guided fractionation of secondary metabolites secreted by antagonist strains reveal that lipopeptides and dimethylpolysulfide volatile organic compounds are among the main antifungal active biomolecules.

P1.1-123

A SEED ENDOPHYTIC TRICHODERMA SP. PROTECTS THE WHEAT PLANT AGAINST INFECTION CAUSED BY THE FUNGAL WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Text
A diverse group of microorganisms inhabiting plant seeds can colonize the host during growth and development. Previous studies have demonstrated the capacity of fungal and bacterial species recovered from wheat seeds to manage plant
disease. Trichoderma species are promising biocontrol agents. They are opportunistic, avirulent plant symbionts or endophytic fungi, and the molecular mechanisms employed by them to control fungal phytopathogens such as mycoparasitism and antibiosis have been previously described. Endophytic Trichoderma spp. have a broad host range with a remarkable ability to promote the host plant's performance. In the current study,
a Trichoderma sp. was isolated from seeds of wheat cv. Tiregan through surface disinfection. Our infection assay under greenhouse conditions revealed that spray application of Trichoderma sp. could reduce Septoria tritici blotch (STB) symptoms caused by the Z. tritici IPO323 on inoculated plants. The percentage leaf area covered in lesions (PLACL) and percentage leaf area covered in pycnidia (PLACP) were reduced by 70% and 18%, respectively, in wheat plants treated with applied Trichoderma sp. compared with control plants. Therefore, this endophytic Trichoderma sp. isolated from wheat seed can reduce symptoms caused by Z. tritici IPO323 under glasshouse conditions.

P1.1-125

EXPLOITING BIODIVERSITY IN PERENNIAL CROPS: EFFECT OF MYCORRHIZAL BASED PRODUCTS ON THE VINEYARD RHIZOSPHERE

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Text

The massive use of chemical products not only affects the application effectiveness due to the risk of resistance, but also affects the environment. For this reason, the viticulture sector is facing a major challenge, which is an ecological transition. In recent years, the development of different vineyard management alternatives are trying to contribute to a sustainable viticulture model. Increasing biodiversity helps to reduce the use of pesticides and increase ecosystem services. The use of arbuscular mycorrhizal fungi improves the tolerance to abiotic stresses and protects the roots against pathogens. The main objective of this work was to analyze the effect on the biodiversity of vine rhizosphere under mycorrhizal products application. For a period of four years, the microbial rhizosphere biodiversity was manipulated in an experimental vineyard using seven different microbial products, with annual inoculations. Soil and root samples were taken annually for bacterial and fungal detection by the Illumina sequencing. For each treatment, the detected microorganisms and their respective number of reads was obtained. The microbial biodiversity was estimated by the indices: Shannon, Simpson, Pielou, species abundance and species richness. Preliminary results show that the products applied influence the rhizosphere biodiversity increasing the richness index and, in some cases, providing a promising induced resistance to downy mildew.

P1.1-126

OCCURRENCE OF NATURAL POPULATIONS OF ENTOMOPATHOGENIC NEMATODE STEINERNEMA FELTIAE IN CEREAL FIELDS OF SOUTHEAST IDAHO

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Text

Wireworms are very destructive in small grain production in the Pacific Northwest and difficult to control with insecticides. In a targeted survey to detect local populations of entomopathogenic nematodes (EPNs) in southern Idaho, USA, six populations of EPNs were isolated from the soils of cereal fields that were heavily infested with larval stage of click beetles (Coleoptera: Elateridae). Initial morphological characteristics placed the collected EPNs into the genus *Steinernema* (Travassos, 1927) which is a known biological control agent, with active nictation, cruiser locomotion, and infection dynamics that can be variable

at intraspecific level. Additional molecular analysis was required as morphological characteristics will not delineate the isolated samples to species level. The sequence analysis of partial ribosomal RNA gene complexes including internal transcribed spacers (ITS1 and ITS2) and D2D3 expansions of 28S large subunit confirmed the occurrence of *S. feltiae*. Phylogenetic relationships inferred from maximum likelihood (ML) analysis of ITS-rRNA sequences distinguished the isolated EPNs to the Feltiae clade. Cytochrome c oxidase I (*COI*) of mitochondrial DNA resolved the phylogenetic relationships to within *S. feltiae* subclade using ML analysis of *COI* mtDNA sequences. This study is the first report and characterization of *Steinernema feltiae* in southeast Idaho. Additional studies on the infection dynamics and bacterial symbiosis for these isolates is required.

P1.1-127

USE OF PSEUDOMONAS RHIZOBACTERIA AS BIOHEBICIDES FOR PROTECTING CROPS AGAINST BROOMRAPES

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Text

Broomrapes (Orobanche and Phelipanche) are root parasitic plants that affect a wide range of economically important crops worldwide (rapeseed, sunflower, etc), thus posing tremendous threats as they can cause heavy yield losses. When broomrapes infest a field, farmers have to reduce their producing area using only non-infested plots. So far, crop protection against parasitic weeds is mostly based on the use of systemic chemical herbicides in combination with the use of crop genotypes that show tolerant behavior against broomrapes.

In conventional agriculture, large scale use of chemical plant protection products has led to the degradation of soil quality and has had a dramatic impact on natural flora and fauna. Living organisms able to protect plants against broomrapes, or any weed management strategies that are more environmentally friendly needs to be found.

We have identified and characterized several Pseudomonas rhizobacteria able to produce compound(s) that stop, under in vitro conditions, the germination of broomrape seeds, thus further affecting the growth of the parasitic plant under greenhouse conditions. One of this compound is a polyketide. It is efficient at concentrations below 25µM against P. ramosa and 50µM against O. cumana. Other Pseudomonas strains that do not produce this polyketide also shared an herbicide activity against these plant parasites, showing that a very wide diversity of novel secondary metabolites from Pseudomonas could be used as bioherbicides.

P1.1-128

USE OF NATURAL COMPOUNDS WITH LOW ENVIRONMENTAL IMPACT FOR THE PROTECTION OF SEED-BEARING ONION AGAINST FUNGAL DISEASES

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Text

Seed-bearing onion is an important crop in several area of production, and it can be affected by several fungal pathogens. Most of these infect the seed, which then become an efficient vehicle to disperse seedborne pathogens over long distances, resulting in potential severe crop losses. The need for high-quality seed and the increasingly stringent restrictions on the use of synthetic plant protection products imposed by the European Union fostered the search for alternative solutions to protect seedbearing vegetable crops from seedborne pathogens. Within the project “CleanSeed” promoted by PSR Marche, Central-Estern Italy, this work aimed to evaluate, on a company scale, the effectiveness of several innovative protection strategies based on the use of basic substances, biocontrol agents, plant extracts and low-risk active substances. Four strategies including chitosan, chito-oligosaccharides and oligo-galacturonides (COS-OGA), a mixture of terpenes, and Bacillus spp. were tested, and an assessment was carried out on the infections of the stem and flowers by Botrytis spp. Chitosan strategy reduced Botrytis spp. McKinney Index compared to untreated control on onion plant by 60%. The results obtained from this study open the way for new protection strategies based on the use of natural substances in the management of fungal diseases of seed-bearing vegetables.

P1.1-129

EVALUATION OF ESSENTIAL OILS OF LOCAL AROMATIC PLANTS AGAINST CHICKPEA BLIGHT IN PAKISTAN.

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Text

The aim of this study was to evaluate the essential oils of Pakistan`s local aromatic plants as biological control of chickpea blight caused by *Ascochyta rabiei*. Another objective was to evaluate the impact of selected essential oils on plant health and growth. Out of 30 plants essential oils only 10 showed the antifungal activities against *A. rabiei*. Cumin (*Cuminum cyminum* L.) essential oil produced the highest fungal mycelial growth and spore germination inhibition and was selected for the further study on planta. The minimal inhibitory concentration was revealed as 0.5 ml/L and the minimal fungicidal concentration was found 1 ml/L. Microscopic examination of *C. cyminum* EO on hyphal morphology revealed that the essential oil caused degenerations, less branching, loss of septations, vesicles formation, shriveling and lysis of hyphae. Cumin essential oil improved the plant growth especially when the seeds were treated with essential oil (≥0.5ml/L) after inducing the priming with water. In greenhouse, cumin essential oil protective spray on chickpea reduced the disease severity was significantly reduced (upto 70%). The biochemical changes and expression of resitance genes in planta due to the treatment by cumin essential is in progress. It will help to understand the possible role of *C. cyminum* essential oil in resistance mechanism in chickpea plants. The results of field experiments and These studies will hopefully be completed and presented in the conference.

P1.1-130

DOES THE BACTERIAL SYMBIONT FRANKIA MODULATE PLANT DEFENCES OF ALNUS AGAINST THE PHYTOPATHOGEN PHYTOPHTHORA ?

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Text

Because they are sessile organisms, plants have developed different strategies to fight pathogen infections. Although the plant immune system has been mainly described for plant-pathogen interactions, commonalities between beneficial microorganism and pathogen infections have been established. Among beneficial interactions, the nitrogen fixing symbiosis of alders with the actinobacteria *Frankia* has been well studied. Establishment of this symbiosis leads to the formation of root nodules. In the other well-known nitrogen-fixing symbiosis between legumes-rhizobia, it is recognized that the plant immune system is modulated in early stages of symbiosis. Much less data is available on *Frankia-Alnus* model and it is still unclear how plant immune system reacts when root nodules are well established and how it impacts plant ability to face pathogen infections.
We hypothesised that the presence of *Frankia* in alder tissues activates the plant immune system and could play a role in the defence against *Phytophthora alni*.
Our objective is to understand whether the alder immune system is activated by the symbiosis with *Frankia* and whether this symbiosis can play a role in modulating the defences against the pathogen *Phytophthora alni*. For this purpose, we developed an experimental system of *Alnus*-*Frankia* interactions including the pathogen *Phytophthora alni*. We performed multi-omics analyses to compare plant defence reactions to face the pathogen when hosting or not its symbiont *Frankia*.

P1.1-131

HARNESSING POTENTIAL ENDOPHYTIC FUNGI TO DEVELOP BIOLOGICAL SOLUTIONS TO MANAGE BYDV & APHID VECTORS IN SPRING BARLEY

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Text

Entomopathogenic fungi (EPF) offer potential biological solutions to manage plant sap-

feeding insect pests, such as aphids. The plant-colonization capability of notable endophytic EPF strains ensures their survival and confers plant protection and growth functions. This work is aimed to discover novel EPF candidates with the potential for effective management of Barley Yellow Dwarf Virus (BYDV) and the aphids that vector the virus in barley crops.
For this study nineteen endophytic fungal candidates were utilized, which were isolated from different plant niches-seed, stem, leaf and root tissues of cereal plants. These endophytic fungi candidates were associated with ten different taxonomic genera. For the screening experiment, the adult aphids were exposed to endophytic fungal spores for three hours and then transferred to the plants. The results under controlled conditions have shown a promising reduction in the aphid population across different time points. There were up to two-fold reductions in the number of adult aphids and nymphs elicited by four different fungal candidates. Interestingly, >30% of the endophytic fungal candidates screened demonstrated the potential to suppress aphids and nymph numbers.
The key findings suggest that diverse representations of these endophytic taxa could potentially offer multi-choice effective biological control agents. Ongoing work is focused on establishing a mechanistic understanding of aphid inhibition by potential endophytes.

P1.1-132

EVALUATION OF THE PATHOGENICITY OF ENTOMOPATHOGENIC NEMATODES ISOLATED IN TAIWAN AGAINST FALL ARMYWORM

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Text

The insect pest Fall armyworm *Spodoptera frugiperda* (FAW) invaded Taiwan in 2019. It is capable of causing severe damage to food crops. Entomopathogenic nematodes (EPNs) and thier symbiotic bacteria have the capacity to parasitize and kill their host. However, no local EPN products are currently available on the market in Taiwan. Therefore, this study aimed to obtain local EPN populations, identify and characterize the EPNs and their symbiotic bacteria, and further evaluate their parasitism efficacy and pathogenicity against FAW. Between 2019 and 2020, four EPN strains were isolated from a survey of 45 soils in Taiwan. The nematodes were identified as *Pristionchus pacificus* (strain 6) and *Oscheius myriophilus* (strains 16, G1A1, and G1B1). The bacteria *Serratia marcescens* and *Achromobacter insuavis* were isolated from *P. pacificus; Cupriavidus spp, Pseudomonas spp. Variovorax spp.* and *Stenotrophomonas spp.* were obtained from *O. myriophilus*. Among them, *C. malaysiensis* and *V. paradoxus* resulted in 41% and 30% mortality rates on FAW 3rd instar larvae, respectively. Moreover, *P. putida*, *C. alkaliphilus* and *C. malaysiensis* reduced the FAW pupae eclosion rate significantly. Further, three concentrations of EPN suspension were examined. As a result, the mortality rate of FAW was between 21-27 %, 21-23%, 18-20%, and 22-26% for the application of strains 6, 16, G1A1, and G1B1, respectively. The damage on leaves was 53-60%, 56-59%, 53-56%, and 53-56% for each EPN strain, respectively.

P1.1-133

SYNERGISM OF TRICHODERMA GENOTYPES FOR MANAGEMENT OF FUSARIUM WILT IN TOMATO

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Text

Trichoderma genus has a good place in biological control market. Trichoderma spp are capable to recognize and attack on several soil borne plant pathogens but during current study, synergistic effects among four species were assessed against Fusarium wilt of tomato. Primarily, a comprehensive survey was conducted, and four dominating species of Trichoderma were identified and purified from different agro-ecological zones of Pakistan. These species were Trichoderma harzianum, T. viride, T. atroviride and T. virens. All these species were successfully cultivated in sets of two with different degree of success. In the present study an isolate of Fusarium oxysporum pv lycopersici was taken form First Fungal Culture Bank of Pakistan. After pathogenicity test, the pathogen was subjected to grow on PDA plates against all four Trichoderma species. In-vitro results showed that, T. harzianum and T. viride gave 92 and 84% reduction in colony growth over control whereas T. virens and atroviride gave 32 and 26% reduction respectively over control. Furthermore, sick plants were treated with all four strains of Trichoderma spp separately and in combinations of two with all possible blends. Statistical analyses revealed that, T. harzianum along with T. atroviride increased their antagonistic potential 3.6 fold where as T. atrovirude and T. viride gave 2.8 fold increase in protection value against Fusarium wilt of tomato with reference to single isolate application.

P1.1-134

MANAGEMENT OF ADULT AND IMMATURE LARGE PINE WEEVIL (HYLOBIUS ABIETIS L.) USING NOVEL LOCAL-PROVENANCE ENTOMOPATHOGENIC FUNGI AND COMMERCIAL ENTOMOPATHOGENIC NEMATODES.

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Text

The large pine weevil (LPW) is a widespread forest pest in conifer clearfell restocking areas. Biological control agents are the best candidates to replace widely used synthetic chemicals with potential side effects on the environment and humans. Entomopathogenic nematodes (EPN) and fungi (EPF) are successful at controlling its immature stages and also present additive effects when applied together to tree stumps. We propose a new approach that also targets the adults that survived the control of immatures. Surviving emerging adults will be lured with volatiles like alpha-pinene to traps containing an EPF. A large-scale soil sampling campaign within various habitats has provided new locally sourced EPF to be used together with commercial EPN strains. High diversity of EPFs has been obtained from soil using a. The local strains of EPF are tested for pathogenicity on LPW to select the best entomopathogenic agent for adult traps and to study their additive/synergistic effects with commercial EPN. To test the efficacy of the approach in the field, mark-recapture

experiments will be conducted, which will inform the development of an efficient chemical-free method to control H. abietis on forest clearfell areas.

P1.1-135

PRODUCTION AND ACTIVITY OF RHIZOBACTERIAL ANTIMICROBIAL-VOLATILES STRONGLY DEPEND ON CULTURE CONDITIONS

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Text

Antimicrobial volatile activity of rhizobacteria has been achieved by growing them on rich media, raising a question of whether the production and activity of antimicrobial volatiles are limited to a few reported media types. Effect of inoculum amount and inoculation method of antagonist on production and activity of antimicrobial volatiles has not been reported. Therefore, antimicrobial activity of Bacillus cabrialesii FA26 against soilborne fungi was investigated under different culture conditions. Results showed that FA26 when grown on NA showed higher suppression against Fusarium oxysporum, Rhizoctonia solani, and Phytophthora capsici by 39.83, 63.57, and 43%, respectively under double-dish chamber. However, FA26 exhibited 56.38% better suppression against Sclerotinia sclerotiorum when grown on LB. Effect of inoculum amount and inoculation method on the production and activity of antimicrobial volatiles of FA26 showed a clear dose-dependent potential and was highly correlated with the increase in the amount of inoculum using either of the inoculation methods; however, spreading mode of inoculation was better than that of the drops. Headspace SPME/GC-MS analysis revealed 26 volatiles' production by FA26. Of these, 8 volatiles completely inhibited one of the four phytopathogens. The study suggests production and activity of rhizobacterial volatiles strongly depend on culture conditions. Furthermore, the results revealed a direct long-distance biocontrol mechanism of FA26.

P1.1-136

EFFICACY OF BOTANICALS AGAINST BROWN LEAF SPOT OF RICE CAUSED BY BIPOLARIS ORYZAE

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Text

Rice (Oryzae sativa L) is suffering from several biotic and abiotic factors. Among biotic factors, brown leaf spot of rice (BLS) is potentially devastating disease of rice causing the severe yield losses up to 100%. The current study was designed to evaluate eco-friendly management strategy towards BLS to avoid environmental and human hazards. For this

purpose, thirty botanicals were screened out under in vitro conditions and five most effective extracts were further demonstrated against the targeted pathogen with three different concentrations (10, 20 and 30%) by using poisoned food technique. The results revealed that Ginger and Eucalyptus showed the strong inhibitory effect against B. oryzae at 30% concentration followed by Mint, Turmeric and Dhatura respectively. The promising extracts (Ginger and Eucalyptus) under lab conditions were further evaluated in vivo against BLS disease by using 3 types of applications i.e. Preventive, Curative and after symptoms appearance. Findings showed that, preventive application was found most effective as compared to other applications, furthermore combination of ginger and eucalyptus showed significant reduction in disease incidence percent as compared to solo applications. The reduction in disease incidence percent in all application methods suggested that these extracts could be used as alternative of synthetic fungicides against BLS of rice.

P1.1-137

TRICHODERMA SPECIES INTERACTION WITH FUSARIUM OXYSPORUM PV LYCOPERSICI AND INDUCED RESISTANCE IN TOMATO

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Text

Trichoderma are versatile beneficial fungi which can stimulate growth and plant resistance to biotic stress. Understanding the Trichoderma species for their diverse modes of action for management of Fusarium oxysporum, pv Lycopersici is a central goal of this research. In our ongoing studies most recently, we have tested the ability of T. harzianum to protect against salinity which adversely affects germination and growth of tomato seedlings. Trichoderma seed treatment improved plant tolerance. A model system for Trichoderma induced resistance to biotic stresses was provided by induction of a systemic response against fusarium wilt of tomato. Firstly 27 strains belonging to three species of Trichoderma were subjected to evaluate against F. oxysporum pv lycopersici through dual culture technique in-vitro. Statistical analysis revealed that eight species showed better antagonism against tested pathogen and were selected for field studies. These eight species were cultivated on sorghum based solid state fermentation on large scale and applied in field on artificially inoculated tomato seedlings. The results of current study showed that, the isolates of T. harzianum isolated from soil sample taken from tomato field expressed promising results and gave 94.6% reduction in disease over control whereas other isolates gave protection value under 62.0%. Therefore, the isolate which showed better performance under way to be characterized and its molecular identification.

P1.1-138

BIOLOGICAL CONTROL OF AFLATOXINS USING NON-TOXIGENIC STRAINS OF ASPERGILLUS FLAVUS

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Text

Mycotoxins are one of the major threats to food and feed safety and quality worldwide. More specifically, aflatoxin AFB1 and its metabolite AFM1, have been classified by the International Agency for Research on Cancer (IARC) among the most carcinogenic compounds for humans. Due to the inability of chemical methods to control aflatoxin levels on maize and pistachios, the use of non-toxigenic strains of *Aspergillus flavus* has been characterized by numerous studies as the most effective control strategy. The purpose of the present study was to evaluate several non-toxigenic strains in terms of their ability to reduce aflatoxin production in situ, on artificially infected corn seeds with a highly toxigenic *A. flavus* strain from the collection of the Laboratory of Phytopathology, Agricultural University of Athens.Our experiments indicated the high effectiveness of these specific non-toxigenic isolates in inhibiting the biosynthesis of aflatoxins on pistachios, reaching reduction rates of aflatoxin levels between 80-90% both in laboratory experiments and in field experiments. In the context of biological management, the most effective of the non-toxigenic isolates in corn kernels were further applied to maize cultivation, under field conditions and their effectiveness was further confirmed at high percentages. Novel formulations of the application of the non-toxigenic strains in the field are also examined.

P1.1-139

METABOLOMIC AND GENOMIC CHARACTERIZATION OF A NEW BIOCONTROL STREPTOMYCES STRAIN

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Text

In the context of reducing the use of synthetic pesticides, one of the solutions consists in using microorganisms. The strains belonging to the *Streptomyces* genus, known to produce a wide variety of antifungal metabolites, constitute a promising alternative to conventional products. To isolate new candidates a collection of 35 strains of *Streptomyces* was screened to detect antifungal and anti-oomycete activities using *Fusarium graminearum* and *Phytophthora capsici*. Five strains inhibited both fungal and oomycete growth among which a top candidate species was selected. This strain was further characterized for its ability to inhibit diverse plant pathogenic oomycete species belonging to *Phytophthora*, *Pythium* and *Aphanomyces* genus. To study protection of the strain against root disease, we performed soil inoculation or seed treatments on the legume model *Medicago truncatula* and on one of the major legume crops, pea, and evaluated the protection against *Aphanomyces euteiches*. To identify the mode of action of the strain, sequencing with PacBio CLR and Nanopore technologies of the genome was performed enabling us to annotate 34 specialized metabolite biosynthetic gene clusters. Untargeted metabolomics performed on culture medium confirmed the production of some candidate metabolites related to these gene clusters known to display as antifungal and anti-

oomycete. Our results suggest that this strain could be promising for the biocontrol of plant disease notably caused by oomycetes.

P1.1-140

EFFICACY OF TRICHODERMA SPP. AGAINST PHYTOPHTHORA CAPSICI, THE CAUSE OF ROOT ROT OF CHILLI

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Text

Root rot caused by *Phytophthora capsici* is a devastating disease of chilli pepper in tropical and subtropical areas of the world causing huge economic losses. Now a days, management of *P. capsici* considered to be a great challenge due to its long-term survival in soil, resistance to fungicides and commercially available chilli varieties. New possibilities to manage phytophthora root rot in chili production are under demand due to emerging fungicide and plant resistance. Therefore, this study aimed to investigate the anti-oomycete activity of three different *Trichoderma* species (*T. viride, T. virens,* and *T. harzianum*) against *P. capsici* both *in-vitro* and *in-planta*. The lab trial, through dual culture plate method indicates that, each tested *Trichoderma* species were significantly inhibiting the mycelial growth of *P. capsici* as compared to control treatment. The mycoparsitic interaction was also observed between *P. capsici* and *Trichoderma* spp. during slide culture assay. On further, *in-planta* evaluation the combined application of *T. virens* and *T. harzianum* expressed the lowest disease incidence (22.56%) with highest control efficacy (72.82%) as compared to individual application of each tested *Trichoderma* spp. The observed disease reduction indicates that these *Trichoderma* spp. could have a significant role in biologically based plant disease management strategies for management of Phytophthora root rot of chili pepper.

P1.1-141

EVALUATING THE ANTAGONISTIC EFFICACY OF PLANT GROWTH-PROMOTING RHIZOBACTERIA AGAINST ALTERNARIA SOLANI-INDUCED EARLY BLIGHT DISEASE IN TOMATO PLANTS

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Text

Present Study investigated the efficacy of PGPR as an alternative to chemical fungicides in controlling Alternaria blight, a severe fungal disease of tomato caused by Alternaria solani. At least 11 fungal isolates were recovered from symptomatic tomato plants and identified the most virulent one through virulence assay. A total of 17 bacterial strains belonging to two potential PGPR species; Bacillus and Pseudomonas were recovered from the rhizospheric soil of healthy tomato plants. Four rhizobacterial strains were selected based on their effectiveness in inhibiting the most virulent strain of A. solani in vitro. Two isolates belonging

to Azotobacter and Rhizobium spp. with proven plant growth promoting traits provided by AARI Faisalabad were tested in vitro for their efficacy against the same virulent strain. Among all the bacterial isolates, Pseudomonas isolates showed the highest mycelial growth inhibition of A. solani. In repeated pot trials, all tested bacterial strains alone and in combination significantly improved seed germination and plant growth and provided substantial protection against early blight disease. The PGPR-pretreated tomato plants also exhibited increased chlorophyll content, total phenol, free proline, total protein, and the activities of peroxidase and polyphenol oxidase. All treatments showed increased levels of indole acetic acid, abscisic acid, salicylic acid, and jasmonic acid compared to the levels in infected plants used as control.

P1.1-142

A DECISION SUPPORT SYSTEM BASED ON LITERATURE REVIEW AND FARMERS' EXPERIENCE TO PROMOTE AN EFFICIENT USE OF MICROBIAL BIOCONTROL AGENTS AGAINST DISEASES

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Text

Microbial biocontrol agents are promising tools to reduce the use of chemical pesticides in agriculture. Due to their characteristics of living organisms, their deployment is more complex than applying chemicals, and result in the variability of their efficacy, which can hinder their adoption. Taking this complexity into account would make their use more reliable.
Thus, it is necessary to develop decision support systems (DSS) based on biological properties of biocontrol agents, those of plant pathogens, and the characteristics of cropping systems. To develop such a DSS, a database has been set up to integrate information collected from scientific and technical literature and synthesize them into easily accessible datasheet.
However, an analysis of the database reveals that the available data are not sufficient, and many information is lacking in particular on the real conditions of use of biocontrol agents and few factors can be apply in commercial situation.
To solve this issue, it seems both necessary and promising to supplement available data with feedbacks from users of biocontrol agents directly from the field. Therefore, an application is currently under development to collect these feedbacks on the use of biocontrol to enrich the database to allow better guideline when using biocontrol product.

P1.1-143

EFFECT OF BIOCONTROL AGENTS AS PRUNING WOUND PROTECTANTS AGAINST GRAPEVINE TRUNK DISEASES

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Text

The world grape industry is seriously affected by grapevine trunk diseases (GTDs) and effective and sustainable management strategies are required. From 2019 to 2022, our laboratory evaluated biological and chemical fungicides as pruning wound protectants against GTD pathogens. In these trials, bacterial and fungal biocontrol agents (BCAs) obtained from healthy grapevine tissues were evaluated and compared to commercial chemical and biological fungicides. BCAs were prepared in liquid culture in the laboratory and applied on fresh pruning wounds of 10-years-old 'Cabernet Franc' vines. Commercial pruning wound protectants were applied at their label rate. After five days, treated pruning wounds were inoculated with spore suspensions (10,000 conidia) of *Neofusicoccum parvum*. After six months, pruning wounds were evaluated by performing isolations on potato dextrose agar. Results showed that fungal BCAs (*Aureobasidium pullulans* and *Trichoderma* spp.) exerted significant pruning wound protection when compared to synthetic chemicals and other biofungicides. In conclusion, fungal BCAs provided better pruning wound protection than bacterial BCAs in field conditions and constitute a suitable and sustainable management alternative for GTD management.

P1.1-144

ITALIAN TRADITIONAL MAIZE LANDRACES AND THEIR MICROBIOME: NEW PERSPECTIVES FOR BIOLOGICAL CONTROL OF FUSARIUM VERTICILLIOIDES

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Text

Maize-associated microorganisms, established through the evolutionary mechanism of selection, change according to certain factors such as genotype, geographical location, and environmental variables. GEMMA project focuses on beneficial endophytic bacteria inhabiting the embryo of four traditional maize landraces (Nero Spinoso Valcamonica, Spinato Gandino, Rostrato Rosso Rovetta, Fiorine Clusone, preserved at CREA Bergamo Genebank) and an inbred line (B73). To highlight the effect of environmental selection and the influence of vertical microbiota inheritance, plants were grown for three years in four different locations (Landriano, Bergamo, Verderio, Carvico) under low-input farming systems. Since recent studies show how certain endophytes can fight pathogens, this study aims also to study the relationship between the isolated endophytes and the most common toxigenic maize fungal pathogen in Lombardy region: Fusarium verticillioides. In vitro and in vivo test antifungal assays show that, out of over 100 isolates, only 2 from Spinato Gandino significantly reduce fungal infection. Instead, field trial results from 2021 experimental fungal inoculations show that Spinato di Gandino is the most susceptible variety out of those

analysed.
Pathogen resistance traits from landraces and their associated microbiome can be of interest for future organic maize production and contribute to more sustainable biotic stress management and higher yields, even in the scenario of climate change.

P1.1-145

CAN PREDATORS MITIGATE SOILBORNE DISEASES?

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Text
Soil predators are of central importance in regulating the interaction between plants, soil, and microbiota via a top-down control of microbes, including plant pathogens, and increased nutrient cycling. Infections by plant pathogens trigger defence, can alter the host metabolism and nutrient flow into the soil, leading to changes which feed-back to the soil microbiome. However, the links between soilborne pathogens, soil predators and the soil microbiome are only starting to be explored. We used the clubroot pathogen *Plasmodiophora brassicae* - a major obstacle for the cultivation of Brassica worldwide with no effective control options - to investigate disease induced changes of the soil microbiome and the role of soil predators in clubroot disease development. We aim to identify potentially disease suppressive and disease conducive predators and microbiome members, including bacteria, fungi and protist and other top-down controller. We combined soil physicochemical analyses with long-amplicon sequencing to decipher underlying drivers of taxonomic and functional changes in the microbiome to clubroot infections in field and greenhouse experiments. Additionally, feeding behaviour of soil predators on clubroot spores was investgated. We present insights of the potential of soil predators to control soilborne diseases such as clubroot that might lead to new biocontrol applications for soilborne pathogens in the future.

P1.1-146

HARNESSING THE SOIL MICROBIOME TO CONTROL ARMILLARIA ROOT ROT ON OLIVE.

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Text

Armillaria root rot caused by *Armillaria mellea* represents a serious threat to several plant species, including olive trees. This fungus is highly pathogenic, and current control strategies are mainly based on the prevention of the disease since curative methods are generally ineffective and may have a significant impact on the environment. In this study we focused on

the use of the soil microbiome as a possible tool to control rots caused by *A. mellea*. Specifically, we mass-selected bacterial isolates with antagonistic activity against several soil-borne pathogens (*Rosellinia necatrix*, *Phytophtora sp*., and *Phytopythium sp*.) from soils with high microbial diversity, and we assessed their *in vitro* antagonism against *A. mellea* in dual-culture assays. The most effective isolates were then evaluated *in-vivo* using potted olive plantlets, testing the efficacy of both single microbial strains or their combinations to contrast A. mellea. Finally, we investigated the impact of selected combinations of strains on the plant and soil microbiomes, in presence or absence of *A. mellea*. Our results contribute to show that the fundamental understanding and the correct management of the soil microbiome can be one of the major tools shaping the future generation of plant protection strategies.

P1.1-147

TAR SPOT DISEASE SEVERITY INFLUENCES PHYLLOSPHERE-ASSOCIATED BACTERIAL AND FUNGAL MICROBIOMES

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Text

Tar spot, caused by the obligate fungal pathogen *Phyllachora maydis*, is a foliar disease of corn that since 2015 has become a major concern in the USA. To test for interactions between other microbes and the tar spot pathogen, phyllosphere microbiomes were compared among corn inbreds with differential tar spot symptoms under natural infestation in the field. Leaf samples from sixteen inbred lines were assessed for tar spot symptoms, and bacterial and fungal microbiomes were characterized. Comparison of the phyllosphere microbiomes revealed distinct bacterial and fungal communities between resistant and susceptible lines. Bacterial and fungal species richness was significantly higher in resistant compared to susceptible inbred lines. In contrast, there were no clear differences in diversity when including evenness of bacterial communities between the resistant and susceptible lines. Diversity of fungal communities differed significantly, particularly between twelve of the fourteen susceptible versus resistant lines. Many of the bacterial and fungal species showed statistically significant correlations with *P. maydis* reads. Those that are positively associated could be mycoparasites that are more common with a more abundant food source. Species with significant negative correlations could be antagonistic with a potential for biocontrol. Further analyses of these distinct microbiota could lead to a better understanding of the potential role of foliar microbiomes on tar spot.

P1.1-148

UNRAVELING THE MODE OF ACTION OF A FUNGICIDAL AND NEMATICIDAL DIPEPTIDE PRODUCED BY BACILLUS VELEZENSIS UMAF6639

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Text

In the last decades, the use of beneficial bacteria has become a promising strategy to combat plant diseases. Previous studies have shown that the Bacillus velezensis UMAF6639 strain had an excellent biocontrol capacity against fungal and bacterial diseases of cucurbits. In addition, it had been observed that it could also be effective against plant parasitic nematodes.
Currently, the application of chemical agents remains the most common method for managing and controlling these pathogens. However, due to the increasing concern about environmental and public health safety issues, many highly toxic chemical compounds have been restricted in their use. Therefore, there is an urgent need to develop more environmentally friendly ecological alternatives for controlling these pathogens.
Therefore, in this study, the identification, characterization, and mode of action description of a molecule produced by Bacillus velezensis UMAF6639, a cyclic dipeptide, which was demonstrated to have nematicidal and fungicidal activity, was carried out. The results indicated that the activity of this molecule was based on a common mechanism capable of altering the physical characteristics of the pathogens plasma membrane, which is key to the physiology and homeostasis of these organisms. This discovery is important because it provides a basis for the development of new biological control agents that are effective against plant diseases caused by pathogens other than fungi and bacteria.

P1.1-149

UNLEASHING CRYPTIC CHEMISTRIES FROM THE BENEFICIAL MICROBE TRICHODERMA HAMATUM HEPA

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Text

Crop inoculation with the eco-friendly strain of *T. hamatum* or their secondary metabolites could induce active biologicals against soilborne pathogens and reduce the use of pesiticides and fertilizers in the agricultural and environmental systems. *Trichoderma* species have garnered interest for decades due to their plant growth promoting and antimicrobial properties. Mycoparasitism by *Trichoderma* was first observed in the 1930s, followed more recently by the identification of small molecules such as peptiabols, gliotoxin and 6-pentyl pyrone. Sequencing of *T. hamatum GD12* revealed that up to 38% of its genome is unique by comparison to other sequenced *Trichoderma* strains, and only 50% of the gene clusters identified by fungiSMASH have homology to clusters from other *Trichoderma* strains. These data combined imply that the genetic potential of *T. hamatum hepA* to produce novel specialised metabolites may be unprecedented within the *Trichoderma* genus. To access the biosynthetic potential of *T. hamatum hepA*, a mutant strain was constructed in which a gene encoding a heterochromatin protein – a global regulator of metabolism – was knocked out. This *hepA* mutant exhibits enhanced growth promotion in assays with lettuce, in addition to antifungal activity against *Sclerotinia sclerotiorum*. By a combination of comparative chromatography-mass spectrometry analyses and bioassays, we have begun to identify the cryptic metabolites responsible for the enhanced antimicrobial phenotype observed.

P1.1-150

MICROBIAL COMMUNITY STRUCTURE ASSOCIATED WITH RICE ROOTS IN CONTRASTING RICE AGROSYSTEMS IN WESTERN BURKINA FASO

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Text

Plants recruit soil microorganisms to provide various functions or protection against pathogens. Crop plants and their associated microbial communities are therefore increasingly studied together. However, the mechanisms that control the assembly of the root-associated microbiome remain difficult to disentangle, especially in rice ecosystems, which are poorly studied. Here, we compare the assembly of rice rootassociated microbiota sampled from 19 smallholder fields in the irrigated and rainfed lowlands of Burkina Faso. Using a 16S rRNA gene amplicon and ITS metabarcoding approach, we show that the rice production system is a major factor in the structure of the microbiome in addition to the expected structure by root compartments (root vs. rhizosphere) and geographic areas. In irrigated systems, we found greater diversity of rhizosphere prokaryotic communities and more complex co-occurrence networks, compared to rainfed lowlands, while fungal communities showed an opposite pattern. The main taxa were different between the two systems, and indicator species were identified: mostly within Bacillaceae in the rainfed lowlands, and within Burkholderiaceae and Moraxellaceae in the irrigated areas. Finally, a higher abundance in rainfed lowlands was found for mycorrhizal fungi. Our results highlight profound differences in the microbiome induced by contrasting rice production systems that should therefore be considered for microbial engineering applications.

P1.1-151

TOWARDS A MORE SUSTAINABLE CONTROL OF ALMOND WOOD DISEASES

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Text

Almond is one the most important nut crops worldwide. The implementation of new management techniques such as high-density cultivation, prune intensification, drip irrigation and fertilization, mechanical harvest, use of more productive varieties, and the cultivation in agronomically and environmentally more favorable cropping areas, has increased the

productivity in last decades. However, this new scenario, together with the current climate change situation, have increased almond diseases such as those caused by fungi of the Botryosphaeriaceae family. Symptoms include cankers on the trunk, extensive gummosis, internal tissue necrosis, and occasional death of the plant. Control methods are based on cultural practices, fungicide application and the search for resistant varieties. In this work, two collections of bacteria, rhizospheric and endophytic, have been obtained and characterized as potential biological control agents (BCA) of almond wood diseases. The in vitro and in planta antagonistic effect of 22 bacterial strains against Botryosphaeria dothidea, Neofusicoccum parvum, Diplodia seriata and Macrophomina phaseolina has been evaluated. Strains of Bacillus velezensis, Pseudomonas aeruginosa, B. mobilis and B. safensis could inhibit the in vitro growth and reduce the length of the lesions caused by these fungi in almond trees. The production of hydrolytic enzymes could be related to the mechanism of action of these potential BCAs

P1.1-152

EVALUATION OF VARIOUS FUNGICIDES AND BOTANICALS AGAINST POSTHARVEST BLUE MOLD OF ONION

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Text

Onion is important vegetable crops in Pakistan. It has special quality, adds taste to the flavor and is being consumed throughout the world. It has been affected by various post-harvest diseases on large scale that results in the reduction of shelf life of onion. Blue mold of onion is one of the detrimental disease in storage. The Experiment was conducted in Plant Pathology laboratory at Balochistan Agriculture College, Quetta to isolate and identify the pathogen and to check the efficiency of various fungicides viz., Acetic acid, Aerosol, Mancozeb and Puslan and three botanical extracts such as Coriander, Mint and Turmeric. These Fungicides were applied in vivo and in vitro conditions to find the most effective chemicals/ fungicide. Disease severity was checked from infected bulbs and data was statistically analyzed. On the basis of morphological characteristics Penicillium expansum was determined to cause blue mold of onion. It was revealed from research that Fungicides/Botanicals brought significant reduction in mycelial growth and spore germination of Penicillium expansum. Within tested fungicide aerosol gave effective result in controlling mycelial growth and spore germination followed by Puslan, Acetic acid and Mancozeb. Amongst Plant extract coriander gave significant result followed by mint and Turmeric.

P1.1-153

BIOLOGICAL CONTROL OF PADDY BUG (OEBALUS POECILUS) IN IN VITRO AND POT CULTURE CONDITION

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Text

The agriculture sector in Guyana as well as in the Caribbean region continues to face new challenges.

Several advanced lines were evaluated and superior strains are under cultivation in Guyana. Among the various factors influencing the quality and quantity of rice produced, paddy bug Oebalus poecilus is known to be the principal insect pest of rice in Guyana
To evaluate potential microbial pesticides as biological control for paddy bug Oebalus poecilus in order to avoid ecological and toxicological hazards for chemical pesticides in Guyana.
The methods are used Screening, isolation and characterization of micro-organisms from soil, Culture medium for screening micro – organisms, Selection of bio pesticide (Microbial pesticide), Preparation of squash mounts from fungal cultures, In vitro- contact kill bioassay to control paddy bug using microbial insecticide, Mortality assessment of paddy bug under pot culture condition against microbial insecticide.

Bio pesticide shown in control of paddy bug both in in vitro and pot culture condition.

P1.1-154

THE MICROBIAL COMMUNITY IN OLIVE (OLEA EUROPAEA L.) RELATED TO GENOTYPE AND PATHOGEN INFECTION

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Text

Olive knot disease caused by the Gram-negative bacterium *Pseudomonas savastanoi* pv. *savastanoi* is one of the most important diseases that affect olive trees (*Olea europaea* L.). The plant genotype has been recognized to be a key determinant of microbial community that is associated with plant health. We assessed the microbial community in phyllosphere and rhizosphere of olive varieties from the Olive Germplasm Collections. Varieties showed different susceptibility to olive knot disease. The trees showing the symptoms were compared with asymptomatic trees to elucidate the potential role of microbiome in protecting the host plants from the disease. DNA was extracted from root and leaf samples and whole metagenome shotgun sequencing of 16S rRNA gene was used to characterize microbiota in olive compartments. With regard to prokaryotic communities, both richness and diversity indices were compared. Bacterial communities were composed by dominant phyla *Proteobacteria*, *Bacteroidota* and *Actinobacteriota* in leaves and by *Proteobacteria*, *Actinobacteriota* and *Chloroflexi* in roots. The overall data suggest that genotype and pathogen infection may result in distinct microbial community structure. These results suggest that highly diverse microbiome may improve the plants ability to resist the effects of

pathogens potentially contributing to plant health.

Biology and paleovirology of the Caulimoviridae

C9.7-1

CHARTING THE INTERACTOME OF CAULIFLOWER MOSAIC VIRUS

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Text

The ability of a virus to infect a plant is dependent on an intricate series of interactions between both the virus and the host. Viruses might appear to be handicapped by their genome size, as the genomes of many plant viruses have a coding capacity limited to less than ten proteins. However as new functions are described for individual virus proteins, the apparent simplicity of the virus genome has been shown to be an illusion that belies the true impact that plant viruses have on host physiology. In this presentation, we will discuss our evolving understanding of the interactome of cauliflower mosaic virus (CaMV), a process that was initiated over forty years ago with the publication of the genome sequence of CaMV. The initial nucleotide sequence revealed that the CaMV genome is composed of seven open reading frames (ORFs), and six proteins were subsequently matched up with their respective ORFs; the seventh putative protein has never been found in infected plants. Since the CaMV genomic sequence was finalized, numerous studies have been completed to determine the functions of the six CaMV proteins, as well as their subcellular localization, and new functions have been characterized for these proteins. In particular, interactome maps have illustrated how individual CaMV proteins interact with each other as well as with host proteins. Interactome maps coupled with subcellular localization studies can be used to reveal new insights into the CaMV disease cycle.

C9.7-2

ORIGIN, SPREAD AND CONTROL OF ENDOGENOUS PARARETROVIRUSES WITHIN PETUNIA

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Text

Petunia is an ideal model system to study endogenous pararetroviruses (EPRVs), particularly Petunia vein clearing virus (PVCV). Bioinformatic analysis of available Petunia genome sequences and small (sm)RNA databases revealed two distinct variants of endogenous PVCV (ePVCV1 and -2) in both wild diploids as well as in today's garden Petunia, *Petunia hybrida*. ePVCV1 was present in all Petunia genomes studied, but ePVCV 2 existed only in *P. axillaris* and P. hybrida. Elements of ePVCV 1 showed 99% sequence identity with episomal PVCV (U95208.2) and homologous smRNAs mapped throughout the viral genome. Therefore, ePVCV 1 copies likely serve as a template for initiating episomal PVCV replication in *P. hybrida*. Sequences of ePVCV2 insertions possessed 74% similarity with PVCV and lacked several regulatory elements and homologous smRNAs. This suggests that ePVCV2 is replication incompetent. Chromosome 3 seems to be a hot spot for EPRV residence in permissive Petunia genomes like *P. axillaris* and *P. hybrida*. *P. parodii*, with no detectable ePVCV, was infected with an infectious PVCV-clone and grown in tissue culture followed by generative propagation via seeds. *De novo* integration of PVCV in the telomeric regions of single chromosomes was identified using FISH. Thus, the telomeric ePVCV localization additionally to the multiple pericentromeric insertions found in the *P. hybrida* line W138, may indicates a recent, transient activation of ePVCV1 resulting in a *de novo* integration.

C9.7-3

DIAGNOSIS AND EPIDEMIOLOGICAL DYNAMICS OF CACAO SWOLLEN SHOOT BADNAVIRUSES IN WEST AFRICA: MAJOR ADVANCES AND GAPS IN KNOWLEDGE

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Text

Cacao swollen shoot disease (CSSD) badnaviruses, family Caulimoviridae, remains a major cacao production limitation in terms of yield loss and mortality of affected cacao plants in West Africa. This presentation highlight achievements from past research efforts such as; purification of viral particles, eradication as a control strategy, the use of tolerant cacao varieties, identification of mealybug vectors, mild strain cross protection, identification of alternative hosts, and shade effect on disease severity. Further research advances including; characterization of new species of the virus and their diversity across the cacao landscape diversity are also highlighted. Pertinent issues of epidemiological importance and diagnostics considered as knowledge gabs include the following; limited information on host-pathogen interactions and its effect on development of resistant planting materials, poor quantification of viral titre in host tissues, and poor primer detection efficiency with PCR based detection assays. Sustained investigations into these grey research areas would provide an updated knowledge on the epidemiological dynamics and diagnostics of the disease for improved molecular detection and characterization of the virus to support breeding of tolerant cacao planting materials, certification of virus-free cacao planting material for distribution to farmers, field surveillance and integrated management of the disease in West Africa.

C9.7-4

IDENTIFICATION AND DISTRIBUTION OF NOVEL BADNAVIRAL SEQUENCES INTEGRATED IN THE GENOME OF CACAO (THEOBROMA CACAO)

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Text

As part of an ongoing study to understand the diversity of the badnavirus complex, responsible for the cacao swollen shoot disease in West Africa, evidence was found recently of virus-like sequences in asymptomatic cacao plants. The present study exploited the wealth of genomic resources in this crop, and combined bioinformatic, molecular, and genetic approaches to report for the first time the presence of integrated badnaviral sequences in most of the cacao genetic groups. These sequences, which we propose to name eTcBV for endogenous Theobroma cacao bacilliform viruses, varied in type with each predominating in a specific cacao genetic group. Additionally to the viral insert of type VI first identified, we recently described, with the help of Oxford Nanopore technology, viral inserts of type I, II, III and V longer than 10kb. A diagnostic multiplex PCR method was developed to identify the homozygous or hemizygous condition of the specific insert of type VI, which was inherited as a single Mendelian trait.
These data suggest that these integration events occurred before or during the species diversification in Central and South America, and prior to its cultivation in other regions. Such evidence of integrated sequences is relevant to the management of cacao quarantine facilities, and may also aid novel methods to reduce the impact of such viruses in this crop.

C9.7-5

MOLECULAR BIOLOGY OF RICE TUNGRO BACILLIFORM VIRUS (TUNGROVIRUS BACILLOORYZAE): NEW LEADS TO CONTROL RICE TUNGRO DISEASE

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Text

Rice tungro bacilliform virus (RTBV), family Caulimoviridae, genus Tungrovirus, is the causative agent for the devastating rice tungro disease (RTD), a potential threat to the regional food security in Asia. The viral dsDNA has a long 5’ untranslated region (5’ UTR) capable of folding into strong secondary structures, and four open reading frames (ORFs), ORF IV being the most variable. ORF III-encoded protein has domains resembling coat protein, aspartate protease (PRT) and reverse transcriptase-RNAseH. Infectious clones of RTBV have been developed, which give rise to mild RTD symptoms upon delivering through

agrobacterium into the crown region of young rice plants. This opens the way for precise mutations to be introduced in RTBV genes to study their effects on pathogenesis. ORF IV-encoded P4 functions as a suppressor of RNA silencing in an isolate from Philippines. Interestingly, the 5' UTR gives rise to a large populationof small RNAs,a potential decoy against RNA silencing. Emerging evidence indicates that PRT of an Indian isolate interacts with a component of RNA silencing machinery, revealing a surprising diversity of function. Rice plants show global changes in gene expression patterns, including hormonal pathways and membrane transport, among others. Using a transgenic approach, an ORF IV-derived ds RNA generating construct gives rise to tolerance against RTBV and reduces the viral levels 103-fold, simultaneously dampening the RTD symptoms.

C9.7-6

UNRAVELLING THE STRUCTURE OF ENDOGENOUS BADNAVIRUSES OF AFRICAN YAM SHED LIGHT ON THE ORIGIN AND DIVERSITY OF YAM CAULIMOVIRIDAE INSERTIONS

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Text

Endogenous viral elements (EVEs) of four distinct badnavirus species were previously partially characterized in the genome of African yam (*Dioscorea cayenensis* and *D. rotundata*), using molecular approaches. In order to fully elucidate the structure of badnaviral EVEs in African yams, the genomes of accessions 'Ti Guinée' (*D. cayenensis*) and 'Msg 5' (*D. rotundata*) were sequenced using HiFi PacBio high-throughput sequencing (HTS) and searched for badnavirus EVEs.

Large contigs of up to 50 Mbp were assembled and combined with optical maps produced by Saphyr system (Bionano), resulting in two high-quality reference sequences for *Dioscorea*. Twelve and six contigs originating from the genomes of 'Ti Guinée' and 'Mgs 5', respectively, contained badnaviral EVEs ranging in size between 124.4 and 17.7 kbp. All badnaviral EVEs were highly rearranged, especially those of *D. cayenensis*, which were also larger, and most contained interspersed sequences originating from distinct badnavirus species or from viruses in different *Caulimoviridae* genera. One EVE from accession 'Ti Guinée' contained badnaviral sequences surrounded by putative *Geminiviridae* sequences. Several EVEs from 'Ti Guinée' contained sequences of a yet unreported *Caulimoviridae* closely related to but distinct from Dioscorea nummularia-associated virus (DNUaV; *Dioscovirus*). Several EVEs originating from either species contained more-than-length copies of badnaviral genomes that may be replication competent and infectious.

Botryosphaeria dieback: which hosts are affected, what we know and how to fight

C8.1-1

BOTRYOSPHAERIACEAE ON CROPS: CURRENTS STATUS OF THE TAXONOMY OF GENERA AND SPECIES

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Text

The Botryosphaeriaceae is the largest family in the Botryosphaeriales (Dothideomycetes) with 22 genera and over 200 species known from culture and for which DNA sequence data is available. Species in this family are widespread globally and include some important pathogens of woody plants. These pathogens are commonly associated with dieback, cankers and fruit rots that impact plant yield and lead to economic losses in several crops. Undeniably, grapevine is the most widely studied crop, with many species of Botryosphaeriaceae being associated with Botryosphaeria dieback. However, apple, avocado, blueberry, citrus, olive, pistachio, mango, and other crops are also affected by these fungi. Of the genera recognised in the Botryosphaeriaceae, Botryosphaeria, Diplodia, Lasiodiplodia and Neofusiccoccum are the most frequently associated with diseases in those crops. Some are also amongst the most species-rich genera in the family. Botryosphaeriaceae have been subjected to taxonomic revisions over time. The impact of DNA sequence data in resolving taxonomic issues is undeniable, but this DNA-based approach has its own set of problems and limitations. Arguably it has contributed to a proliferation of species names and to some taxonomic confusion. Thus, there are aspects that require attention. Some of the larger genera are clearly in need of revision. The fast growing of fungal genomics represents a potential tool to assist in the challenging task of delimiting genera and species.

C8.1-2

OCCURRENCE OF BOTRYOSPHAERIACEAE SPECIES PATHOGENS IN AGRICULTURE AND FOREST SYSTEMS IN CALIFORNIA

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Text
California produces nearly half of U.S.-grown fruit and nuts and contains 33 million acres of forested land. Pathogens in the Botryosphaeriaceae cause a wide range of diseases on crops and forest tree species in California, including “Bot” canker on woody plants and fruit stem-end and crown rots, resulting in significant ecological and economic losses across heterogeneous landscapes. Here, we review case studies of disease-causing pathogens in the Botryosphaeriaceae family in California. Since 2008, we identified over 30 species causing severe dieback on important agricultural and forest tree species in California (e.g., avocado, grapevine, citrus, tejocote, coast live oak, coast redwood, sycamore, willow, and strawberry). In addition, we recently identified a new pine ghost canker disease complex causing severe dieback on several pine species within a 40-ha urban forest in Southern California. Many Botryosphaeriaceae species are recognized as endophytes that become pathogenic following the onset of plant stress. The striking increase of observed diseases caused by these pathogens following prolonged drought in California highlights the impact of climate change on host-pathogen interactions.

C8.1-3

THE LATENT PINE PATHOGEN DIPLODIA SAPINEA CONTAINS TWO DISPENSABLE CHROMOSOMES WITH DISTINCT GENOMIC CHARACTERISTICS

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Text
Diplodia sapinea is a pathogen of conifers that predominantly infects *Pinus* spp. It typically remains in a latent phase in infected trees until the onset of stress when disease symptoms such as shoot blight, stem cankers, root disease or die-back develop. Isolates of *D. sapinea* display varying degrees of aggressiveness, but little is known regarding the genetic basis for this variation. Using a combination of Nanopore and Illumina technologies we sequenced the genomes of three *D. sapinea* isolates. Comparing these genomes revealed the existence of two dispensable chromosomes (DCs) in two isolates, 0.46Mb and 0.64Mb in size, of which the former occurred in two isolates and the latter in a single isolate. Low coverage Illumina sequencing of seven additional isolates from various countries, of which six contained the 0.46Mb DC and one contained the 0.64Mb DC. These DCs were found to encode for 80 and 152 proteins, respectively, and had lower gene density and higher proportions of transposable elements compared to the core chromosomes. Sequence analysis indicated that genes on the DCs are rapidly evolving, probably owing to the low selection pressure. Gene ontology enrichment analysis showed that the DCs have enriched GO terms associated with transposable elements and pathogenicity. Pathogenicity trials conducted on *Pinus patula* seedlings, however, showed no obvious association between the DCs and isolate aggressiveness. The biological roles of the DC’s remain to be identified.

C8.1-4

BOTRYOSPHAERIACEAE CANKERS OF ALMOND IN CALIFORNIA: SPECIES DIVERSITY, MOLECULAR DETECTION AND BIOLOGICAL CONTROL

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Text

Botryosphaeriaceae cankers represent some of the most common canker diseases affecting almond in California. To date at least 12 Botryosphaeriaceae spp. have been isolated from almond cankers, including *Neofusicoccum parvum*, *N. mediterraneum*, *Botryosphaeria dothidea* and *Neoscytalidium dimidiatum* as the most common species. Recently, our laboratory developed PCR-based assays for the specific detection of Botryosphaeriaceae pathogens directly from infected almond tissues. Eight species-specific primers were designed by exploiting sequence differences in the translation elongation factor or β-tubulin gene. We also investigated the efficacy of various biocontrol agents to protect almond pruning wounds from infection by these pathogens. Field assays indicated that *Trichoderma atroviride* SC1, *T. paratroviride* RTFT014, and *Clonostachys rosea* J1446 provided significant protection of pruning wounds from infection by *N. parvum*, with levels of disease control similar to those reached by the conventional chemical thiophanate-methyl. We also investigated the duration of pruning wound susceptibility to Botryosphaeriaceae infections according to the month of pruning, i.e. September, October, November, December and January. Pruning in January showed reduced wound susceptibility to Botryosphaeriaceae. Overall pruning wound susceptibility decreased sharply after one- and two-weeks following pruning.

C8.1-5

BOTRYOSPHAERIA PANICLE AND SHOOT BLIGHT OF PISTACHIO: FROM A DILEMMA OF THE CALIFORNIA PISTACHIO INDUSTRY TO A SUCCESS STORY

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Text

Botryosphaeria panicle and shoot blight (BPSB) was first observed and reported in California pistachio in 1985. It was discovered in a few sprinkler-irrigated orchards in Butte County, CA, causing severe damage. Initially the pathogen was identified as *Botryosphaeria dothidea*. By 1990, erroneous opinions among industry representatives resulted in discontinuing any research on this disease, since the disease was considered very localized and of minor importance. As the acreage of pistachio has increased over the years and some years were really rainy in the spring, the disease became widespread, creating a major epidemic during 1997-1999 in our pistachios. The disease caused severe blighting and killing of clusters, shoots, leaves, and buds at a time when there was no known control, with the exception of an expensive sanitation by severely pruning and removing cankered branches, shoots, and infected rachises. Desperate growers started cutting down pistachio trees and planting

walnuts in the same locations, not knowing at that time that about 10 years later Botryosphaeria would become a major disease of walnut as well. The BPSB created a major dilemma among the pistachio growers, necessitating an urgent emergency action, which was an increase in research funding to quickly solve this major problem. Thus, this accelerated research from 1999 to 2005 helped determine the etiology, epidemiology, prediction, and successful disease management of BPSB of pistachio in California.

C8.1-6

NEOFUSICOCCUM PARVUM: A PLANT PATHOGEN OF GLOBAL SIGNIFICANCE

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Text
Neofusicoccum parvum is an important pathogen with a global distribution and for which our knowledge has expanded rapidly in the past decade. We have synthesised research showing that it has a host range of more than 200 plant species residing in at least 64 families. These hosts include numerous important crops, urban and forest tree species. The most common disease symptoms caused by *N. parvum* are die-back and branch or stem cankers. *Neofusicoccum parvum* is also very commonly encountered as an endophyte in healthy plant tissues, and become pathogenic once the host is subjected to biotic or abiotic stress. Historical inaccurate taxonomic classifications, as well as difficulties in distinguishing it from a number of closely related species continue to be a problem. Our recent analyses of sequence data show that the global population of *N. parvum* is dominated by a few closely related haplotypes with little structure based on geography or host. These globally distributed populations have most likely arisen from inter-continental dispersal through the movement of infected plant material, which urgently requires characterization of common pathways. In addition, genomics and transcriptomics are beginning to provide insights into the molecular mechanisms that underpin *N. parvum* infections, their persistence as endophytes and disease development. A holistic perspective considering all these fields is needed to inform strategies to manage this pathogen of growing global importance.

P8.1-001

FUNGAL TRUNK DISEASES OF FRUIT TREES IN EUROPE: PATHOGENS, SPREAD AND FUTURE DIRECTIONS

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Text

The European production of major crops such as pome and stone fruit, nut crops, berry fruit, citrus, grapevine, and olive is increasingly threatened by fungal trunk diseases (FTD). These diseases and the consequent production losses represent a relevant issue. Several fungi infect host wood mainly through wounds and subsequent colonization of tissues, causing symptoms such as cankers, gummosis, wood rotting, blight and dieback. Some of these fungal pathogens live as endophytes in hosts and their spread occurs through propagative plant material such as rootstock, seedlings and fruit. Abiotic factors are strongly involved in disease development. High planting densities combined with plant nutrient programmes, global warming and climate change favour stress to the cultivated plants. Wounds caused by pruning or mechanical shaking of trunks for fruit harvesting can promote the risk of infections through possible airborne pathogen entry points. The European working group presenting this work recently published an article to review literature on FTD, with a particular focus on the European situation of their causal agents, distribution and host associations, particularly relating to case studies on apple, citrus, grapevine, berry, nut and stone fruit, and olive trees. Moreover, epidemiology and hypotheses on the increase of FTD incidence were discussed. Future prospects and direction of FTD research are presented with the purpose of achieving sustainable disease management.

P8.1-002

ROLE OF SIX EFFECTORS WITH ARSENIC AFFINITY ON THE PATHOGENICITY OF NEOFUSICOCCUM PARVUM

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Text

Grapevine Trunk Diseases (GTDs), which affect 12% of French vineyards, have had no efficient treatment since Sodium Arsenite was banned in 2001. *Neofusicoccum parvum* is one of the most aggressive and prevalent fungi causing GTDs (namely Botryosphaeria dieback), but its infecting mechanisms are still not fully resolved. To control GTDs, understanding the virulence factors involved in grapevine-microbe interaction, and leading to wood degradation is crucial. This project aims to investigate the role of six secreted proteins of *N. parvum*, identified according to their affinity to arsenic, in the pathogenicity of two isolates causing differential symptom expression in detached wood cane assays. Kinetic observation of effectors expression was performed by RT-qPCR on grapevine cane, and the

impact on plant defense pathways was studied by agroinfiltration in *Nicotiana benthamiana* leaves. Notably, the two isolates have non-synonymous sequences for the genes coding for the six effectors. Preliminary results show that inoculation of grapevine wood by the fungi induces early expression of some effectors compared to damaged non-inoculated plants. Meanwhile, transient expression of at least one of these virulence factors in *N. benthamiana* leaves triggered cell death and modulated plant defense responses by targeting specific pathways. The effector's apoplastic localization seems particularly important to induce a complete necrotic response.

P8.1-003

GENOME SEQUENCING OF NEOFUSICOCCUM PARVUM (TELEOMORPH: BOTRYOSPHAERIA PARVA) FROM HEMP (CANNABIS SATIVA) OFFERS CLUES INTO MOLECULAR MECHANISMS OF PATHOGENESIS

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Text

Neofusicoccum parvum (Np) (Botryosphaeria parva) is a globally important fungal pathogen/endophyte of diverse native forest species and woody agricultural and ornamental hosts. Np was first identified as a pathogen of hemp (Cannabis sativa) in the U.S. in Arkansas in 2019. The molecular bases of endophytism and pathogenicity of Np, including its broad host range, are poorly understood. To address this gap, draft genome sequences were obtained and analyzed from two hemp reference isolates of Np (ON1 – MT093349 and MT141110; ON2 – MT093349). Genomes were sequenced at a predicted depth of ~80x coverage with DNBseq (BGI Americas) and assembled with CLC Genomics Workbench (Qiagen). The predicted genome sizes were 42.62 Mb and 42.69 Mb for ON1 and ON2, respectively, and similar to a previous report of 42.59 Mb for an isolate of Np from grape (UCD646So). Whole-genome alignments incorporating draft genomes from ON1 and ON2 (collected from two different years and two locations), indicated they are possibly clonal. Also, isolates of Np from diverse hosts and geographic origins revealed surprisingly high levels of genomic synteny and identity. The lack of molecular diversity among the disparate Np isolates examined suggests that some clonal lineages may predominate worldwide.

P8.1-004

BOTRYOSPHAERIACEAE INVOLVED IN RECENT WALNUT DIEBACK IN FRANCE AND LEVEL OF THEIR POPULATIONS DIVERSITY

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Text

Fungi of the Botryosphaeriaceae family are responsible for wood diseases worldwide, including the *Botryosphaeria* dieback associated with blight and cankers in grapevine and nut crops. Since 2013, new unseen symptoms, including branch dieback, fruit necrosis and blight, have appeared in French walnut orchards although widespread in Mediterranean-climate countries, such as California and Spain. To unravel the pathobiome associated with these symptoms, symptomatic husks and twigs were collected in 12 French orchards from the two main production areas and analyzed by culturing and metabarcoding. In addition to *B. dothidea* (Bd) and *Neofusicoccum parvum* (Np), the two main Botryosphaeriaceae, *Diaporthe eres*, *Colletotrichum fioriniae*, *C. godetiae* and *Fusarium juglandicola* were also predominant by both methods, suggesting that Bd and Np were part of a complex pathobiome. Simple Sequence Repeats (SSR) sequencing was then used to explore the diversity and structure of Bd (n=190) and Np (n=285) populations, including isolates from Californian walnut orchards (n=28) and French vineyards (n=62). First results revealed a low degree of genetic diversity of Np populations (28 markers, 100 haplotypes) which were not structured based on geographic origin or host, confirming an asexual reproduction mode. The level of genetic diversity between isolates from French symptomatic husks and twigs will be further compared, and Bd population structure (11 markers, 23 haplotypes) will also be analyzed.

P8.1-005

EMERGING PECAN LEAF DIEBACK DISEASE CAUSED BY NEOFUSICOCCUM CARYIGENUM: RESEARCH UPDATE AND IMPLICATIONS FOR MANAGEMENT

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Text

Pecan leaf dieback (PLD) is caused by the *Neofusicoccum caryigenum* pathogen and was first reported in 2021. PLD symptoms include darkening of leaves, dead leaves remaining on trees, and early defoliation, which can negatively affect tree health and productivity of pecan (*Carya Illinoinensis*). Research on the newly identified pathogen is needed to develop management strategies. Since 2021, Texas pecan orchards have been surveyed to determine the distribution of PLD. Over sixty *N. caryigenum* isolates were obtained, and greenhouse assays showed that *N. caryigenum* can cause disease on pecan seedlings effectively with petiole wounds. To better understand the genetic diversity of *N. caryigenum*, the whole genome of one isolate was sequenced, resulting in approximately 43 Mbp with a GC content of 56%. This sequencing data will be used to develop simple sequence repeat (SSR) markers for further pathogen population genetic diversity study. Understanding pathogen epidemiology and genetic diversity is crucial for developing effective management

strategies for PLD. This new information will provide a foundation for developing chemical and cultural management practices to prevent the spread of the disease and minimize its impact on pecan trees and the pecan industry. Further research is needed to combat this emerging disease and ensure the continued health and productivity of pecan trees.

P8.1-006

INCIDENCE AND SEVERITY OF MANGO TREES DECLINE IN CÔTE D'IVOIRE AND CHARACTERISATION OF LASIODIPLODIA SPECIES ASSOCIATED

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Text

Mango tree (*Mangifera indica* L.) decline caused by Botryosphaeraceae fungi is an important threat to mangoes production worldwide. This study aimed to assess the incidence and severity of mango trees decline in Côte d'Ivoire, determine the impact of climate parameters and farmers' cultural practices on the disease, and identify its causal pathogen. Four surveys were conducted during two years in the dry and rainy seasons in 42 mango orchards distributed in four agroecological zones (AEZ) of Côte d'Ivoire and 2,100 mango trees were evaluated. Mango symptomatic organs were collected in each orchard and proceeded to the laboratory. Mango decline incidence varied from 30 to 100 % depending on AEZ and severity ranged from 25 to 80 % in orchards. However, disease severity evaluation could be affected by seasonal variations and pruning. Fungi isolated from diseased mango samples were identified using morphological characteristics and phylogenetic analyses based on the internal transcribed spacer (ITS) of rDNA and elongation translation factor 1-alpha (tef1-α) partial DNA sequences. Fungal isolates showed macro- and micro-morphological features of Lasiodiplodia spp. The phylogenetic analysis grouped mango isolates with *Lasiodiplodia theobromae*, *Lasiodiplodia euphorbicola* or *Lasiodiplodia brasiliense*. The pathogenicity tests revealed that all isolates were pathogenic to mango seedlings. These data will help for the control and prevention of mango trees decline in Côte d'Ivoire.

P8.1-007

DIVERSITY OF LASIODIPLODIA SPECIES ASSOCIATED WITH MANGO (MANGIFERA INDICA L.) DECLINE IN BURKINA FASO AND INFLUENCE OF CLIMATIC FACTORS

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Text

Mango (Mangifera indica L.) is one of the most important fruit tree in tropical and subtropical regions of the world. Mango decline, caused by Lasiodiploadia spp., is a major disease of mango in Burkina Faso. The main objective of this study was to identify Lasiodiplodia species associated with the mango decline in the main mango-producing areas of the country. In addition, incidence and severity of mango decline was determined and climatic and edaphic factors affecting the geographic distribution of the disease were identified. The genetic diversity of 47 Lasiodiplodia isolates was studied on the basis of sequence data of the translation elongation factor 1-alpha gene (tef1-a) and the rDNA internal transcribed spacer region (ITS). Phylogeny analyses grouped the isolates with reference isolates of Lasiodiplodia brasiliensis, Lasiodiplodia caatingensis, Lasiodiplodia crassispora, Lasiodiplodia euphorbicola, Lasiodiplodia pseudotheobromae and Lasiodiplodia theobromae. Lasiodiplodia isolates tested on mango seedlings (cv. Amelie) induced the typical dieback symptoms, including necrotic bark lesions and wilting. The incidence and severity were strongly associated between each other and were generally higher in the eastern regions of the country, where the weather patterns are also drier and warmer than in the western regions. This study is of paramount importance for the establishment of strategies to control mango decline in Burkina Faso.

P8.1-008

STATISTICAL IMAGE SEGMENTATION OF VINES' WOOD COLONIZED BY NEOFUSICOCCUM PARVUM IN FLUORESCENCE MICROSCOPY

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Text

Grapevine young decline associated with wood decays is a major threat for the grapevine industry. Understanding the fungal colonization is crucial to develop sustainable cures. Graftlings samples of vines inoculated with the esca-associated fungus *Neofusicoccum parvum* were observed under a wide field fluorescent microscope after a post staining treatment with WGA-fitc fluorescent marker. We aim at developing a tool to separate the pathogen from its auto fluorescent textured background and then quantify its colonization in woody tissues.
This classical task in image processing is hampered by the blur encountered in the images. Because the blur depends of the depth of the mycelium in images, the inverse problem is

twofold: estimate a mycelium map, as well as its depth. The blur is encoded in a point spread function that describes the degree of spreading of a point object, and measures imaging system quality. We introduce a dedicated statistical model encoding three elements: 1) the observation; 2) the “hidden” mycelium map to estimate and 3) the depth of the observation at each pixel. Assuming a pixelwise smoothness in the image (mycelium, and depth, do not vary rapidly from one pixel to its neighbors) this model belongs to the Triplet Markov Field family.
We propose an alternating Bayesian scheme to estimate jointly the hidden fields, and the model’s parameter (such as average fungi / wood colors) from the observation alone, i.e. without training database.

P8.1-009

BOTRYOSPHAERIA DIEBACK IN WALNUT ORCHARDS IN AUSTRALIA

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Text
An emerging challenge for the productivity of the Australian walnut industry is yield losses caused by dieback of branches and fruiting spurs. Species of Botryosphaeriaceae that have been implicated in dieback in many horticultural crops worldwide have been reported in walnut orchards in Australia, yet systematic studies on the species present and control strategies are incipient. This is the first systematic study to investigate the epidemiology and management of Botryosphaeria dieback in walnuts in Australia. DNA sequencing of fungi isolated from walnut tissues collected from major walnut growing regions of Australia confirmed the presence of five Botryosphaeriaceae species, namely Diplodia seriata, Dothiorella omnivora, Neofusicoccum parvum, N. macroclavatum and Spencermartinsia viticola. Of these, D. seriata and N. parvum were the most prevalent species. Pathogenicity studies using detached stems and potted plants in the glasshouse indicated that N. parvum was the most virulent, causing lesions that were three times greater than D. seriata. Pruning wounds were susceptible to N. parvum and D. seriata for up to four months and two months respectively, with the highest disease incidence occurring in the first week following pruning. The green shoots and younger tissues were more susceptible than the older stems to the isolates selected for pathogenicity studies. Further glasshouse experiments on disease progression and field trials on control strategies are in progress.

P8.1-010

FUNGAL TRUNK PATHOGENS IN HAZELNUT ORCHARDS IN CHILE

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Text

Hazelnut is one of the most important fruit crops in Chile (37.905 h) and is affected by trunk diseases. The objective was to determine their etiology, pathogenicity, epidemiology and susceptibility to pruning paintings. Wood samples (n=292) were collected from 46 orchards showing dieback and cankers, mostly from cvs. Giffoni, Barcelona, Lewis and Yamhill. Sections were cut, disinfected and plated on a quarter-strength acidified potato dextrose agar, incubated and purified on PDA. Fungal isolates (n=182) were morphologically identified. DNA was extracted and specific genes were amplified by PCR, identifying mostly Botryosphaeriaceae, Diaporthaceae, Nectriaceae and Basidiomycete. Mycelial plugs of representative isolates (n=25) were inoculated on injuries of healthy cuttings cv. Lewis and incubated for 72-d in flowing water at 22°C. The most virulent ones were inoculated on fresh pruning cuts of 3-y potted plants cv. Lewis and incubated for 112-d at shadehouse. For both tests, internal necrosis on inoculated twigs was measured, compared and fungi reisolated, finding that *Neofusicoccum*, *Diaporthe* and *Chondrostereum* were the most virulent. To study the epidemiology, a 9-y orchard cv. Lewis was weekly monitored for 12-m analysing the inoculum on glass spore traps by qPCR. Once the inoculum presence was confirmed, healthy plants were pruned and painted with commercial paintings (n=9) and control. After 10-m, internal necrosis varied from 6.5-17cm compared to the control (22,3cm).

P8.1-011

DIFFERENTIAL CARBOHYDRATE-ACTIVE ENZYMES AND SECONDARY METABOLITE PRODUCTIONS BY THE GRAPEVINE TRUNK PATHOGEN NEOFUSICOCCUM PARVUM BT-67 GROWN ON A HOST AND NON-HOST BIOMASS

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Text

Neofusicoccum parvum is one of the most aggressive Botryosphaeriaceae species associated with grapevine trunk diseases. This species may secrete enzymes, such as cellulases, hemicellulases and oxidoreductases, capable of overcoming the plant cell wall barriers. Furthermore, N. parvum produces toxic secondary metabolites that may contribute to its virulence. To increase knowledge of the mechanisms underlying pathogenicity and virulence, we evaluated the N. parvum Bt-67 capacity in producing lignocellulolytic enzymes and secondary metabolites when grown in vitro on two biomasses: grapevine canes (GP) and wheat straw (WS). We performed a multiphasic study combining enzymology, transcriptomic and metabolomic analyses. Enzymatic activity assays showed higher xylanase, xylosidase, arabinosidase and glucosidase activities when the fungus was grown on WS in contrast with GP. Infrared spectroscopy confirmed the lignocellulosic biomass degradation caused by the secreted enzymes. Transcriptomics revealed up-regulation of 134 Carbohydrate Active Enzymes (CAZymes)-coding genes, where 94 were expressed in both biomass growth

conditions. Lytic polysaccharide monooxygenases, glucosidases and endoglucanases were the most represented CAZymes. The secondary metabolites diversity was variable depending on the carbon source. This diversity was higher when growth occurred with GP. Our results provide insight into the influence of lignocellulosic biomass on virulence factor expression.

P8.1-012

HOW TO MANAGE NEOFUSICOCCUM PARVUM IN PROTECTING GRAPEVINE: COMBINING THE BENEFICIAL EFFECTS OF BACILLUS SUBTILIS PTA-271 AND TRICHODERMA ATROVIRIDE SC1.

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Text

Botryosphaeria dieback (BD) is one of the big threat for global viticulture. Without effective sustainable treatments, biocontrol strategies are developed as alternatives to better cope with environmental concerns. A combination of biological control agents (BCAs) is tested as promising for managing BD through complementary ways of protection.
Indeed, Bacillus subtilis (Bs) PTA-271 and/or Trichoderma atroviride (Ta) SC1 efficiently protect Chardonnay and Tempranillo rootlings against Neofusicoccum parvum Bt67, an aggressive pathogen associated to BD. Indirect benefits offered by each BCA and their combination were then characterized in planta, as well as their direct benefits in vitro. Results provide evidence that the cultivar contributes to the beneficial effects of Bs and Ta against N. parvum, and that the in vitro BCA mutual antagonism switches to a strongest fungistatic effect toward NpBt67 in a three-way confrontation test. We also report for the first time the beneficial potential of a combination of BCA against NpBt67 especially in Tempranillo. Our findings highlight a common feature for both cultivars: salicylic acid (SA)-dependent defences were strongly decreased in plants protected by the BCA, in contrast with symptomatic ones. We thus suggest that the high basal expression of SA-dependent defences in Tempranillo explains its highest susceptibility to N. parvum, and that the cultivar-specific responses to the beneficial Bs and Ta remain to be further investigated.

P8.1-013

MANAGEMENT OF BOTRYOSPHAERIA DIEBACK PATHOGENS IN GRAPEVINE PROPAGATION MATERIAL COMBINING BACILLUS SUBTILIS PTA-271 AND TRICHODERMA ATROVIRIDE SC1.

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Text
Fungal grapevine trunk diseases (GTDs) still represent a threat to viticulture, leading to important economic losses worldwide. In nurseries, grapevine planting material is very susceptible to infection by GTDs pathogens due to several cuts and wounds made during the different steps in the propagation process. Without effective chemical treatments, a combination of biological control agents (BCAs), could improve the plant material protection against GTDs pathogens in the nursery process. In this study, we evaluated the effect of single or combined treatments with Bacillus subtilis PTA-271 (Bs) and Trichoderma atroviride SC1 (Ta) to reduce infections caused by fungal pathogens belonging to the family Botryosphaeriaceae in grapevine planting material during the propagation process. Our results showed a reduction in Botryosphaeria dieback incidence and severity on grapevine propagation material treated with Ta and the combination Ta + Bs. Therefore, the Ta SC1 and Bs PTA-271 combination, showed the potential to reduce infections caused by these pathogens in the nursery propagation process. These biological treatments may be relevant components of an integrated approach, using complementary management strategies to limit infection by GTD pathogens. Further research is still needed to elucidate the effectiveness of Bs PTA-271 and the benefits of simultaneous application with Ta SC1 for the control of GTD pathogens in nurseries.

Controlling Globally Developing Disease Threats of Banana

C8.5-1

FUSARIUM WILT OF BANANA CAUSED BY TROPICAL RACE 4: PROBLEMS AND PROSPECTS

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Text
Banana is the world's top fruit and one of the most important staple foods. Cavendish clones dominate total global banana production (> 50%) and export (>95%). Their importance goes back to the first epidemic of Fusarium wilt of banana (FWB), a soilborne disease that wiped out Gros Michel between the 1920s and 1950s. This epidemic, caused by the so-called Race 1, wreaked havoc on banana production in Central America and impacted millions of households. Cavendish clones boomed as they could be cultivated on heavily Race 1 - infested soils on which Gros Michel succumbed. However, FWB already re-emerged in Cavendish clones in the 1960s in Taiwan. The Fusarium strain that caused the disease was called Tropical Race 4 (TR4) and surged throughout Southeast Asia for decades, decimating Cavendish plantations and affecting manifold other banana varieties until its occurrence in Jordan in 2014. The rest is history. Since then, TR4 has disseminated to 14 new countries in all major banana-producing regions of the world with the latest incursion in Venezuela. What

next? Host resistance is the cornerstone of disease control and Cavendish's resistance to Race 1 - that still holds - is the ultimate benchmark of the required level of resistance to manage FWB caused by TR4, but foremost the crop needs to be diversified. Furthermore, all options for early warning, disease management, and innovation of cultivation systems need to align to sustainably secure this important crop.

C8.5-2

FROM GENE DISCOVERY TO COMMERCIAL RELEASE: A GM CAVENDISH BANANA HIGHLY RESISTANT TO FUSARIUM WILT TROPICAL RACE 4

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Text

Around 20 years ago, we started the search for a banana gene that would provide resistance to Fusarium wilt (Panama Disease) tropical race 4 (TR4). At that time, TR4 had a limited distribution, confined primarily to south east Asia and northern Australia. It is now present and spreading in five continents and is considered the greatest threat to banana production worldwide. We identified a NBS-LRR gene in a TR4 resistant wild diploid banana that had characteristics consistent with a potential TR4 resistance gene. We transferred this gene to Cavendish bananas and regenerated several transgenic lines. These lines, together with controls, were transferred to a field with a history of high TR4 disease incidence. From the first small scale field trial, we identified four lines with varying levels of resistance and these were progressed into a large scale field trial. After three years and 5 crop cycles, one line, QCAV-4, was shown to be highly resistant to TR4 with only 2% of plants infected compared with 66% of the non-GM controls. Importantly, there was virtually no yield penalty for QCAV-4 compared with uninfected non-GM controls. We have now fully characterised this event and have submitted an application to the regulators in Australia towards commercial release of this line. Further, we have recorded the history of each plant of the four initial lines as well as non-GM controls and this is providing significant insights into the progression of the disease in a plantation.

C8.5-3

LIMITING FUNGICIDE USE IN THE MANAGEMENT OF BANANA LEAF SPOT DISEASES

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Text

Three related fungi cause similar leaf diseases of banana and form the sigatoka leaf spot complex: Pseudocercospora fijiensis, P. musae et P. eumusae. The more aggressive species P. fijiensis has been recently spread throughout the world leading to a massive

systematic use of fungicides in most countries, especially in industrial export plantations. Research work has been conducted to limit the use of fungicides. The development of forecasting strategies and cultural practices (such as necrotic deleafing) first allowed to reduce significantly the number of applications. Other studies have been recently conducted at landscape or farm scale to quantify the effects on epidemics of hedgerows or association with other crops. In parallel, genetic improvement programs are being conducted to create new resistant varieties. However, as for fungicides, it has been shown that pathogen populations can adapt and breakdown or erode banana resistances after 5 years of monoculture. A more durable deployment strategy would be to combine resistances with antagonistic interactions in order to constrain and limit the adaptation of pathogen populations. Studies on populations pathogen adaptation and host-pathogen interactions are thus underway. Finally, a generic model has being adapted to P. fijiensis to test the efficiency and durability of various resistance deployment strategies. The last results obtained from all these studies will be presented.

C8.5-4

EFFECT OF A PLANT-BASED BIOLOGICAL CONTROL OF FUSARIUM WILT ON THE SOIL MICROBIOME

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Text

Plant root exudates exert strong influences on the soil microbiome, and rhizospheres are among the most diverse microbial communities. In agriculture, there is growing interest in the manipulation of the soil microbiome through root exudate management, for example for disease suppression. Fusarium Wilt is an important soil-borne pathogen of certain banana (Musa spp.) cultivars. Fusarium Wilt is difficult to eradicate from soils and currently there are no economically viable controls. Observations of apparent disease suppression in some Chinese banana plantations have resulted in the identification of a culinary understorey plant, Allium tuberosum (colloquially Chinese leek), as producing root exudates that kill Fusarium spores and inhibit disease development. However, the wider, non-target effects of Allium root exudates on soil microbes are unknown. Potentially, strong general antimicrobial activity could impact soil ecosystem function. Here, we investigate the influence of banana and Chinese leek roots on soil microbes in a pot trial. We compared the bulk soil and rhizosphere microbiome of Allium and bananas and evaluated the effect of their co-cultivation under greenhouse conditions. Our results indicate that Allium induces a significant shift in soil microbe community, however, this effect is outweighed by the presence of banana roots. Therefore, Allium is a potential plant-based biocontrol for Fusarium that does not significantly alter the soil microbiome in general.

C8.5-5

BANANA BUNCHY TOP VIRUS – MANAGING THIS EVER-EXPANDING THREAT

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Text

Banana bunchy top virus (BBTV) causes the most serious virus disease of banana (Musa sp.) worldwide. Though not yet present in the Americas, its distribution continues to spread in most other banana growing regions. Most of the production of this important crop (85%) is grown for domestic, often subsistence, consumption. No edible cultivars of banana are immune to BBTV, but differences in susceptibility and tolerance are found among the 1000 known cultivars. Combating this disease threat requires a multi-pronged approach. Exclusion through quarantine remains the best strategy for currently disease free-areas. In affected areas, eradication and the use of healthy planting material in low input situations can keep the disease at low, manageable levels. Growing less susceptible cultivars can reduce the rate of spread of epidemics. Apparent immunity has been uncovered in wild, seeded progenitor Musa sp. paving the way for introgression of resistance into conventional breeding lines or exploitation through genetic manipulation. Although several attempts have been made using an RNAi strategy to transform banana for BBTV resistance, adequate field resistance has not yet been demonstrated. However, RNAi strategies with the banana plant or the aphid vector and gene editing strategies still show promise for control of BBTV.

C8.5-6

AN OPTIMISED SAMPLING AND DETECTION PROTOCOL FOR FUSARIUM OXYSPORUM F. SP. CUBENSE TROPICAL RACE 4 FROM ENVIRONMENTAL SOIL

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Text

The soilborne pathogen *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) is a major threat to banana production worldwide. Once introduced, Foc TR4 can persist in the soil as resistant chlamydospores for decades. Accurate and sensitive protocols are thus needed to detect and measure Foc TR4 inoculum levels in soil. This, however, is challenging due to the low concentration of pathogen DNA, the heterogenous distribution, and the presence of potentially inhibitory compounds that might prevent detection. In this study, a sampling and detection protocol was optimised to determine the presence and quantify of Foc TR4 inoculum in infested soil. Samples were collected between plants (bulk soil) and from the root rhizosphere at a commercial banana plantation in northern Mozambique. Higher levels of Foc TR4 inoculum were present in the root rhizosphere than in bulk soil, suggesting that bulk soil sampling needs a larger number of samples to be reliable. Concentrating spores with a soil washing protocol before extraction improved the limit of quantitative PCR detection of the pathogen, and pre-culturing soil on artificial media had an enriching effect and improved the number of positive samples detected. The optimised protocol can be used to determine the efficacy of integrated disease management strategies, or at least the spread of the pathogen in infested areas.

P8.5-001

UNRAVELLING THE PLANT DEFENSE MECHANISMS OF DIFFERENT BANANA CULTIVARS AGAINST FUSARIUM WILT TR4, INCLUDING RESPONSES TO ELICITOR APPLICATION

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Text

To determine the effect of elicitor isotianil on Fusarium wilt of banana TR4, a greenhouse experiment was showed that isotianil could significantly alleviate the symptoms of TR4, enhanced disease control on two cultivars with control effect 50.14% and 56.14%, respectively. The results showed that TR4 hyphae could rapidly penetrate the cortex into the root vascular bundle for colonization, and the colonization capacity in Brazilian was significantly higher than that in Yunjiao No.1.The accumulation of a large number of starch grains was observed in corms cells, and further analysis showed that the starch content in Yunjiao No. 1 as resistant cultivar was significantly higher than that in Brazilian as susceptible cultivar. Besides, a mass of tyloses were observed in the roots and corms and these tyloses increased after application with isotianil. Furthermore, the total starch and tyloses contents and the control effect in the corms of Yunjiao No.1 was higher than that in the Brazilian. These results suggest that there are significant differences between cultivars in response to TR4 invasion and plant reactions with respect to starch accumulation, tyloses formation and the expression of plant resistance induction and starch synthesis related genes. Isotianil application may contribute to disease control by inducing host plants to defend against TR4 infection and could be potentially used together with resistant cultivar as integrated approach to manage this destructive disease.

P8.5-002

MOLECULAR DIAGNOSIS AND VEGETATIVE COMPATIBILITY GROUP ANALYSIS OF FUSARIUM WILT OF BANANA IN NEPAL

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Text

Fusarium wilt of banana (FWB) caused by Fusarium oxysporum f. sp. cubense (Foc) is the most important constraint of banana industry globally. In Nepal, epidemics resembling FWB have been increasingly observed on the cultivar Malbhog in the past several years. However, the disease has not been officially reported yet and consequently little is known about the pathogen present across the country. In this study, we characterized 13 fungal strains isolated from banana plants of the cultivar Malbhog (Silk, AAB) showing symptoms similar to FWB in banana plantations in Nepal. All the strains were typed as belonging to the F. oxysporum and caused FWB symptoms when inoculated in Malbhog and Cachaco (Bluggoe, ABB) cultivars. No symptoms were observed in the cultivar William (Cavendish, AAA). Vegetative compatibility group (VCG) analysis classified the strains as VCG0124 or

VCG0125. PCR analyses conducted with primers specific for Foc race 1 (Foc R1) or Foc tropical race 4 (TR4) revealed that all the strains reacted positively for Foc R1 and none for TR4. Altogether, our results demonstrated that the pathogen populations causing FWB of the cultivar Malbhog in Nepal are Foc R1. This work reports for the first time the occurrence of FWB in Nepal. Further studies with larger Foc populations are needed to better understand disease epidemiology to design sustainable disease management strategies.

P8.5-003

SECURING THE FUTURE OF ECUADORIAN BANANAS: AN INTEGRATED APPROACH TO MITIGATING FUSARIUM WILT TR4

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Text

Consumption of bananas is crucial to the nutritional well-being of more than 400 million people around the world. There is, however, a continuing threat to global production caused by a soil-borne fungus known as Fusarium oxysporum f.sp. cubense, which causes wilting of bananas. Tropical Race 4 (FocTR4), a highly virulent strain of this fungus, has affected more than 20 countries, including Colombia, Peru, and Venezuela in South America. FocTR4 has now put Ecuador, the largest banana exporter, at increased risk. With no silver bullet in sight, producers are calling for a unified front to protect their farms-the backbone of the second most prosperous industry in the country, which has suffered millions of losses due to adverse climate, war conflicts, and other factors. This work will present the current efforts to secure the future of bananas in Ecuador, a significant contributor to the national economy and source of livelihoods and nutrition. Our presentation includes strategies for prevention, but also preparing for the worst-case scenario of an incursion. Some of the initiatives to be discussed include priming bananas with local microorganisms guided by exploratory microbiome studies, modulating soil changes using organic amendments to increase suppression, application of cloud-based technology to increase training, detection and biosecurity efforts, and first steps towards improving banana resistance using gene editing and nuclear energy.

P8.5-004

INVESTIGATING THE POTENTIAL OF TRICHODERMA ASPERELLUM AGAINST FUSARIUM WILT DISEASE IN BANANA PLANTS

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Text

In order to combat the threat posed by the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*) tropical race-4 (TR4) to the economically important Cavendish banana cultivar, researchers are exploring various integrated pest management (IPM) strategies. These strategies include the use of biological control organisms (BCOs) that can exhibit direct antimicrobial activity or indirectly induce resistance in plants against pathogens. Our research focuses on investigating the direct and indirect activity of the BCO, *Trichoderma asperellum*, against *Foc* TR4 in Cavendish cultivars. Dual culture assays revealed that *T. asperellum* could directly inhibit the growth of the pathogen under *in vitro* conditions. *In planta* bioassays in the greenhouse with multiple applications of *T. asperellum* resulted in a significant decrease in Fusarium wilt disease symptoms. In a separate greenhouse experiment, we also evaluated the effect of *T. asperellum* on banana plant growth. Results revealed a significant increase in plant growth parameters such as banana pseudostem height and leaf surface area for the *T. asperellum* treated plants compared to the uninoculated control plants. In order to distinguish between the direct and indirect activity of the BCO against the pathogen, a split root assay is currently being performed.

P8.5-005

CURRENT STATUS OF BANANA BUNCHY TOP DISEASE IN INDONESIA AND ITS ALTERNATIVE CONTROL STRATEGY

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Text

Indonesia is known as one of the centers of origin of banana (*Musa* spp.). Various banana cultivars with different genome types are grown across the country to meet domestic needs and for export purposes. Banana bunchy top disease (BBTD) is an important disease that has the potential to effect banana production in Indonesia. Field surveys conducted in recent years indicated that most of the banana cultivars are susceptible to BBTD. Several wild banana species native to Indonesia are moderately resistant to BBTD. One of the strategies to manage BBTD is to increase the resistance of banana cultivars to BBTD through the provision of priming agents. Two separate experiments were conducted to evaluate the effectiveness of the priming agents, i.e. (1) liquid smoke treatment on in vitro banana plantlets and (2) application of *Pseudomonas fluorescen*s and guano filtrate. The application of liquid smoke was given at several concentration levels and time during *in vitro* propagation. Liquid smoke treatment had a significant effect on the number of shoot multiplication and growth of banana plantlets; and it reduced disease incidence. *P. fluorescens* and guano filtrate was able to significantly reduce the intensity of the disease only when applied before BBTV infection occurred. Treatment of liquid smoke on banana tissue culture and the use of beneficial microbes can be recommended as part of BBTD control strategy.

P8.5-006

THE VULNERABILITY OF THE CUBAN BANANA PRODUCTION TO FUSARIUM WILT CAUSED BY TROPICAL RACE 4

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Text

Bananas are major agricultural commodities in Cuba. One of the main constraints of banana production worldwide is Fusarium wilt of banana (FWB). Recent outbreaks in Colombia, Perú and Venezuela raised widespread concern in Latin America due the potential devastating impact on the sustainability of banana production, food security and livelihoods of millions of people in the region. Here, we phenotyped 18 important Cuban banana and plantain varieties with two *Fusarium* strains; Tropical Race 4 (TR4, aka *F. odoratissimum*) and Race 1 (R1, aka *F. oxysporum*) under greenhouse conditions. These varieties represent more than 70% of the total Cuban area planted to bananas and are also widely distributed in Latin America and Caribbean region. A broad range of disease responses from resistant to very susceptible was observed against Race 1. Contrary, not a single banana variety was resistant to TR4. These results underscore that TR4 potentially threatens nearly 56% of contemporary Cuban banana production area, which is planted with susceptible and very susceptible varieties, and call for a preemptive evaluation of new varieties obtained in the national breeding program and the strengthening of quarantine measures to prevent the introduction of TR4 into the country.

P8.5-008

ANAEROBIC SOIL DISINFESTATION FOR FUSARIUM WILT DISEASE CONTROL IN BANANA

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Text

Fusarium wilt of bananas caused by the soil-born pathogen, *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4), has been causing a severe problem in banana-producing countries. In most cases, the suffering field is abandoned once the disease occurs because of no control method.
To control the disease, we applied anaerobic soil disinfestation (ASD) using low-concentration EtOH in the Philippines. We prepared nit mutant of TR4 as a marker strain to

utilize selective detection and adjusted soil to 9 x 10^7 cfu/g. The soil was then packed inside mesh sachets and was buried under 15 and 30-cm depths (0.5 x 1 m/plot). One % and 0.5% EtOH were douched to the soil as a carbon source to activate microorganisms and covered with plastic sheets to make an anaerobic environment. Water and non-treated (dry) plots were prepared as controls. Three sachets were set at each depth in plots and repeated twice.
TR4 was not detected on selective media (MMCPA) from the 1.0 and 0.5% EtOH plots. While at the water plot, 1x10^2-4.3 x10^3 and 4 x10^2-2.6 x10^3 cfu/g of TR4 were detected at 15 and 30-cm depths, respectively. At the dry plots, 2 x 10^3-6.3 x 10^4 and 4 x 10^3-2.8 x 10^4 cfu/g of TR4 were detected at 15 and 30-cm depths, respectively. The oxidation-reduction potential at 10 cm depth at 1.0 and 0.5% EtOH plots, water, and dry plots decreased to -485, 11, 42, and, 137 mv, respectively. The results indicate that applying ASD using 1.0% and 0.5% EtOH effectively reduced TR4 in the soil.

P8.5-009

FUSARIUM MUSAE, A PATHOGEN CROSSING “BORDERS”

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Text
Fusarium musae is an agent of crown rot in banana fruits and was recently isolated as cause of keratitis and nail infection as well as cause of systemic infections in immunocompromised patients. It is a sister species to *F. verticillioides*. To confirm the hypothesis that *F. musae* is a cross kingdom pathogen, strains of *F. musae* (n=19), isolated worldwide from banana fruits and human patients were characterised in comparative analyses. Plant and human strains did not group according to their host origin by morphological, nuclear and mitochondrial molecular analyses. *In vitro* sensitivity to azoles widely used in agricultural and clinical settings showed that *F. musae* is less sensitive than its “sister species” *F. verticillioides*. No significant differences were observed between human and banana strains. *In vivo* infection on banana fruits and *Galleria mellonella* (as “human proxy”) demonstrated that both, banana and human strains, are able to invade both pathosystems causing comparable levels of infection. Complete genomes of 2 representative strains were assembled on chromosomal level, and these strains were used to generate fluorescent and luminescent *F. musae* reporter strains that will be used to study the interaction with both hosts. A survey from bananas sold on local market confirmed that *F. musae* can be isolated from fruit shipped from different producing countries. Our work proved that *F. musae* is a cross-kingdom pathogen likely originating in agricultural settings.

P8.5-010

DIVERSITY OF FUSARIUM SPECIES ASSOCIATED WITH THE CAVENDISH BANANA WILT DISEASE IN DAVAO REGION, MINDANAO ISLAND, PHILIPPINES

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Text

The Philippines is the main exporter of banana from the Asian region. However, several plantations in Mindanao are affected by the problem of wilting disease, particularly in Davao, the largest banana producing region in the country. The epidemic predisposition of *Fusarium oxysporum* f. sp. *cubense* TR4 which causes Panama wilt in Cavendish banana cultivars has led this race to be widely studied particularly aimed at managing the disease. This idea delimits the banana wilting problem to be attributed solely to the race belonging to *F. oxysporum* species complex, which is a problem in the disease management. In this study, the taxonomic characteristics, phylogenetic position, and pathogenic traits of the *Fusarium* isolates collected from wilted Cavendish banana were analyzed. The isolates were collected from different production areas in Davao City, Davao De Oro, and Davao Del Norte. Phylogenetic analyses using trees based from the internal transcribed spacer 5.8S rRNA, translation elongation factor-1alpha, and the RNA polymerase II second largest subunit show that the *Fusarium* isolates fall into the *F. dimerum, F. incarnatum-equiseti, F. fujikuroi, F. oxysporum* and *F. solani* species complexes. The morphology of the isolates was further characterized and selected isolates from each species complex were tested for their pathogenicity to the Dwarf Cavendish banana cultivar following Koch's postulates.

P8.5-011

EMERGENCY RESPONSE ACTION TO CONTAIN THE BANANA BUNCHY TOP VIRUS OUTBREAK IN EAST AFRICA

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Text

A severe outbreak of banana bunchy top virus (BBTV), responsible for the bunchy top disease of bananas, was reported in Tanzania and Uganda in 2021. BBTV spreads through infected planting material and by the aphid Pentalonia nigronervosa, which is widespread in all banana production areas. Uganda and Tanzania are leading producers and together contribute over 22% of all bananas grown in Africa. The first outbreak in Tanzania was noticed in the Kigoma region. Surveys in 2021-22 revealed that the virus had reached

several districts in Tanzania, including Dar es Salaam, Kilimanjaro, Mwanza, Pwani, and Rukwa. The virus was first reported in Uganda in Arua City, West Nile. Delimiting surveys conducted in October 2022 revealed widespread BBTV in the West Nile region and some other districts in Western Uganda. In many farms, virus infection led to a 90-100% production loss, a 100% to 150% increase in banana price, and a loss of jobs, income, and banana biodiversity. The drivers of BBTV spread include the lack of awareness that impeded early detection, lack of capacity for surveillance, diagnostics, eradication, and access to virus-free planting materials. This presentation will appraise the status of BBTV in East Africa and highlight steps taken to implement a response plan, including efforts to build a regional alliance to coordinate surveillance and early detection and implement actions to curb virus spread and recover banana production.

P8.5-012

EVALUATION OF THE BIOCONTROL CAPACITY OF NATIVE MICROORGANISMS AGAINST FUSARIUM OXYSPORUM F. SP. CUBENSE

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Text

Fusarium Wilt is an economically important disease of bananas caused by the fungus Fusarium oxysporum f. sp. cubense (Foc). It causes severe losses in the yield and quality of bananas and is extremely difficult to control conventionally using chemical fungicide. Biological control offers an eco-friendly alternative for sustainable plant disease management. In this context, the objective of this research is the determination of the biocontrol capacity of native microorganisms against Fusarium oxysporum f. sp. cubense. For the isolation of native rhizospheric microorganisms, samplings were carried out in the region of Perené and Satipo in the central jungle of Peru. Thirty rhizobacterial isolates were screened for antagonistic activity in dual culture, and isolate 27 showed the highest antagonistic activity (81,52% mycelial growth inhibition) against Foc. The metabolites of isolate 27 inhibited mycelial growth of Foc by 80%. Based on the morphological, physiological and phylogeny analysis with 16S rRNA sequence the isolate 27 was identified as Burkholderia sp. This is a preliminary study of the SATREPS project (Japan - Peru): "The Project on establishment of an alert system for Fusarium oxysporum f. sp. cubense the banana and plantain wilt pathogen, and biological mitigation strategy of the pathogen"

P8.5-013

A POLYPHASIC APPROACH REVEALS NOVEL GENOTYPES AND UPDATES THE GENETIC STRUCTURE OF THE BANANA FUSARIUM WILT PATHOGEN

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Text

Fusarium oxysporum f. sp. *cubense* (Foc) is a soil-borne fungus that causes Fusarium wilt, a destructive plant disease that has resulted in devastating economic losses to banana production worldwide. The fungus has a complex evolutionary history and taxonomic repute and consists of three pathogenic races and at least 24 vegetative compatibility groups (VCGs). Surveys conducted in Asia, Africa, the Sultanate of Oman and Mauritius encountered isolates of *F. oxysporum* pathogenic to banana that were not compatible to any of the known Foc VCGs. Genetic relatedness between the undescribed and known Foc VCGs were determined using a multi-gene phylogeny and diversity array technology (DArT) sequencing. The presence of putative effector genes, the *secreted in xylem* (*SIX*) genes, were also determined. Fourteen novel Foc VCGs and 17 single-member VCGs were identified. The multi-gene tree was congruent with the DArT-seq phylogeny and divided the novel VCGs into three clades. Clustering analysis of the DArT-seq data supported the separation of Foc isolates into eight distinct clusters, with the suite of *SIX* genes mostly conserved within these clusters. Results from this study indicates that Foc is more diverse than hitherto assumed.

P8.5-014

ADVANCES IN PLANT VIRUS DISEASE MANAGEMENT IN SUB-SAHARAN AFRICA – THE CASE OF BUNCH TOP DISEASE

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Text

Plant viruses have caused several devastating epidemics on annual and perennial crops in sub-Saharan Africa (SSA). All the effectively managed virus diseases in SSA relied on host-plant resistance. For viruses lacking durable host resistance, management relies on integrated methods (vector control, clean seed, habitat management, etc.) that are less effective, especially in preventing the transboundary spread of viruses due to weak phytosanitary capacity. This presentation discusses the case of emergence and spread of the banana bunchy top virus (BBTV) responsible for the devastating bunchy top disease for which durable host resistance is unavailable. First detected in the 1960s in the Democratic Republic of Congo, BBTV spread to 16 countries between 1990 and 2022. The virus transmitted by an aphid, *Pentalonia nigronervosa*, and vegetative propagation is increasingly becoming a serious threat due to the expansion into new regions, more recently to Tanzania and Uganda. BBTV management depends on preventing virus spread, eradicating infected plants, and replanting with virus-free planting materials. We will highlight the progress and status of the recent advances to control BBTV in SSA, including the search for host-plant resistance for BBTV and its vector, surveillance using remote sensing and machine learning methods, RPA-based rapid diagnostics, biocontrol for aphids, and ALLIANCE model for coordinated control of BBTV in the continent.

P8.5-015

THE NEW PROPOSAL FOR A DIAGRAMMATIC SCALE TO BLACK LEAF STREAK DISEASE ASSESSMENT FOR BANANA

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Text

Black sigatoka or black leaf streak (BLS) is the most important foliar disease of Musa production and is caused by Pseudocercospora fijiensis. The use of a diagrammatic scale helps people more effectively evaluate severity and improves disease measurement by evaluators. Our goal was to develop a new scale with colored pictures and seven disease levels 0 (0%), 1 (0.1 – 5.0%), 2 (5.01 – 13.0%), 3 (13.01 – 23.0 %), 4 (23.01 – 40.0%), 5 (40.01 – 65.0%) 6 (>65.0%) and then compare the severity results of BLS on banana leaves with those based on other scale. Three evaluations were performed by thirteen different evaluators and the evaluation was performed at seven-day intervals. The first assessment was performed without a scale. Raters performed four assessments with each of the two diagrammatic scales. We analyzed the statistics with linear regression and Lin's concordance correlation. The evaluators using the proposed scale improved the precision, accuracy and reproducibility of the evaluations and reduced residual distribution when compared to the evaluators who did not use the proposed diagrammatic scale or who used the other scale. Overall, the proposed diagrammatic scale is a tool that can assist users in producing a disease estimate close to the real value of BLS on banana leaves. Keywords: Banana, Pseudocercospora fijiensis, Diagrammatic Scale, Precision, Accuracy

CRISPR crops: plant Genome Editing Toward Disease Resistance

C9.1-1

RESISTANCE TO VIRUSES BASED ON EIF4E: FROM NATURAL VARIATION TO EDITED GENES

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Text

eIF4E translation initiation factors have emerged as major susceptibility factors for RNA viruses. Natural eIF4E-based resistance alleles are found in many species and are mostly variants that maintain the translation function of the protein. eIF4E genes represent therefore targets for engineering viral resistance, and gene-editing technologies can be used to make up for the lack of natural resistance alleles in some crops. However, redundancy among eIF4E genes can restrict the efficient use of knockout alleles in breeding. Using Arabidopsis,

we previously showed how gene-editing technologies can be used to design de novo functional alleles, using knowledge about the natural evolution of eIF4E genes in different species, to drive resistance to viruses without affecting plant physiology. Here, we will also present how this knowledge can be applied to a crop, tomato, using CRISPR-Cas9 base editing. We will show that there is a trade-off to find between resistance and functionality, and discuss these results in the light of resistance durability.

C9.1-2

GENOME EDITING OF A RICE CDP-DAG SYNTHASE CONFERS BROAD-SPECTRUM DISEASE RESISTANCE

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Text

The discovery and application of genome editing introduce a new era of plant breeding, giving researchers efficient tools for the precise engineering of crop genomes1. Here, we demonstrate the power of genome editing for engineering broad-spectrum disease resistance in rice (Oryza sativa). We first isolated a lesion mimic mutant (LMM) from a mutagenized rice population, demonstrated that a 29-bp deletion in a gene we named RESISTANCE TO BLAST1 (RBL1) caused this phenotype and showed that this mutation caused a ca. 20-fold reduction in yield. RBL1 encodes a cytidine diphosphate diacylglycerol (CDP-DAG) synthase required for phospholipid biosynthesis2. Mutation of RBL1 results in reduced levels of phosphatidylinositol (PI) and its derivative PI(4,5)P2. Rice PI(4,5)P2 is enriched in cellular structures specifically associated with effector secretion and fungal infection, suggesting a role as a disease susceptibility factor3. Using targeted mutagenesis, we obtained an allele of RBL1, named RBL12, which confers broad-spectrum resistance but does not decrease yield in a model rice variety as assessed in small-scale field trials. Because RBL1 is conserved in plants, editing of RBL1 homologs is likely applicable to diverse crops.

C9.1-3

APPLICATIONS OF GENE EDITING FOR HIGH-THROUGHPUT GENE FUNCTION DISCOVERY AND DISEASE RESISTANCE IMPROVEMENT IN RICE

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Text

Gene editing via the CRISPR/Cas9 system is revolutionizing plant research and crop breeding. Here, we present an effective and streamlined pipeline for arrayed CRISPR library construction in plants. This pipeline introduces artificial PCR fragment-length markers for distinguishing guide RNAs (gRNAs) (named FLASH tags), and a group of 12 constructs harboring different FLASH tags are cotransformed into plants each time. Therefore, the identities of gRNAs in *Agrobacterium* mixtures and transgenic plants can be easily read out by detecting the FLASH tags, which only requires conventional PCR and gel electrophoresis rather than sequencing. We generated an arrayed CRISPR library targeting all 1,072 *receptor-like kinases* (*RLK*s) in rice using this pipeline, which resulted in a mutant population covering gRNAs targeting 955 RLKs. Our results indicate that the FLASH tags *bona fide* surrogate the gRNAs and tightly (92.1%) associate with frameshift mutations of intended target genes. Owing to their high editing efficiencies, these CRISPR lines enable fast identification of defense-related RLK genes. Additionally, we used *OsCPK18* (Calcium-dependent protein kinase) as an example that editing the phosphorylation motif can simultaneously improve rice disease resistance and yield. Together, we present a novel pipeline for arrayed CRISPR screening in plants and propose a gene editing strategy to improve rice disease resistance without yield tradeoff.

C9.1-4

TAPPING INTO PLANT IMMUNE PRIMING BY GENOME EDITING-TOWARDS AGRICULTURALLY IMPROVED CROPS

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Text

Pathogens are the cause of devastating diseases and significant tomato crop losses worldwide. While resistance traits exist in wild tomato populations, transferal of this resistance to cultivated tomato has thus far had limited success. A well-known concept in the world of plant immunity is that of induced resistance (IR). We propose that tapping into plant immune priming by genetically manipulating plant immunity, generating constitutively immuno-activated plants, can result in increased pathogen resistance. Here we present two examples of constitutively primed plants that we obtained using CRISPR-Cas9 genome editing of defense receptors. In the first case, we knocked out an immune-decoy receptor, LeEIX1. LeEIX1 normally attenuates the plants' response to the Trichoderma elicitor EIX (Ethylene Inducing Xylanase). LeEix1 knockout plants have amplified systemic immunity, are more responsive to Trichoderma, and are more resistant to pathogens. In the second case, we constitutively activated an intra-cellular immunity-signaling hub by generating gain-of-function mutants in NRC family NLR receptors. NRC mutants also exhibited enhanced defense and biotic resistance. All our edited lines possess broad-spectrum resistance to plant pathogens without impairing fertility or agricultural traits, indicating this is a promising environmentally friendly approach for combating plant disease, which may be superior to priming, leading to better reproducibility and agricultural performance.

C9.1-5

GENOME EDITING OF AN AFRICAN ELITE RICE VARIETY CONFERS RESISTANCE AGAINST ENDEMIC AND EMERGING XANTHOMONAS ORYZAE PV. ORYZAE STRAINS

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Text

Bacterial leaf blight (BB) of rice, caused by Xanthomonas oryzae pv. oryzae (Xoo), threatens global food security and the livelihood of small-scale rice producers. Analyses of Xoo collections from Asia, Africa and the Americas demonstrated complete continental segregation, despite robust global rice trade. Here, we report unprecedented BB outbreaks in Tanzania. The causative strains, unlike endemic African Xoo, carry Asian-type TAL effectors targeting the sucrose transporter SWEET11a and iTALes suppressing Xa1. Phylogenomics clustered these strains with Xoo from Southern-China. African rice varieties do not carry effective resistance. To protect African rice production against this emerging threat, we developed a hybrid CRISPR-Cas9/Cpf1 system to edit all known TALe-binding elements in three SWEET promoters of the East African elite variety Komboka. The edited lines show broad-spectrum resistance against Asian and African strains of Xoo, including strains recently discovered in Tanzania. The strategy could help to protect global rice crops from BB pandemics.

C9.1-6

DISEASE RESISTANT GM AND GE CROPS: A REGULATORY TRIP AROUND THE WORLD

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Text

Disease resistance has become one of the most important and desirable traits across all cultivated crops, for conventionally-bred, as well as transgenic (GM) and gene-edited (GE) crops. For over two decades, the largest cultivated transgenic crops focused on insect and herbicide resistance. Disease resistance presents a more complex task due to the sheer diversity of pathogens and infection mechanisms. Genome editing offers a precise, efficient, and affordable technique resulting in new GE crops entering regulatory schemes for commercialisation. We aver that the law is not static and must adjust to the *mores* of society, informed by the experiences of over 25 years of cultivation and regulation of GM crops. This work consolidates the global legislative landscape on GM crops, as well as specifically addressing how GE crops fit into the existing frameworks. Our presentation aims to highlight key regulatory developments that may impact the future of GE research and ultimately, GE products. To this end, countries leading in cultivating and exporting traditional GM crops are quickly adopting disease resistant GE products as non-GMO. This legislative development,

first implemented in Argentina, and soon followed by many, demonstrates considerable shifts in the landscape of agrobiotech products. We further illustrate the complex legal frameworks using specific examples of disease challenges such as potato late blight, and possible solutions.

P9.1-001

CONTRIBUTION OF DISEASE RESISTANT CROPS DERIVED FROM GENE EDITING AND CISGENESIS TO EU SUSTAINABILITY OBJECTIVES

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Text

The European Commission aims to reducing the use and risk of chemical pesticides by 50 percent and the use of more hazardous pesticides by 50 percent, by 2030. The use of varieties with improved biotic resistances or tolerances is a key tool to achieve these objectives. Work on a new proposal for legislation addressing crops obtained by targeted mutagenesis and cisgenesis is ongoing in the EU. Here, we present a market outlook for applications of targeted mutagenesis and cisgenesis on disease-resistant crops using a database compiled recently (1). The database comprises 113 entries of projects on disease resistances, mostly using targeted nucleases (67% CRISPR/Cas, 7% TALEN, 2.5% ZFN) . We also look in detail at 2 case studies and develop a methodology for estimating the farm level impacts (economic and pesticide use impacts) , the market impacts and the spatial environmental impacts of cultivating these varieties. The case studies selected are potato varieties with stacked resistance genes for late blight via cisgenesis and an apple variety with resistance to scab (2).

(1) Parisi, C. and Rodriguez Cerezo, E., Current and future market applications of new genomic techniques, EUR 30589 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-30206-3, doi:10.2760/02472, JRC123830.
(2) Schneider et al (2023). Economic and environmental impacts of disease resistant crops developed with cisgenesis (in preparation).

P9.1-002

TARGETED AND UNTARGETED EPIGENETIC MODIFICATIONS TO CONTROL PLANT PATHOGENS

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Text

Plants use epigenetics to regulate gene expression during development and environmental stress to achieve plasticity and rapid adaptation.
For a successful infection, pathogens must interact with host factors termed susceptibility factors, and these susceptibility factors are a valuable source of obtaining resistant plants.

We used epigenetics to silence susceptibility genes for different viruses and powdery mildew in Arabidopsis and tobacco plants, respectively. We used virus-induced gene silencing (VIGS) and the SunTag system coupled with the Nicotiana tabacum DRM methyltransferase catalytic domain [1] to stably methylate the promoter of selected host genes representing susceptibility factors. We investigated the mobilization of transposons as a source of phenotypic variation for pathogen resistance screening using epigenetics [2].
Our results show that epigenetics modifications can successfully downregulate the expression of the susceptibility factors providing resistance. Even in the absence of the transgene, the next generation of plants inherited the epigenetic effects.

References
[1] Papikian, et al. 2019. Site-specific manipulation of Arabidopsis loci using CRISPR-Cas9 SunTag systems. Nat Commun 10, 729. doi: 10.1038/s41467-019-08736-7
[2] Thieme et al. 2017. Inhibition of RNA polymerase II allows controlled mobilisation of retrotransposons for plant breeding. Genome Biol 2017;18:134. doi:10.1186/s13059-017-1265-4

P9.1-003

EDITING LADY FINGER BANANAS FOR STR4 RESISTANCE USING CRISPR-CAS9

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Text
Fusarium wilt is a devastating fungal disease that continues to threaten the production of banana crops world-wide. *Fusarium oxysporum* f. sp. *cubense* subtropical race 4 (STR4) is a variant that infects banana plants predisposed to environmental stressors such as cool temperatures and drought. Many commercially and locally important cultivars in subtropical regions, including Lady finger, are susceptible to the pathogen, causing significant loss to farmers and industry. Management of Fusarium wilt relies on strict biosecurity regulations as no control measures are currently available. The discovery of plant-derived disease susceptibility genes has potentially created new opportunities for engineered plant disease management. Identification of fungal susceptibility genes in banana and development of gene-edited bananas with resistance to STR4 would future-proof this important crop against the pathogen. In this present study, we have identified four susceptibility gene targets in Lady finger through differential expression analysis and edited these in Lady finger bananas using CRISPR-Cas9. An average of four lines per gene target have been characterised for edits. An optimised STR4 bioassay will challenge edited lines to assess whether deletion of these susceptibility genes confers resistance to STR4.

P9.1-004

AN ITERATIVE GENE-EDITING STRATEGY BROADENS EIF4E1 GENETIC DIVERSITY IN SOLANUM LYCOPERSICUM AND GENERATES RESISTANCE TO MULTIPLE POTYVIRUS ISOLATES

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Text

Resistance to potyviruses in plants have been largely provided by the selection of natural variant alleles of *eukaryotic translation initiation factors (eIF) 4E* in many crops. However, the sources of such variability for breeding can be limited for certain crop species, while new virus strains continue to emerge. Different methods of mutagenesis have been applied to inactivate the *eIF4E* genes to generate virus resistance, but with limited success due to the physiological importance of translation factors and their redundancy. Here, we employed genome editing approaches at the base level to induce nonsynonymous mutations in the *eIF4E1* gene and create genetic diversity in cherry tomato (*Solanum lycopersicum* var. *cerasiforme*). We sequentially edited the genomic sequences coding for two regions of eIF4E1, located around the cap-binding pocket and known to be important for susceptibility to potyviruses. We show that the editing of only one region, by gene knock-in and base-editing, respectively, is not sufficient to provide resistance. However, combining amino acid mutations in both regions resulted in resistance to multiple potyviruses without costing its functionality in translation initiation. Altogether, our work demonstrates that precision editing allows to design plant factors based on the knowledge on the evolutionarily selected alleles and enlarge the gene pool to potentially provide advantageous phenotypes such as pathogen resistance.

P9.1-005

SALICYLIC ACID IS REQUIRED FOR BROAD-SPECTRUM DISEASE RESISTANCE IN RICE

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Text

Rice plants contain high basal levels of salicylic acid (SA), but some of its functions remain elusive. Here, we characterized four rice SA hydroxylase (OsSAH) genes and verified their roles in disease resistance. Knockout OsSAH (sahKO) genes conferred enhanced resistance to both hemibiotrophic and necrotrophic pathogens, whereas overexpression of each OsSAH gene increased susceptibility to the pathogens. sahKO mutants showed increased SA and jasmonate, and decreased indole-3-acetic acid levels compared to those of the wild-type and OsSAH-overexpressing plants. Analysis of OsSAH1 and OsSAH3 promoters indicated that the induction of these genes was mainly restricted around Magnaporthe oryzae infection sites. Recombinant OsSAH3 protein showed only SA 5-hydroxylase (SA5H) activity, which was remarkably higher than that of other OsSAHs that presented both SA3H and SA5H activities. Amino acid substitutions revealed that three amino acids in the binding pocket affected enzyme activity and/or specificity. Taken together, our findings indicate that SA plays a vital role in immune signaling. Moreover, fine-tuning SA homeostasis through suppression of SA metabolism provides an effective approach to study broad-spectrum

disease resistance in rice.

P9.1-006

THE IN-FRAME-DELETION ALLELE-ENCODED EIF4E PROTEIN CONFERS RESISTANCE TO CUCUMBER MOSAIC VIRUS BY INHIBITING 2B IN TOMATO

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Text

Both the host organisms and the infecting viruses rely on the eukaryotic translation initiation factor 4E (eIF4E) for gene expression. Plants utilize the genes from the eIF4E family to develop antiviral resistance. The mechanisms of resistance previously studied are attributed to the lack of proper interactions of the resistant eIF4E protein with viral proteins, hindering the ability of targeted viruses to infect. Our research has demonstrated an alternative mode of eIF4E-mediated resistance. We used CRISPR/Cas9 to create tomato eIF4E1 alleles, including one with an in-frame 9-nucleotide deletion (9DEL) within the eIF4E1 coding sequence. Unlike a 1-nucleotide insertion knockout allele, we showed that the 9DEL deletion was associated with a significant reduction in susceptibility to cucumber mosaic virus (CMV). Here, we present new data shedding light on the molecular mechanism behind the 9DEL-mediated resistance to CMV. The 9DEL-encoded mutant eIF4E1 was found to bind to the CMV-encoded protein 2b through a strong affinity to the C-terminal of 2b. We have previously reported that 2b is a target of autophagy, a primary proteolytic pathway. Our results suggest that the 9DEL appears to enhance the 2b degradation. Consistently, the RNA silencing suppressor activity of 2b was weakened when co-expressed with 9DEL. Since 2b was essential for systemic CMV infection in tomato, we propose that the 9DEL mutation confers resistance to CMV on tomatoes by binding to and inhibiting 2b.

P9.1-007

EMPLOYING MOLECULAR TECHNIQUES TO CONFER RESISTANCE AGAINST PHYTOPHTHORA INFESTANS IN POTATO

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Text

Phytophthora infestans (*Pi*) is an Oomycyte pathogen known to cause blight in potato. Late blight is considered the most serious disease of potatoes because it can destroy the entire

crop in a span of days. Therefore, molecular studies might help pave the way for sustainable *Pi* resistance. miRNAs are known to actively regulate physiological processes as well as biotic and abiotic stress tolerance. The *miR396* gene in potato is known to promote the colonization of *Pi*. In addition, reduced invasiveness has been reported on tubers with downregulated polyphenol oxidase (PPO) genes when subjected to *Pi*, associating the defense to phenolic compounds.
Based on the strong literature evidence, we designed CRISPR/Cas targets to the *miR396* gene as well as for root- and tuber-specific *PPO* genes in potato and generated edited lines in the commercial cultivars 'Botond' for miR396, and 'Desirée' and 'Balatoni Rózsa' for *PPO* genes. The leaves of three *miR396* complete mutant lines along with the control are being evaluated for *Pi* resistance. Metabolomics analysis of the mutant PPO lines indicate higher levels of jasmonic acid and salicylic acid both in the leaves and roots compared to the control lines. This increase has been suggested to aid in conferring resistance.
The project has been supported by grants NKFIH K-132829 and ATK-MATE 0205K0036P.

P9.1-008

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF A UDP-ARABINOPYRANOSE MUTASES GENE TAUAM1 IN WHEAT

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Text
UDP-arabinopyranose mutases family is a key enzyme in arabinose biosynthesis and plays an important role in plant morphogenesis and response to stress. However, little work has been reported on UDP-arabinopyranose mutases in wheat. In the present, a full length cDNA of a UDP-arabinopyranose mutases gene (named TaUAM1) was isolated from wheat. The sequence analysis results showed that the full length of the open reading frame of TaUAM1 was 1107 bp encoding 368 amino acids. RNAi-based stable silencing of TaUAM1 resulted in decreased resistance to Pst. In addition, CRISPR-mediated genome editing (GE) of TaUAM1 enhanced susceptibility of wheat to Pst or compromised disease resistance accompanied by increased fungal growth and decreased H2O2 production in plant tissues. Moreover, the transcript levels of pathogenesis-related (PR) genes and ROS-generating genes were down-regulated in both the RNAi and CRISPR-edited plants, while the ROS-scavenging gene was up-regulated. Therefore, TaUAM1 positively regulates the resistance of wheat to Pst.

P9.1-009

GENERATION OF RYMV-RESISTANT ORYZA SATIVA LINES BY EDITION OF SUSCEPTIBILITY FACTORS

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Text

Rice yellow mottle virus (RYMV) causes one of the most devastating rice diseases in Africa. Genetic resistance is the most effective control strategy. However most of the known resistance genes originate from the African cultivated rice *Oryza glaberrima* and their transfer to *O. sativa*, high yielding and world-grown, is hampered by interspecific sterility barriers. We tested wether CRISPR/Cas9 genome editing of the susceptibility genes *RYMV1* and *RYMV2*, encoding a translation initiation factor (OseIF(iso)4G.1) and a nucleoporine (OsCPR5.1) respectively, is effective in generating resistance alleles in the *O. sativa* Kitaake variety. We developed lines with knock-out mutations or in-frame deletions or substitutions in *RYMV1*, *RYMV2* and their paralogs and we analysed them for resistance, resistance durability and possible growth defect. Our results indicated that genome editing is a promising way to develop RYMV-resistant elite *O. sativa* lines and that some mutations may even increase resistance durability compared to the alleles found in the natural diversity. Besides, the comparison of the effect of the mutations obtained in the resistance genes and their paralogs will help understanding the specificity of the interactions between the virus and the host susceptibility factors.

P9.1-010

DEVELOPMENT OF BROAD-SPECTRUM RESISTANCE BY INTROGRESSION OF SEXTUPLE SWEET EBE MUTATIONS AND MAJOR RESISTANCE GENES IN AFRICAN RICE CULTIVARS

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Text

Bacterial leaf blight (BLB) of rice caused by Xanthomonas oryzae pv. oryzae (Xoo) is one of the major diseases affecting rice cultivation in Africa and Asia. BB causes substantial yield loss in rice. Xoo injects transcription activator-like effectors (TALes), which bind effector binding elements (EBE) in the promoters of the sucrose uniporter genes OsSWEET11a, OsSWEET13 and OsSWEET14, likely triggering sucrose efflux into the xylem sap. Six major SWEET-inducing TAL effectors have been identified: PthXo1 for OsSWEET11a, PthXo2 for OsSWEET13, PthXo3, AvrXa7, TalF and TalC for OsSWEET14. Elite lines with CRISPR-Cas9-induced EBE mutations in five EBEs were highly resistant to a wide range of Xoo strains. In countries with appropriate regulations, edited rice lines (SDN-1) that do not carry transgenes can possibly be introduced to protect from BLB. However, for countries without suitable regulations, an alternative approach is required. Here we introgressed a combination of six EBE mutations in the promoters of OsSWEET11a, OsSWEET13 and OsSWEET14 into FARO-44 and NERICA-4, and two major BLB resistance genes xa13 and Xa21 into Komboka, FARO-44 and NERICA-4 through marker assisted backcross breeding, respectively. Independent crosses were performed between donors and recipients to generate F1 generations. Derived F1 were confirmed by genotyping and sequencing.

Generation of stable homozygous BC2F4 and BC3F4 plants with broad spectrum resistance against BLB is under progress.

P9.1-011

GENOME EDITING OF BANANA FOR RESISTANCE TO BACTERIAL WILT DISEASE

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Text

Banana is an important staple food crop and a source of income for resource-poor farmers in more than 136 tropical countries. Many diseases and pests severely constrain banana production, particularly where many pathogens co-exist. Banana Xanthomonas wilt (BXW) caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) is among the most destructive banana diseases in East Africa. All the cultivated banana varieties are susceptible, and only the wild-type progenitor, *Musa balbisiana,* is resistant to BXW disease. Disease-resistant varieties are one of the most effective strategies for managing banana diseases. CRISPR/Cas9-based genome editing can accelerate the breeding of bananas for disease resistance traits. IITA is currently advancing the application of genome editing to control BXW disease by disrupting the function of disease-causing susceptibility ('S') genes, nutrient transporters, or negative regulators of plant defense. The target genes have been identified through literature or comparative transcriptomics of BXW-resistant wild progenitor banana 'Musa balbisiana' and BXW-susceptible banana cultivar during early infection with Xcm. Genome-edited crops can be released to the market without going through the same time-consuming regulatory process required for transgenic crops in several countries. This paper will present a synopsis of recent advancements in the application of genome editing to improve bananas for BXW disease resistance.

P9.1-013

GENETIC EDITING OF CML GENES IN POTATO SOLANUM TUBEROSUM

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Text

The vast array of tools to generate or stimulate plant resistance to biotic stresses, such as bacteria, fungi, and viruses, is an important means for genetic improvement. However, using these tools in an open biogeocoenosis may brake the natural interconnections between consumer, producer and reducer organisms. Therefore, it is preferable to induce or enhance the defence mechanisms in the crop plants. Such measures may include the (increased)

expression or knockout of certain plant genes.
We selected as candidates members of the CML (calmodulin-like) gene family. These gene may act both as positive and negative regulators of plant defence. Knockouts of CML genes are predicted to reveal their specific physiological roles and may also lead to agronomically advantageous mutant phenotypes.
A literature review and bioinformatics analysis were performed to find target genes in potato for knockout by the CRISPR/Cas system. Two genes were found, the sequence of which was identified as potential targets. Two plasmid vectors were then constructd and transferred into the potato varieties 'Désirée'. At the present stage, mutant lines of potatoes were obtained, in which the knockout of the CML30 gene was proven. Currently, research is being conducted on the resistance of these plants to pathogen Phytophthora infestans.

Current and emerging forest pathology issues

C5.3-1

A GLOBAL ACCOUNT OF THE DOTHISTROMA NEEDLE BLIGHT PATHOGENS AND RISKS POSED BY EMERGING NEW LINEAGES

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Text
Dothistroma needle blight (DNB) is caused by two pathogens; *D. septosporum* and *D. pini*. Questions regarding their origin, global distribution, evolutionary history, pathogenicity and their potential to hybridise are being elucidated through population genetics and genomic comparisons. The only areas where the two species are known to co-occur are Canada and some European countries. Whereas *D. septosporum* has a global distribution, *D. pini* is only present in certain Northern Hemisphere countries and distinct lineages are observed in populations of both species in North America compared to those found in Europe. Recently, genetic comparisons of strains collected from Central America, the area where *Dothistroma* is believed to be native, have revealed novel haplotypes of *D. septosporum* as well as lineages that may represent new *Dothistroma* species. The similarity of some of these strains with isolates collected from recent DNB outbreaks on Central American *Pinus* spp. in Colombia, suggests a possible route of movement out of their native area, similar to that of *Lecanosticta pharomachri*. The presence of these novel *D. septosporum* haplotypes on *P. patula* and *P. maximinoi*, that are both considered as species tolerant to infection, poses a risk to plantation forestry in countries utilising these pine species. Furthermore, this now emerges as an important global forest pathology issue given the fact that their impact on Northern Hemisphere *Pinus* species is unknown.

C5.3-2

IMPORTANCE OF HEALTHY CARRIERS IN ASH DIEBACK

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Text

Ash dieback, induced by an invasive ascomycete, Hymenoscyphus fraxineus, has emerged in the late 1990s as a severe disease threatening ash populations in Europe. Future prospects for ash are improved by the existence of natural genetic resistance / tolerance to the disease and by limited disease impact in many environmental conditions where ash is common. Nevertheless, it was suggested that, even in those conditions, ash trees are infected and enable pathogen transmission. We studied the influence of the environment on the ability of H. fraxineus to infect, be transmitted and cause damage on its host. We showed that healthy carriers, i.e. individuals showing no dieback but carrying H. fraxineus, exist and play a significant role in ash dieback epidemiology. The environment parameters influencing H. fraxineus are different at different life cycle stage. The ability of H. fraxineus to establish on ash leaves and to reproduce on the leaf debris in the litter mainly depended on total precipitation in July-August and was not influenced by local tree cover. By contrast, damage to the host (shoot mortality) was strongly reduced by high summer temperature and by high autumn average temperature. Thus, ash trees are often infected and enable H. fraxineus transmission while showing very limited damage. We also observed a decreasing trend of severity (shoot mortality) with the time of disease presence in a plot that could be significant for the future of ash dieback.

C5.3-3

CURRENT AND EMERGING ISSUES FOR MANAGING PHYTOPHTHORA IN A LANDSCAPE OF RAPID CHANGE

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Text

Phytophthora pathogens are serious threats to forest health around the world. Of the 38 species of Phytophthora currently noted as present in New Zealand, 16 have been found to be present in forests and natural ecosystems with variable impacts on forest health. One of the most devastating pathogens is Phytophthora agathidicida, which threatens Agathis australis (kauri), a foundational tree species of key northern forest systems. In addition to P. agathidicida, pathogens *P. cinnamomi* and *P. multivora* are also prevalent in these forest systems and are commonly associated with dying kauri. Similarly, in exotic pine plantations pathogens *P. pluvialis, P. kernoviae* and *P. aleotoria* all impact productivity either at

establishment or throughout the rotation and can co-occur within trees. Finally, the emergence of *P. podocarpi*, a species pathogenic to native t?tara (*Podocarpus totara*) is putatively associated with changes in climatic conditions. Given the increased intensity of weather events resulting from climate change, the resilience of forest systems to Phytophthora infection is of great concern. This talk will discuss the observed and projected impacts of a changing climate and extreme weather events and implications of Phytophthora on the health of forests and natural ecosystems.

C5.3-4

GENOMIC BIOSURVEILLANCE OF INVASIVE ALIEN TREE PATHOGENS CAN DETECT VARIANTS, HYBRIDS AND REVEAL SUPER-SPREADER EVENTS

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Text

Invasive alien tree pathogens often have complex invasion histories. Understanding the source and pathways of invasion is crucial to improve prevention. Genomic biosurveillance can help untangle the invasion history. The BioSAFE project sequenced and analysed more than 500 genomes of a global collection of the pathogen responsible for the sudden oak death, the sudden larch death and Ramorum blight (*Phytophthora ramorum*). Variants within clonal lineages of *P. ramorum* were often geographically and/or chronologically restricted. We detected a shift in variants of the EU1 and NA2 lineages of *P. ramorum* in nurseries in British Columbia, Canada. One of the EU1 variants replaced all previous variants and spread to many nurseries, a signature of a potential super-spreader event. We also identified interlineage hybrids that are F1 progenies and produce viable sporangia and chlamydospores and are infectious to rhododendron, a common host. Comparison of variant composition in nurseries following treatment revealed both instances of eradication success and failure. For rapid and high-throughput biosurveillance, we have developed a tool, SODseq, that generates high-throughput sequence data for 355 informative amplicons that recapitulate the patterns obtained with whole genome sequencing. This tool can be used with DNA extracted from cultures or directly from environmental samples or infected host tissues and provide useful genomic data that can inform regulation and management.

C5.3-5

LATENT PATHOGENS TRIGGERED BY CLIMATE CHANGE ARE CAUSING LARGE-SCALE TREE DECLINE IN CALIFORNIA

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Text
Large-scale tree mortality is thought to be caused by the emergence of invasive pathogens or by outbreaks of secondary organisms such as insects. Conversely, variability in resilience to climate-driven stressors may limit the scale of mortality driven exclusively by climate change. Until recently, disease caused by latent pathogens, i.e. pathogens that alternate between an endophytic , a pathogenic and a saprobic phase, has been thought to occur only in localized situations of acute stress. Since 2015, large-scale mortality of trees and shrubs, both native and exotic, has been occurring throughout Northern California. Isolations revealed that disease of each of eight tree/shrub species studied was associated with the widespread presence of latent pathogens in the genera *Botryosphaeria*, *Diplodia*, *Neofusicoccum*, *Dothiorella*, *Diaporthe* and *Pseudosysdowia*. Although most of the fungi identified are generalists, different pathogen species were dominant and widespread on different tree species. For unreported pathogen x host combinations, inoculation studies confirmed these fungi can cause disease, although at different rates, and showed that lack of water and increasing temperatures, can lead to higher disease severity. Together, our field and lab data suggest that physiological stress caused by changing climate is triggering true outbreaks of latent pathogens, regarded now as an additional class of agents causing large scale mortality in tree populations at the regional scale.

C5.3-6

EXOGENOUS APPLICATION OF DOUBLE-STRANDED RNA TO CONTROL FUSARIUM CIRCINATUM IN PINES

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Text
Fusarium circinatum is an invasive fungal pathogen that affects coniferous species, resulting in significant socio-economic impact. This fungus can affect all stages of tree development, causing seedling mortality in nurseries and cankers and dieback in adult trees. So far, there is no effective way to control this pathogen and phytosanitary products cannot be applied in forests. SIGS (Spray-Induced Gene Silencing) could be used as an environmentally friendly method to control *F. circinatum*. This strategy is based on RNA interference (RNAi), a conserved eukaryotic gene-silencing mechanism naturally occurring in cells. It is initiated by double-stranded RNA (dsRNA), which triggers the degradation of homologous mRNAs. By applying exogenous dsRNA, the pathogenic fungus uptake the molecules and trigger RNAi. In this study, we show that *F. circinatum* is able to uptake dsRNA and we designed dsRNA molecules targeting essential genes of the pathogen, successfully blocking important pathways for its survival. The application of dsRNAs to pine seedlings resulted in the inhibition of fungal growth and delayed symptoms. Furthermore, the efficacy of the dsRNAs has been tested on the wheat pathogen *Fusarium graminearum*, successfully reducing the infection. Although this strategy is being developed intensively for mainly agricultural pathogens, it has been less investigated in forestry. These results suggest that SIGS could be a sustainable method for disease management in forests.

F5.3-1

CANDIDATE EFFECTOR PROTEINS CONSERVED ACROSS FUNGAL AND OOMYCETE FOLIAR PINE PATHOGENS

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Text

Dothistroma needle blight, Cyclaneusma needle cast and red needle cast are devastating *Pinus radiata* diseases, caused by the fungi *Dothistroma septosporum*, *Cyclaneusma minus* and oomycete *Phytophthora pluvialis*, respectively. These pathogens colonize the apoplastic host environment, secreting effector proteins to promote disease. If these effectors are recognized by corresponding host resistance proteins, they activate the plant immune system to stop pathogen growth. Two *D. septosporum* candidate effectors, Ds69335 and Ds131885, were identified with orthologues in both *C. minus* and *P. pluvialis*. Their protein structures were analysed using AlphaFold2 and the corresponding genes disrupted through CRISPR/Cas9 to study their function during pine infection. Ds69335 is structurally similar to proteins with known roles in fungal virulence and *Ds69335*-disrupted strains showed decreased fungal biomass *in planta* compared to wild-type (WT). Ds131885, a cell death elicitor in *Nicotiana* species and *P. radiata*, showed structural similarity to a cross-kingdom PAMP that was recognized by a *Nicotiana benthamiana* immune receptor, triggering defence responses. Disruption of *Ds131885* did not convincingly alter fungal biomass. Unexpectedly, none of the complementation strains restored WT fungal biomass. Despite these ambiguous results, these candidate cross-kingdom effectors deserve further exploration as they might ultimately hold the key to selection for broad spectrum resistance in pines.

P5.3-001

LAUREL WILT DISEASE MANAGEMENT: CURRENT STRATEGIES AND ONGOING LINES OF RESEARCH

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Text

Laurel wilt (LW), caused by Harringtonia lauricola, is a deadly vascular disease affecting numerous hosts in the Lauraceae, including ecological important forest species and avocado trees. The fungal pathogen is spread by beetle-vectors, through root grafts, and human-mediated transport of infested wood. LW is present in 12 U.S. states and continues to move towards major avocado-producing areas. The destructive nature of this disease, in combination with its multiple hosts and potential vectors, makes it an eminent threat to avocado production worldwide. The potential loss could be devastating, as LW has led to the

destruction of at least 300,000 fruit bearing trees in Florida alone. In forested ecosystems, LW has decimated entire populations of dominant and ecologically important tree species likely changing ecosystem functioning and compromising important reservoirs of biodiversity. Current management strategies are limited and rely on the implementation of cultural practices, such as sanitation and pruning (light management). The prophylactic injection of Tilt® was once recommended; however, our recent work has proven it only delays the death of the tree while remaining a source of inoculum. This presentation will provide evidence for the need of active ingredients with lower fungicidal thresholds, longer half-life, and higher xylem mobility. We will also present the progress our group has made on the screening of avocado germplasm for traits associated to LW tolerance.

P5.3-002

EXTENT OF DALBERGIA SISSOO (SHISHAM) DECLINE IN DIFFERENT AGRO-ECOLOGICAL ZONES AND ITS INTEGRATED MANAGEMENT

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Text

Shisham (Dalbergia sissoo) has been attacked by various pathogens responsible for 80% mortality in Pakistan. Hence a study was designed to identify a better approach to manage shisham decline caused by Fusarium solani, Botryodiplodia theobromae, Curvularia lunata and Ganoderma lucidum. Primarily, efficacy of 8 fungicides were evaluated by the poisoned agar technique against colony growth of all 4 pathogens in-vitro. Among tested chemicals four (Score 250SC, Topsin-M 70%WP, Avito 480SC and Carbendazim 50%WP) were significantly inhibited pathogen growth. Simultaneously, four isolates of Trichoderma harzianum were also evaluated through dual culture technique against all pathogens and one isolate was selected for further study. Subsequently, four fungicides and an isolate of T. harzianum were appraised on mature fully effected diseased plants. During January 2022, five regions were selected from different agro-ecological zones and were classified as healthy, partially and fully affected. All four chemicals with different doses (50, 100, 200, 400PPM) and a biocontrol isolate were applied together on fully affected diseased trees in all regions. The mean results of field trials indicated that Topsin-M 70%WP gave 98.4%, while Carbendazim 50%WP, Score 250SC and Avito 480SC showed 72.8, 64.0 and 48.4% protection value respectively in the field with integration of T. harzianum after 6 months. Topsin-M 70%WP was most effective when integrated with T. harzianum at all doses.

P5.3-003

OCCURRENCE OF CYPRESS CANKER PATHOGENS ON CUPRESSACEAE IN SOUTH AFRICA

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Text

Trees and shrubs in the Cupressaceae are widely planted in gardens and public spaces globally. Several Cupressaceae, particularly planted varieties of *Cupressus*, are highly susceptible to infection by various *Seiridium* species, causing a disease collectively known as cypress canker. The global distribution of these plants has led to many examples of pathogenic *Seiridium* species being introduced into new environments. These include well-documented examples of the pathogens undergoing host-jumps to native Cupressaceae. Most recently, cypress canker caused by *S. neocupressi* has been reported on native *Widdringtonia nodiflora* cedars in South Africa. This prompted a study to consider the distribution of *Seiridium* species in South Africa and the possible source of *S. neocupressi*. A large collection was assembled by isolating *Seiridium* from cankers on *Cupressus* and including isolates available in our culture collection. Isolates were identified by sequencing the *RPB2* gene and performing phylogenetic analyses. At least five different *Seiridium* species were present on Cupressaceae in South Africa. Of these, the well-known *S. cardinale* was most abundant. Importantly, *S. neocupressi,* that has evidently invaded native *W. nodiflora* forest, was rarely found on non-native *Cupressus* species, but had a wide distribution. This study emphasizes the fact that the global movement of *Seiridium* species, strongly linked to the nursery trade, is a growing threat to native Cupressaceae globally.

P5.3-004

OCCURRENCE OF SOME EMERGING POWDERY MILDEWS ON ORNAMENTAL AND FOREST TREES IN ALGERIA: A THREAT TO PARKS, AVENUES AND PUBLIC RECREATION AREAS.

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Text

During the last two decades, outbreaks of powdery mildew have been emerging on ornamental and forest trees in different regions of Algeria, thus constituting a threat to parks and recreation areas. On plane trees, the symptoms appear in early spring on both sides of the leaves, whereas on mulberry and ash they occur later on only the lower side of the leaves. Attacks on the three species of mulberry, Morus alba, M. nigra and M. platanifolia, are severe on the first two species while the last one seems immune. Microscopic observations of samples collected from several sites in Algiers showed the presence of both types of fungal fruiting bodies: solitary spindle-shaped conidia and chasmothecia with characteristic appendages. The morphology of these fruiting bodies enabled us to identify three species: Erysiphe platani on plane trees, Phyllactinia moricola on mulberry trees, and P. fraxini on ash trees. Due to the high morphological similarities of the fungal fruiting bodies of the pathogen on mulberry and ash trees and the proximity of sampling on these two host plants, the use of specific molecular markers (ITS sequencing) to confirm the identity of these two species was needed, to discriminate the species. These observations constitute a first signalisation of these species in Algeria where, so far, only ash powdery mildew was reported by Patouillard (1901). An evaluation of the frequency and incidence of these

pathogens is necessary to take measures to control them.

P5.3-005

CLIMATIC DRIVERS OF PHYTOPHTHORA PLUVIALIS INFECTION AND SPORULATION ON PINUS RADIATA

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Text

Red needle cast (RNC), primarily caused by *Phytophthora pluvialis*, is a foliar disease of radiata pine. Outbreaks of RNC in New Zealand typically occur in winter and have been associated with cooler temperatures and higher rainfall. To quantify the impact of these climatic factors, we completed controlled studies on the effect of temperature and needle wetness on infection of detached radiata pine needles by *P. pluvialis*, as well as sporulation. Infection, as detected by qPCR, was highest in needles inoculated between 5 and 25ºC and when needles remained wet for 12 or more hours. Sporulation occurred at temperatures between 5 and 23ºC. Latent period was shortest at 20ºC, however, the greatest production of sporangia within 14 days occurred at 15ºC. Incubation at 23ºC resulted in the production of abnormal sporangia in vitro and no sporangia in planta. Incubation at 25ºC also restricted sporangia production, however, only when infected needle remained at 25ºC for more than 4 hours per day. Pilot studies suggest that needle wetness is required for sporulation. Infected needles expressed symptoms typical of RNC when incubated in wet or dry conditions, but sporangia were only observed under wet conditions. These findings improve our understanding of key processes in the RNC disease cycle and their relationship with temperature and wetness. These results advance our ability to predict RNC outbreaks, with plans to integrate temperature response curves into an infection risk tool.

P5.3-006

BEECH LEAF DISEASE: A EMERGING ISSUE OF INTERNATIONAL CONCERN

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Text

Beech leaf disease (BLD) was first noted as an unknown ailment of American beech, Fagus grandifolia, near Cleveland, Ohio, U.S.A. in 2012. The disease is characterized by very uncommon symptoms for a forest tree disease. It is now accepted that a foliar nematode, Lytilenchus crenatae mccannii, is a necessary, if not sufficient, condition to cause BLD, making it the first case worldwide of a major forest disease associated with a foliar nematode. Due to the explosive nature and specific dynamics of the North American epidemic, it is hypothesized that the nematode is a non-native pathogen. The disease

eventually leads to abortion of new buds and subsequent failure to produce new leaves, leading to progressive decline and death over several years, especially of younger understory trees. The disease has now spread throughout the Northeastern U.S.A. and threatens the entire distribution range of this iconic and ecologically significant tree species, from the Great Lakes to Georgia, and East of the Mississippi to New England. Furthermore, it has been demonstrated that European beech, F. sylvatica, is also susceptible, which has put Europe on edge as they are dealing with several concurrent forest disease epidemics of major significance. In this talk I will provide an excursus of the discovery and spread of the disease, significant research development in the last 10 years, and an informed opinion on where we are heading with this emerging forest epidemic.

P5.3-007

IMPACTS OF ENHANCED CO2 ON OAK DEFENCES AGAINST POWDERY MILDEW

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Text

Rising levels of atmospheric Carbon Dioxide (CO2), have been demonstrated as harmful for environment and human health. Different strategies are in place to mitigate it such as reducing deforestation and increasing forested areas. In the UK, planting strategies include oak species. However, young oak are highly susceptible to *Erysiphe alphitoides*, the causal agent of oak powdery mildew (PM), which is considered a limiting factor in oak woodland regeneration. Previous work in our group has shown that elevated CO2 (eCO2) causes seedlings to become more susceptible to the infection, which could have devastating future impacts. However, mature oaks are able to tolerate annual powdery mildew infection. We aim to understand how eCO2 impacts mature oaks. For this, monthly leaf samples from the canopy were collected from May-September. Leaf-metabolites were subjected to LC-MS/MS. Spectra were filtered using the XCMS R script and MarVis was employed to putatively identify metabolites and pathways. Mature trees showed low differentially expressed metabolites amounts within the same month, however changes occurred gradually over the months also demonstrating highly different patterns of expression between the months where the PM is present from those without. Comparisons between mature oak trees and an earlier experiment on oak seedlings were performed. Seedling profiles showed unique expression patterns but many of these compound groups are shared between young and mature trees.

P5.3-008

ERYSIPHE LONICERIGENA SP. NOV., A POWDERY MILDEW SPECIES FOUND ON LONICERA HARAE

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Text
A powdery mildew (Erysiphaceae) has been continuously collected on the leaves of Loncera harae from the southern part of the Korean Peninsula, where this shrub is indigenous. Microscopic examination of asexual morphs revealed that the current isolate is differentiated from the all known Erysiphe-Lonicera associations by its longer conidiophores and longer conidia. Although the morphology of the chasmothecia is reminiscent of Erysiphe ehrenbergii and E. lonicerae, but the isolate is differentiated from them by having smaller ascospores. A phylogenetic tree generated from a combined dataset of the internal transcribed spacer region and 28S rDNA gene sequences demonstrates that sequences obtained from three powdery mildew collections on L. harae clustered together as an independent clade with high bootstrap values and placed distinctly from other Lonicera-Erysiphe combinations, representing a species of its own. Based on morphological differences and molecular-phylogenetic results, the powdery mildew on L. harae was proposed as a new species, Erysiphe lonicerigena, and the holomorph of the fungus was described and illustrated in this study.

P5.3-009

EARLY DETECTION METHODS TO PREVENT THE SPREAD OF FOREST PATHOGENS

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Text
Plant diseases caused by pathogenic microorganisms represent a serious threat to plant productivity and natural ecosystems. Early warning and a rapid response are crucial for a successful eradication or mitigation of the impacts and of the possible further spread of the invasive organism. For these reasons, early detection tools play an important role in monitoring plant health, surveillance, and quantitative pathogen risk assessment, thus improving best practices to mitigate and prevent plant pathogens threats.
Recent advances in nucleic acid-based methods, to detect plant pathogens, offer increased specificity and sensitivity over traditional microbiological approaches. The potential benefit of nucleic acid-based methods is reduced time to diagnosis, high throughput, and accurate and reliable results.
Considering prevention to be the best strategy to protect plants from diseases, this contribution focuses on fast and reliable molecular methods to detect forest pathogens by using both in field laboratory techniques, even at early stage of disease development before symptoms occur in the host.
Here we present a series of examples of tools, protocols and assays that shown to be or that, if used, could be useful early detection tools for a successful prevention, eradication or control of threatening both emerging and non-native pathogens.

P5.3-010

RNA VIRUSES IN DECLINING MEDITERRANEAN FORESTS

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Text

Global change alters forestry habitats and facilitates the entry of new pathogens that don't share a co-evolution history with the forest. Due to urban massification, the human population is particularly exposed to new infectious diseases, such as the coronavirus pandemic. For this reason, the study of RNA viruses is essential to understand how viral flow across different hosts might occur, and to prevent possible outbreaks of human diseases in the future. In this work the viral diversity found in trees, insects and fungi from Spanish Mediterranean forests is described. To this extent, three habitats (Quercus ilex, Castanea sativa and Pinus radiata) were sampled and RNAseq was performed on tree tissues, insects and fungi. 161 viral sequences were detected by searching for matches to conserved motifs of the RNA-dependent RNA polymerase (RdRP) using Palmscan. Up to 15 viral families were identified, with Botourmiaviridae (25%) and Partitiviridae (6%) being the most abundant. Viruses belonging to families with cross-kingdom capabilities such as Hypoviridae (1), Mitoviridae (7) and Narnaviridae (5) were also found. Distribution of viruses across ecosystem was: Q.ilex (57%), P.radiata (27%) and C.sativa (16%). Interestingly, 40% of RdRP sequences had no matches in available viral databases, thus constituting a starting point to search for novel viruses that might be participating in unknown infectious pathways within forests and potentially posing a threat to the human being.

P5.3-011

VERTICILLIUM WILT AND MORTALITY OF AILANTHUS ALTISSIMA IN CATALONIA (NORTHEASTERN SPAIN). DISEASE DISTRIBUTION AND PATHOGEN CHARACTERIZATION.

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Text

Ailanthus altissima (Mill.) is a highly invasive tree established worldwide, included into the European Union list of invasive alien species. *Verticillium dahliae* Kleb. and *V. nonalfalfae* Inderb. *et al*. (formerly *V.albo-atrum* Reinke & Berthold) have been reported as the causal agents of Verticillium wilt and mortality of *Ailanthus*. In the last decade, ailanthus trees showing Verticillium wilt disease symptoms have been observed in forests in Catalonia (Spain). Aimed at establishing the disease aetiology and its impact in the ailanthus trees in Girona area, the disease progress was monitored yearly since 2018. Morphological, molecular and pathogenic characterization was performed on about 100 isolates recovered from symptomatic samples.

Based on morphology, 77% of the isolates were identified as *V.dahliae* and 23% as *V.albo-atrum/V.nonalfalfae*. BLASTn study based on ITS sequences showed high identity to *V.dahliae, V.albo-atrum* and *V.nonalfalfae* type strains, depending on isolates. Regarding to pathogenicity, 100% of the tested isolates caused chlorosis, wilting or dying on ailanthus inoculated plants, whereas control plants remained healthy. As far as we know, this is the first report of *V.dahliae* and *V.albo-atrum* as causal agents of Verticillium wilt of *Ailanthus* in Spain.

This research was funded by Diputació de Girona (grants 2017/8719-2019/3091-2020/7565-2021/1468-2022/3571) and Carol, J. was recipient of a fellowship from AGAUR, Generalitat de Catalunya (2021FISDUR00102).

P5.3-012

BLAST FROM THE PAST: A STUDY OF DECADES-OLD FUNGAL CULTURES RESOLVES A LONG-STANDING TREE DISEASE MYSTERY

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Text

A root disease in plantations of *Pinus radiata* and *Pinus pinaster,* where trees died in distinct patches, was present in the Western Cape province of South Africa during the 1970s and 1980s. The disease was originally believed to be caused by *Phytophthora cinnamomi*, but was later attributed to *Leptographium serpens* (Ophiostomatales), an insect-associated fungus. However, doubt regarding *L. serpens* as the causal agent was raised because most *Leptographium* spp., particularly those, like *L. serpens*, which colonise ray parenchyma tissues, are not typically primary pathogens. In the present study, we revived cultures collected from dying trees almost 40 years ago and identified them using DNA sequencing methods, which were not available when the disease was first studied. These cultures were identified as the pyrophillic pathogen *Rhizina undulata*, well-known to cause patch death of conifers in South Africa and elsewhere in the world. Unfortunately, the patches of dying trees no longer exist and thus cannot be further investigated, however it is most likely that *R. undulata* was the primary cause of the patch death observed in the *Pinus* plantations. The study provides a vivid example of the value of preserving cultures of fungi for later study and the power of modern techniques to identify fungal pathogens.

P5.3-013

THE OPHIOSTOMATOID FUNGI IN KOREA

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Text

The ophiostomatoid fungi includes important fungal pathogens, some of which cause serious diseases on agricultural and forestry crops worldwide. Of those, *Raffaelea quercus-*

mongolicae is one of the ophiostomatoid fungi occurring in Korea, which is believed to be associated with oak mortality in the country. Despite the fact that the oak mortality has continued to spread across the country since its first discovery of the disease in 2004 and the significant impact that the pathogen has on forest ecosystem in the country, little is known regarding its biology of the fungus. In addition, wound-associated fungi that belongs to Ophiostomatales and Microascales were first found occurring on Korean native trees via a survey to assess the diversity of the ophiostomatoid fungi, especially including Dutch Elm Disease. These results clearly emphasize the general lack of information on fungal diversity, especially for species of Ceratocystidaceae or other insect related fungi such as the Ophiostomatales in Korea, requiring more surveys on this group being conducted.

P5.3-014

PHYTOPHTHORA SPECIES ASSEMBLAGES IN KAURI FORESTS: COMPARING ISOLATION THROUGH BAITING AND METABARCODING

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Text

Phytophthora species are serious pathogens of many foundation and keystone forest tree species globally. In New Zealand, *Agathis australis* (kauri), a threatened foundation tree species, is under attack by *Phytophthora agathidicida.* This root-rot pathogen causes canopy dieback, creates massive basal lesions and eventually leads to tree death. During routine surveillance programmes, several other *Phytophthora* species have been detected from kauri trees, although their role in kauri dieback is unknown. Our research aimed to characterise the *Phytophthora* species assemblages from randomly selected kauri in the Waitakere Ranges, Auckland. Seven species were detected with metabarcoding and confirmed through both baiting isolation and qPCR analysis of the soils. This is the first time metabarcoding has been used to detect *Phytophthora* in kauri forests. A qPCR assay was developed to validate the metabarcoding results and confirmed that the enrichment approach used for the metabarcoding was useful for giving a qualitative description of the *Phytophthora* species within environmental soil samples. *P. cinnamomi* was the most abundant species, found in 59.2% of all samples, followed by *P. agathidicida* in 10.9% of samples. There were 5 and 51 samples that were positive with sequencing only for *P. agathidicida* and *P. cinnamomi*, respectively. We recommend using both traditional isolation methods and high-throughput sequencing to detect *Phytophthora* populations.

P5.3-015

LOW GENETIC DIVERSITY IN COLOMBIA FUSARIUM CIRCINATUM POPULATION

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Text

The pitch canker fungus, *Fusarium circinatum* is one of the most important pathogens of *Pinus* species globally. It now occurs in plantations and production nurseries in most regions where non-native Pinus species are propagated commercially. In Colombia, *F. circinatum* was first reported in 2005, both from nursery seedlings with root disease and established plantation trees displaying pitch canker symptoms. Since then, disease associated with this pathogen has gradually increased, but information regarding its population biology remains lacking. The aim of this study was to consider the mode of reproduction, population diversity and structure of *F. circinatum* isolates in Colombia. This was achieved using 10 microsatellite markers to analyse a collection of 94 isolates obtained from multiple geographic regions, collected at different time points and from different *Pinus* species. The population in Colombia was found to be predominantly clonal, with no evidence of sexual recombination (P = 0.0001) occurring and all isolates were of the MAT 1-1 mating type. Generally, this limited diversity was structured based on date of collection and *Pinus* host from which isolates were recovered. The findings support the view that following an initial introduction of *F. circinatum* into Colombia, its genetic diversity has not changed greatly over time. Its spread and the increasing incidence of disease is largely due to forestry operations such as movement of infected planting material.

P5.3-016

MULTIVARIATE ANALYSIS AND MODELLING OF SCOTS PINE BLISTER RUST DISTRIBUTION IN CENTRAL AND NORTHERN SWEDEN

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Text

Scots pine (*Pinus sylvestris* L.) is one of the most common tree species in Sweden, it formed 33% of the total volume of the Swedish forest. Scots pine blister rust/resin top disease is caused by the rust pathogen *Cronartium pini*. Severe *C. pini* infection can girdle the stem and cause the death of the top or the entire pine tree. This pathogen is native to Fennoscandia, but its recent epidemics in the central and northern Swedish forests, especially young Scots pine forest stands, caused significant economic and ecological losses in forestry. To understand the distribution of *C. pini* in the Swedish forest and the biotic and abiotic factors associated with Scots pine blister rust, forest surveys were executed in 2021 and 2022. The Surveys covered 3373 plots from 421 forest stands in Norrbotten, Västerbotten, Jämtland and Västernorrland County (62° N to 67° N). Topographical, vegetation, and climate data, and the binary data of *C. pini* occurrence were analyzed in multiple logistic regression with generalized linear mixed (GLMM) models. Results showed *C. pini* has aggregated geographical distribution in Northern Sweden, and it is associated with the distribution of the alternate host (*Melampyrum* spp.), and warmer and more humid weather from May to July. At last, we used the GLMM model to predict the current C. pini occurrence in central and

northern Sweden, and discussed the change of *C. pini* distribution under future climate scenarios.

P5.3-017

A STUDY OF TREE DISEASE ON ST HELENA

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Text

Situated in the South Atlantic Ocean, St Helena is an island home to an abundance of endemic flora and fauna. The cloud forest established at the high peaks of the central ridge support a complex ecosystem for biodiversity to thrive. However, human activity, animal introduction and deforestation have reduced naturally occurring vegetation, and fragmented communities of ecological importance. In conjunction with changes to the climate, the introduction of invasive plants and an increased risk of pests and diseases endanger this unique ecosystem. One of the keystone species to the forest, the black cabbage tree (Melanodendron integrifolium) as well as the other endemic trees, are now threatened by sudden dieback due to an unknown disease causing agent. Other symptoms such as leaf wilt, yellowing, spotting and root rot are also seen across the islands plant nurseries and field gene banks. To identify a causal agent of this disease(s) the microbiota associated with these plants have been surveyed and several candidates for disease have been identified. Pathogenicity testing will be carried out to identify the host range of the candidates and genomic analysis of the candidates will be done to develop a diagnostics tool which will build local capacity to monitor disease on the island. These findings may help prevent the spread of disease and inform management decisions by characterisation of the causal agent(s) and through communication with locals, tourists and land owners.

P5.3-018

INVESTIGATING THE VARIATION IN VIRULENCE OF HYMENOSCYPHUS FRAXINEUS, THE CAUSAL AGENT OF ASH DIEBACK

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Text

The causal agent of ash dieback, *Hymenoscyphus fraxineus* [synonym: *H. pseudoalbidus*], is an introduced species in Europe that causes damage on European ash (*Fraxinus excelsior*).

The necrotic lesions and shoot dieback reduce vitality, timber quality, and can cause tree mortality. Consequently, the pathogen is associated with significant economic and ecological damages, and continues to threaten the survival of ash populations in Europe.

As part of the FraxForFuture project, we are analysing pathogen virulence using infection trials and microsatellite analysis. Initially, we investigated *H. fraxineus* virulence using amended culture media and host reintroductions. We also tested the impact of different inoculation protocols on disease development *in-planta*. We hypothesised isolation, storage and inoculation protocols could influence disease development, and subsequent determination of strain virulence and host resistance. We then established an infection trial to assess virulence for newly isolated *H. fraxineus* strains from across Germany. We hypothesised virulence may vary with the geographical origin and/ or starting substrate (leaves, shoots, stem base) of strains. We are also examining the population structures of these strains using microsatellite analysis. Overall, these results will provide important insights into pathogen evolution and disease development, which we expect will facilitate the development of improved strategies for forest health and disease management.

P5.3-019

INSIGHTS INTO THE BIOLOGY, HOST RANGE AND POTENTIAL PATHWAYS OF PHYTOPHTHORA PLUVIALIS IN BRITAIN

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Text

Phytophthora pluvialis was detected in England in 2021 on Tsuga heterophylla. This was a new UK record for the pathogen, and symptoms of resinous bleeding cankers on T. heterophylla were a first worldwide. Initially described in 2013, P. pluvialis was previously only known from the USA and New Zealand, mainly causing a needle disease on Pseudotsuga menziesii and Pinus radiata, with some records on Notholithocarpus densiflorus and occasionally other pine species. A risk assessment was conducted in the UK, and P. pluvialis was classified as a quarantine pest for regulatory purposes. Official surveys, monitoring programs and research were carried out to establish the symptomatology, distribution, dispersal, hosts, association with timber and potential pathways of P. pluvialis. Between 2021 to early 2023, over 2000 forest tree samples were tested for the pathogen, with findings in England, Scotland and Wales on both T. heterophylla and Ps. menziesii. A symptom guide was created to aid surveillance activities and staff training. Over 15 different hosts were exposed under or near infected trees and all tested negative. Rainwater traps and stream baiting were used as a monitoring system on a bi-weekly basis for one year at several locations which identified optimal times for pathogen detection and a range of successful baiting hosts. All the results will be presented and a life cycle for the pathogen will be proposed

P5.3-020

CHEMOTYPING EUROPEAN AND ASIAN FRAXINUS: UNDERSTANDING HOST DEFENCE MECHANISMS ASSOCIATED WITH RESISTANCE AGAINST THE ASH DIEBACK PATHOGEN HYMENOSCYPHUS FRAXINEUS.

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Text

European ash (*Fraxinus excelsior*) has suffered large-scale decline and mortality due to the invasive fungal pathogen *Hymenoscyphus fraxineus*. The fungus is native to East Asia where it is non-pathogenic on native ash in its natural distribution range. As with most introduced pests and pathogens where the host plant lacks a history of co-evolution, the damage is devastating.
Breeding for resistance is the most preferred method to ensure European ash survival. There is evidence that a small proportion of ash genotypes show tolerance to the disease. Studies conducted around Europe show that low susceptibility to the disease is strongly genotypically controlled, inheritable, polygenic, and stable over time. However, traditional breeding practices are time-consuming and highly costly.
Research shows that chemical fingerprints have been uniquely associated with resistant phenotypes and can be useful as markers for selection and breeding. Studies on defence phytochemicals and their role in non-native ash species, particularly Asian species of *Fraxinus* that have co-evolved with the fungus can be valuable to understand mechanisms of resistance to *H. fraxineus*. In this study, we aimed to i) characterize the metabolite-basis of host defence in congeneric species of *Fraxinus* that differ in their evolutionary history and susceptibility to the fungus *H. fraxineus*, and ii) identify chemical biomarkers associated with disease resistance.

P5.3-021

CERATOCYSTIS IN GREECE; THE EXPANSION

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Text

Fig (*Ficus carica*) and plane (*Platanus* sp.) are two important tree species in the Mediterranean basin. Members of *Ceratocystis* affect both plant species, causing lethal canker-wilt diseases. *C. platani* invaded Europe during World War II, causing important losses in *Platanus* sp.; in Greece, it was first reported in 2003 and spread quickly in mainland natural riparian ecosystems of *Platanus orientalis*, having caused an ecological disaster. Since 2018, *C.*

ficicola has been reported affecting fig orchards in Greece. Both fungal pathogens are soil-born and mostly spread by human activity. Due to the fact of co-existence of two different invading *Ceratocystis* species in Greece, a new collection of isolates and molecular studies took place in the affected areas of the country. Differentiation in molecular level was found to rise in both species based on sequence variation in ITS and FG1093, MS204 and RPB2 genes. Furthermore, in 2019, another pathogenic species of *Ceratocystis* was isolated from fig orchards on Euboea Island, Greece. Based on conducted molecular, morphological and pathogenicity studies, this isolate could not be included in a previously known species. In 2022, *C. ficicola* has entered into EPPO Alert List, and it is crucial to prevent it from generally spreading in fig orchards. Preliminary studies in Greece have demonstrated that different varieties of *F. carica* show different susceptibility when artificially inoculated with *C. ficicola.*

P5.3-022

THE TRAVAILS OF TROUBLESOME TRAVELERS: DISTRIBUTION OF THOUSAND CANKERS DISEASE COMPLEX MEMBERS INTO EXPANDED RANGES ALTERS THE GENETIC SIGNATURES OF THEIR POPULATIONS

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Text

The Thousand Cankers Disease (TCD) disease complex includes the fungal pathogen, Geosmithia morbida, walnut twig beetle (WTB; Pityophthorus juglandis) as a principal insect vector, and Juglans spp. or Pterocarya spp. host plants. TCD has been spread from the western to the eastern USA and into Italy, where it affects several walnut species, including J. nigra (Eastern black walnut) and J. regia (English walnut). Previous work has elucidated pathogen and vector genetic diversity, yet understanding about potential spread, spatial distribution, dispersal pathways, and host-parasite co-adaptation is largely unknown. This study evaluates knowledge gaps in diversity patterns and possible pathways of range expansion from the hypothesized western USA center-of-origin. Using microsatellite loci, we evaluated 807 G. morbida and 1,714 WTB individuals that were genotyped across subpopulations in the western USA. To reduce dataset over-representation of California (CA)-sourced samples for G. morbida (n=501/807) and WTB (n=1188/1714), data were analyzed with four approaches: CA group versus southwestern USA group (that included fewer CA samples) for each of beetle and pathogen datasets. Our results indicate generally high genetic diversity, weak but significant overall linkage disequilibrium, and the presence of population structure in all but the CA WTB dataset. Our data support prior hypothesis that the pathogen and vector are long-standing associates with southwestern USA host plants.

P5.3-023

BRAZILIAN RESEARCH ABOUT SANITY IN FOREST SEEDS

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Text
The sanity of Brazilian forest seed is a theme few explored in the literature. The forest planted is growing in Brazil and seed sanity is more important to this establishment. Then, this research has the aim of resuming information about the sanity of forest seed from Brazilian Research. For this research, on Web of Science platform were inscribed the keywords: seeds, forest, pathogen, and Brazil. As a result, only eighteen papers were found about this theme. The prevalent area was Plant pathology, and Dr. Alvaro F. dos Santos (Brazilian Agricultural Research Corporation) and Dr. Acelino C. Alfenas (Viçosa Federal University) were the most important authors. The most relevant plant species analyzed were Cedrela fissilis, Anadenanthera macrocarpa, Aspidosperma polyneuron, Enterolobium contortisiliquum, Jacaranda spp., Pinus spp., Cacao spp., and others. The themes from these papers are the Blotter test seed analysis method for fungi identification, parasitism of pathogens in commercial forest seeds, and methods to sanity in Brazilian forest seeds. The research about Brazilian forest seed pathology diagnosis is an important and few-studied theme, more research can be made to better the seed sanity in Brazil.

P5.3-024

GANODERMA ROOT ROT: AN EMERGING THREAT TO EUCALYPTUS PLANTATIONS IN INDONESIA

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Text
Ganoderma Root Rot (GRR) caused by *Ganoderma* spp. is a common disease occurring sporadically in native tree species around the world. In the past few years, mortality caused by GRR has emerged in Indonesian *Eucalyptus* plantations. Disease symptoms are mostly detected on the roots and root collar regions. Infected roots are characterized by the presence of reddish rhizomorphs on the bark surface and yellowish-white mycelium under the bark. Affected trees display yellowing canopies after which they wilt and die. Aerial surveys followed by field evaluation have revealed that GRR occurs in patches and spreads from tree to tree, mostly within the planting lines via root contacts between diseased and healthy trees. Plant mortality occurs on a variety of sites and at different tree ages beginning at six months and progressing up to rotation age. Preliminary data reveal approximately 12.5% mortality in a susceptible clone up to 4-years-old. Fungus fruiting bodies are rarely seen on dead trees and stumps from previous rotation, but the pathogen can easily be isolated from freshly infected roots. Based on morphological and DNA sequence analyses, the causal agent has been identified as *Ganoderma philippii*. As a soil-borne pathogen, disease management is challenging and losses can be important. For this reason, GRR

management strategies including a robust survey system to quantify the impact and sylvicultural activities to reduce losses will be increasingly important in the future.

P5.3-025

POPULATION STUDIES SUGGEST MULTIPLE INTRODUCTORY EVENTS OF DOTHISTROMA PINI INTO FRANCE

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Text

Dothistroma needle blight (DNB) is an important disease of *Pinus* spp. in many parts of the world. Although a single disease, it can be caused by two different pathogens, *Dothistroma septosporum* and *D. pini*. The earliest occurrence of DNB in France was recorded in 1860 in the central part of the country. Although both species of Dothistroma occur in France, more recent DNB outbreaks in central France have been attributed to *D. pini*. Previous population genetic studies of *D. pini* strains from central France revealed an established population with high genetic diversity. Outbreaks of DNB have also occurred in southern France, but these populations have not been investigated. The aim of this study was to investigate the genetic relatedness of the *D. pini* populations from central- and southern France using microsatellite markers. Significantly higher levels of diversity were found in central France compared to southern France where populations were clonal and structured. There were no shared haplotypes between central- and southern France and there was little support for geneflow occurring between these regions. The results suggest that there have been multiple introductions of the pathogen into southern France but not originating from central France. One possibility is that *D. pini* has been introduced to southern France from neighbouring Spain, where DNB is also known to occur.

P5.3-026

CLIMATE DETERMINES OOMYCETE PLANT PATHOGEN BIOGEOGRAPHY AT A CONTINENTAL SCALE IN EUROPE

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Text
Understanding the role of climate on the distribution of plant pathogens is paramount owing to the upraising effects of climate change. While this has been studied for other plant-associated microbes, such as bacteria and mycorrhizal and endophytic fungi, the effect of climate on oomycetes has been rarely studied from a community ecology perspective. Oomycetes include the genus Phytophthora which comprises some of the most damaging plant pathogens of agricultural, forestry and natural ecosystems. We explored the role of climate in the assembly of Phytophthora species at >250 river sites across two gradients, a latitudinal gradient spanning from Mediterranean to Arctic conditions, and an altitudinal gradient including the Spanish Pyrenees. Phytophthora communities were obtained by metabarcoding river filtrates. Climate and not host distribution determined Phytophthora biogeography. Two key processes determined species assembly. In southern latitudes, dry climate posed an environmental filter for Phytophthora communities resulting in a lower functional diversity due to communities dominated by drought-tolerant species with thick oospores and high optimum growth temperatures. In northern regions, species diversity decreased and communities were dominated by few species adapted to cold and with the ability to form enduring survival structures.

P5.3-027

NEW INSIGHTS INTO DATE PALM INFLORESCENCE ROT BIOLOGY AND CONTROL IN MOROCCO

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Text
Date palm inflorescence rot (DPIR) is a significant fungal disease in Moroccan date producing areas. However, research into determining disease prevalence and management measures has received little attention. We conducted a three-year study (2019-2022) to assess the occurrence of DPIR across 9 locations, find out factors associated with its spread, and develop an effective chemical management method. Disease incidence varied among years and locations (5.64 – 27.51%). Intercropping and high planting density combined with frequent irrigations created ideal conditions for disease development, especially in young date palms. Farmers' cultural practices and lack of exclusion methods, specifically the use of diseased male inflorescence in pollination, contribute significantly to pathogen spread. Four pathogenic fungus were isolated from symptomatic inflorescences among which Mauginiella scaettae was the dominant species. In vitro assay and field experiments showed that out of five fungicides two pulverizations of copper oxychloride (400 g/ha) at the beginning of December and January decrease incidence by 96.8%. Overall, our findings shed light on the biology of DPIR and pave the road for future studies on management methods of this disease.

P5.3-028

IN VITRO ACTIVITY OF BLAD AGAINST CORK OAK PATHOGENS AND ECTOMYCORRHIZAL FUNGI SPECIES

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Text
BLAD is a polypeptide isolated during the growth of *Lupinus* seedlings and is a breakdown product of β-conglutin catabolism. Among its properties, BLAD showed anti-fungal, anti-oomycete, and biostimulant activity and is already commercially available as a fungicide targeting over twenty diseases of several agricultural crops.
We performed a set of *in vitro* assays testing the effect of BLAD in cork oak decline-related pathogens such as *Biscogniauxia mediterranea*, *Diplodia corticola*, and the root rot agent, *Phytophthora cinnamomi*.
A particular emphasis was given to *P. cinnamomi* comparing five isolates and studying several parameters: mycelium growth rate, sporangia, chlamydospores, and zoospores production.
Four species of ectomycorrhizal fungi (*Amanita citrina*, *Amanita pantherina*, *Suillus bovinus* and *Tricholoma sulphureum*) were also submitted to BLAD action.
These tests clearly illustrated a disturbance in all tested species vs BLAD concentrations, namely in the pathogens, reaching, in some cases, an almost complete growth inhibition with higher BLAD concentrations (15-20 gL-1).
Ectomycorrhizal fungi species exhibited a higher resistance to BLAD activity in the mycelium growth rate parameter, except the *Suillus bovinus* case with a similar reaction to those of the pathogens.

Development of Molecular Diagnostic Tools for Plant Pathogens in a Globalizing World

C4.2-1

NEXT GENERATION SEQUENCING AND ITS IMPACT ON PLANT HEALTH RISK ASSESSMENT AND PHYTOSANITARY MEASURES

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Text
In the last decade, next generation sequencing has accelerated the identification of new species of plant pathogens. Dealing with species newly described is a plant health challenge for both risk assessment and risk management. In commodity risk assessment and in horizon scanning, hundreds of new species of plant pathogens are identified when screening

scientific reports, albeit only for a few information is sufficient to complete the assessment. New plant pests identified as potential threats for the European Union territory, by the commodity risk assessment as well as by the horizon scanning processes, are then further assessed by conducting pest categorisations or, when needed, quantitative probabilistic pest risk assessments. Even in these further steps, knowledge gaps may impede conclusions or cause high uncertainties. This presentation will summarise the challenges encountered when dealing with plant pathogens newly identified by next generation sequencing, for both the plant health risk assessment processes (commodity risk assessment, horizon scanning, pest categorisation, quantitative pest risk assessment) and the phytosanitary legislation.

C4.2-2

USING MARPLE DIAGNOSTICS TO ASSESS THE GENETIC DIVERSITY OF PUCCINIA STRIIFORMIS F. SP. TRITICI (PST) IN THE HIMALAYAN FOOTHILLS OF NEPAL

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Text

Wheat yellow rust, caused by the fungus *Puccinia striiformis f. sp. tritici* (*Pst*), is a constant threat to wheat production in Nepal, leading to significant loss of wheat yields each year. However, in recent years the number of new pathotypes of *Pst* being detected each year has significantly increased, with these new emerging races having broad virulence profiles, being more aggressive and tolerant to warmer temperatures. This has led to rapid failure of established and newly released wheat varieties in Nepal. To track the rapid changes in *Pst* strains in this region, we have begun to implement real-time genotyping using the Mobile And Real-time PLant disEase diagnostics methodology, based on nanopore sequencing technology. This approach has helped to gain a better understanding of the Nepalese *Pst* population diversity. For instance, revealing a recent incursion of unique genotypes of *Pst* in Pyuthan and Kailali districts of Nepal in 2021. The phylogenetic analysis indicates these isolates are close to *Pst*6 reported from Pakistan and also East Africa. Our results also revealed the *Pst* population in Nepal is quite diverse and most strains analyzed are close to races that were previously found in Central Asia. These results indicate the Himalayan foothill could be a center of diversity for *Pst* and that more in-depth studies are essential to decipher the mechanism of new race evolution in Nepal.

C4.2-3

A PANGENOME APPROACH TO STUDY DIVERSITY AND FUNCTIONAL MARKERS AND ITS APPLICATIONS TO ENHANCE DIAGNOSTICS.

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Text
Advances in next-generation sequencing technologies (NGS) made the reconstruction of genomes easier and more accessible over the years. The diversity that was found among strains of the same species resulted in the concept of a pangenome, which reflects a species more accurately than any single member can. The pangenome is an abstract representation of the genomes of all the strains that are present in the population, species or genus. The graphical pangenomic data analysis platform, PanTools [1], has a hierarchical data structure, integrating sequence data (represented as a localized, compressed De Bruijn graph), structural/functional annotations, and crosslinks between DNA and protein sequences and annotations. Recently this platform was extended with modules to study phylogeny and to include and use phenotypic data [2]. Incorporating these allows assessment of functional diversity and population genomic analysis of plant pathogens. This facilitates the selection of target regions or SNP's that are diagnostic for subpopulations that originate from a particular geographical location or that show specific phenotypes such as specificity to a particular host or disease expression. These regions/SNP's can then be used to design efficient and very specific diagnostic methods for different plant pathogens.

[1] PanTools is implemented in Java 8 https://git.wur.nl/bioinformatics/pantools.
[2] Jonkheer et al. Bioinformatics, 2022, PanTools v3:

C4.2-4

IMPROVING AND DEVELOPING DIAGNOSTICS FOR HIGH THROUGHPUT IDENTIFICATION OF VIRUSES, WITH A FOCUS ON BEGOMOVIURSES.

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Text
Begomoviruses are a large group of over 400 plant viruses that infect many dicot vegetables and crops throughout the subtropical and temperate regions of the world. Australia currently has only five known begomovirus species. Thus, the biosecurity threat posed by potential incursions is significant. To date, detection of the different species and strains relies on several different generic PCR's. To simplify begomovirus detection, we set out to develop assays with the capacity for in-field diagnostics. We developed a novel tissue blot hybridization chain reaction (TB-HCR). In this assay, plant stems are blotted onto nitrocellulose membrane tissue blots and then probed with specific probes designed to bind to either a family or group of viruses or specific viruses. During the assay one probe or several different probes can be used for screening at the same time. After the probe binds to the virus, labelled RNA hairpins (HP) are added and bind to the probe initiating a hybridization chain reaction. We have also developed a generic begomovirus Recombinase Polymerase Amplification (RPA) lateral-flow assay which to date, has successfully detected eleven different begomoviruses from Australia, Timor-Leste and PNG. We have also tested specific LAMP assays for agriculturally important begomoviruses in Australia. Our results

with begomoviruses and other virus species, and the advantages and disadvantages of these novel assays will be discussed.

C4.2-5

ADVANCEMENT IN PLANT PATHOGEN DIAGNOSTICS IN HIGH THROUGHPUT SEQUENCING ERA

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Text

New strains/species of plant pathogens are emerging and threatening economically important crops worldwide. Accurate detection and identification of a pathogen is the key to successful and timely disease management and prevention of further incursion of exotic pathogens. Therefore, advanced tools capable of accurately detecting and differentiating plant pathogens is a critical need. PCR or isothermal technologies mostly target single species and cannot detect, identify, or differentiate emerging species/strains. Use of High Throughput Sequencing (HTS), along with ELISA and PCR, has become one of the most significant advances in molecular diagnostics. The HTS-based methods are not simply for pathogen detection but, can also be used to understand the strain's phylogeny, global dissemination, and evolutionary heritage. The HTS-based pipelines are available, including EDNA, Nanopore EP2ME WIMP, and PhytoPipe but, most of them use public databases. In our lab, we have developed a new pipeline "BacPath" that can rapidly and accurately detect pathogens in infected samples using our highly curated complete genome database. The pipeline is compatible with both long- and short-reads. The processing cost per sample can substantially be reduced by barcoding multiple samples in a single run. The HTS-based pipelines and high-quality genome database with accurate metadata will enhance our capabilities in diagnostics and national and international crop biosecurity.

C4.2-6

CRISPR-CAS DETECTION COUPLED WITH ISOTHERMAL AMPLIFICATION OF BURSAPHELENCHUS XYLOPHILUS

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Text

Pine wilt disease (PWD) caused by the nematode *Bursaphelenchus xylophilus* is the most destructive threat to pine forests worldwide, particularly in China, Korea, and Japan. Ultrasensitive diagnosis of *B. xylophilus* at an early stage is therefore imperative for effectively monitoring PWD in pine forests. Herein, we established two isothermal diagnostics methods based on clustered regularly interspaced short palindromic repeats (CRISPR)-based platforms (CRISPR/Cas12a and CRISPR/Cas13a) for *B. xylophilus*–specific detection. The guide RNA

(gRNA) and CRISPR RNA (crRNA) were designed to target the 5S rDNA intergenic spacer sequences (IGS) region of *B. xylophilus*. Recombinase-aided amplification (RAA) was used for pre-amplification whose reaction condition was 37°C for 15 min. The sensitivity of CRISPR/Cas12a could reach 2.33 copies/μl of purified genomic DNA (gDNA) within 45 min at 37°C, while the sensitivity of CRISPR/Cas13a was 100 times higher than that of CRISPR/Cas12a at the minimum reaction time of 4 min via fluorescence measurement. The CRISPR/Cas12a assay enabled the detection of 0.01 PWNs/100 mg of pine wood, 10 times higher than that of the CRISPR/Cas13a assay. At the same time, CRISPR/Cas13a direct test was able to realize the detection of single-headed live nematodes. Therefore, this work could eventually facilitate the protection of healthy pines from *B. xylophilus* infection and enable accurate diagnosis of quarantine nematode species in the pine wood trade.

F4.2-1

ALTERNARIA ALTERNATA AND STRAINS OF THE A. ARBORESCENS SPECIES COMPLEX ARE RESPONSIBLE OF AN UPSURGE OF THE APPLE LEAF BLOTCH DISEASE IN FRANCE

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Text

Leaf blotch and defoliations have been observed for some years in apple orchards of France. These symptoms have raised serious concerns for both the French plant health authorities and apple growers, as it could be caused by the *Alternaria* 'apple pathotype', a fungus that was until recently considered as a quarantine pest in the European Union. This pathogen has the particularity to produce toxins involved in host specificity (AMT toxins). The apple pathotype had so far only been reported twice in Europe: in the Balkans and in Italy. However, recent studies have shown that similar symptoms can also be caused by *Alternaria* that not produce these toxins. As *Alternaria* is a genus with a complex taxonomy, the identification of isolates requires the study of several genes. Our project had four objectives: I. To identify strains isolated from France, II. To track the presence of the apple pathotype in France, III. To assess the pathogenicity of the strains *in vitro*, and IV. To identify, by comparative genomics, candidate regions for the development of specific tests able to detect the *Alternaria* involved in this disease. Our results showed that the apple pathotype is not present in France and that two genetically related taxa are responsible of leaf blotch and defoliations: *A. alternata* and *A. arborescens*. Finally, the analysis of the complete genomes allowed the identification of candidate loci allowing a precise identification of *Alternaria* taxa involved in apple defoliation.

F4.2-2

APPLICATION OF CONVOLUTIONAL NEURAL NETWORK MODEL FOR DETECTION OF CHILI ANTHRACNOSE

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Text

Pepper (*Capsicum annuum*) is an important crop due to its massive consumption as a seasoning vegetable in Korea and many other countries. Anthracnose disease in chili pepper has caused serious damage to plant growth and reduced yield with apparent symptoms and signs on the fruits. In this study, we report a deep learning-enabled detection model for chili anthracnose among chili pepper disease based on a computer-vision algorithm. The model was developed based on a deep learning architecture based on a Convolutional Neural Network (CNN) that specializes in extracting features from image datasets. Large datasets of expert pre-screened pepper disease images were collected from 'AI Hub', a platform of AI infrastructure. We examined the effectiveness of image preprocessing and data augmentation to create a balanced dataset. The implemented model achieves higher than 90% classification accuracy compared with training and validation dataset. Our results showed that CNN could be the deployable method for digital disease detection. This meaningful success makes the model a useful disease detection tool, and this research could be further extended to develop a mobile application to help millions of farmers directly in the fields. Further results for the detection of chili pepper disease will be discussed.

F4.2-3

COMPARISON OF MOLECULAR DIAGNOSTIC TOOLS FOR FUNGAL PATHOGEN DETECTION IN SOIL

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Text

Soil-borne plant pathogens can cause significant economic losses due to reduction in plant establishment and seedling death. Diagnosis of these pathogens in the soil is therefore important for effective disease control in the form of cultural and chemical methods to protect seedling health. In this study a series of soil DNA extraction methods and diagnostic techniques were compared to identify the easiest to use and most effective method for the detection of Fusarium graminearum, Microdochium nivale, M. majus, Rhizoctonia solani and Pythium spp. in soils sampled from UK and Europe. Diagnostic methods used for comparison included DNA quantification using real-time qPCR, LAMP and MinION oxford nanopore technology. Results showed that soil sample size affects the efficiency of extraction for pathogen DNA detection. The LAMP assay showed potential trade-offs between sensitivity and specificity but was identified as the most easily deployed method for commercial testing for pathogen detection and semi-quantification. LAMP and Nanopore sequencing analyses were comparable to qPCR analysis for F. graminearum, R. solani and Pythium spp. Nanopore sequencing provided further information on the wider network of pathogens present in the soil. The findings from these studies can be utilised for further development of field-based rapid diagnostics for key soil-borne pathogens.

P4.2-001

FUSARIUM SPECIES ON IMPORTED VEGETABLES IN THE UK

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Text

Three Fusarium species: Fusarium graminearum, Fusarium chlamydosporum and Fusarium oxysporum were tentatively identified among fungi isolated from green beans (Phaseolus vulgaris) and okra (Abelmoschus esculentus), imported into the UK from Kenya and Thailand, respectively. Isolates were identified using a combination of morphological characters on potato dextrose agar and ITS sequencing. Fusarium chlamydosporum was isolated from 35 and 50% of the green beans and okra, respectively, where as F. graminearum and Fusarium oxysporum were isolated only from green beans at 75 and 50%, respectively. These Fusarium species are well-known pathogens of many important crops plants. The presence of these potentially damaging pathogens on imported crops demonstrates the threat posed to local agriculture and horticulture enterprises by international trade pathway in plants and plant products.

P4.2-002

ABUNDANCE, DIVERSITY, AND PHYLOGENETIC STUDY OF THE FABA BEAN FOOT AND ROOT ROT DISEASE COMPLEX IN THE UNITED KINGDOM

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Text

The root rot disease complex comprises a group of organisms (Fungi, Bacteria, viruses and oomycetes) that affect a very broad spectrum of crops worldwide.
The United Kingdom is one of the most important faba bean producers after Ethiopia and Australia. Foot and root rot of faba bean is an important disease that is reported as a limiting factor for faba bean production in many areas, including the UK. The causal agents are not fully characterised, and progress on control measures such as identifying resistant germplasm through to predictive diagnostics for land management are hampered as a result. Infected plant samples and soil samples will be collected from faba bean growing areas in the United Kingdom. Putative pathogens will be isolated, and pathogenicity will be tested for the isolates obtained. DNA barcoding using ITS and TEF1α will be used to identify the pathogenic organisms and species-specific qPCR assays will be developed to detect the essential and dominant fungal species in the UK. qPCR will be used to perform a larger survey of faba bean growing areas. In addition, the qPCR will form an essential component of a soil risk prediction tool to enable growers to understand the risk of disease on new land in production.

P4.2-003

THE POWER OF ELECTROCHEMICAL BIOSENSORS FOR BOTRYTIS SSP. DIAGNOSTICS AND APPLICATION IN IDM PRACTICES

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Text

Botrytis cinerea is a necrotrophic fungus that causes botrytis grey mould (BGM), a widespread and destructive disease affecting many broadacre and horticultural species. The pathogen travels particularly quickly through mature and close-canopy crops during conducive cool and wet growing seasons, leading to multiple fungicide applications and frequent crop loss. Therefore, early, fast and accurate diagnosis of B. cinerea in the field is essential for best informed disease management practices, to avoid wrongly timed and costly under or over-spraying. To aid in decision-making on optimal spray timing, an electrochemical molecular biosensor device for detecting, quantifying and discriminating B. cinerea, and to discriminate it from the co-occurring B. fabae pathogen was developed. The device detected the target Botrytis ssp. down to picogram levels in three field locations in southern Australia. This has significant advantages over Loop-mediated isothermal amplification (LAMP) and other portable diagnostics devices, since no amplification is required, and a quantitative result is realised within minutes at extremely low application and resource cost. In summary, this new device represents a powerful tool for future on-farm informed disease management scenarios with potential for broad application.

P4.2-004

GENOME-WIDE PROFILING OF OSDRB1-ASSOCIATED RNAS USING TARGETED RNA EDITING IN RICE

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Text

RNA-binding proteins (RBPs) play essential roles in regulating gene expression. However, the RNA ligands of RBPs are poorly understood in plants, not least due to the lack of efficient tools for genome-wide identification of RBP-bound RNAs. An RBP-fused ADAR (adenosine

deaminase acting on RNA) can edit RBP-bound RNAs, which allows efficient identification of RNA ligands of RBPs in vivo. Here, we report the RNA editing activities of ADARdd (ADAR deaminase domain) in plants and then engineered ADARdd to identify the RNA ligands of rice Double-stranded RNA Binding Protein 1 (OsDRB1), which is closely related to the rice responses to multiple (a)biotic stress. Protoplast experiments indicated that RBP-ADARdd fusions efficiently edit adenosines within 41 nt of their binding sites. Overexpressing the OsDRB1-ADARdd fusion protein in rice introduced thousands of A-to-G and T-to-C RNA-DNA variants (RDVs). We developed a stringent bioinformatic approach to identify A-to-I RNA edits from RDVs, which removed 99.7%-100% of background single nucleotide variants in RNA-seq data. Small RNA sequencing also identified 191 A-to-I RNA edits in miRNAs and other sRNAs, confirming that OsDRB1 is involved in sRNA biogenesis and/or function. Our study presents a valuable tool for genome-wide profiling of RNA ligands of RBPs in plants and provides a global view of OsDRB1-binding RNAs, offering a novel insights into RBPs in rice immunity against pathogens and other stresses.

P4.2-005

CHARACTERIZATION AND DIVERSITY OF PECTOBACTERIUM AND DICKEYA SPECIES IN THE NETHERLANDS

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Text

Many members of the genera *Pectobacterium* and *Dickeya* cause important plant diseases on a large range of host plants. Accurate taxonomic determination is necessary to perform epidemiological studies and to take the right measures to restrict pathogen spread. Surveys performed in the last two decades on various host plants in the Netherlands resulted in a high number of representatives of these genera. Their accurate identification in the past was rather complicated due to lack of specific tests. This, in combination with recent taxonomic changes made it hard to get insight in the epidemiology of these soft rot pathogens. This study focusses on the characterization of 70 of these isolates. Using Illumina sequencing, whole genome sequences were generated and the assembled genomes were compared with the genomes of the type strains that were available at NCBI or were generated as part of this project. This analyses revealed that many *Pectobacterium* and *Dickeya* species do occur in the Netherlands and that for some species high intra-species variation exists. Although none of these isolates originated from potato, several of them belong to species that are known potato pathogens. Based on their phylogenetic relationship, isolates have been selected and tested for their virulence on potato. Additionally, the use of MALDI-TOF MS for the reliable identification of isolates belonging to *Pectobacterium* and *Dickeya* species has been investigated. Preliminary results will be discussed.

P4.2-006

EARLY DETECTION OF PHYLLACTINIA GUTTATA, THE CAUSAL AGENT OF POWDERY MILDEW, THROUGH THE USE OF SPORE HUNTING AND PCR SPECIFIC PRIMERS IN EUROPEAN HAZELNUT (CORYLUS AVELLANA L.) ORCHARDS

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Text

European hazelnut occupies an approximate area of 30.000 ha between the Maule and La Araucanía Regions in Chile. In recent seasons there has been a greater recurrence of powdery mildew, caused by the fungus *Phyllactinia guttata*, an obligate parasite that reduces the photosynthetic rate of the tree and influences the aging of the plants. Currently, there are no registered control alternatives for this disease in European hazelnut in Chile, then developing integrated and sustainable management strategies to management powdery mildew is required. Specific PCR primer sets were validated for early detection of the fungus in the field. Through the comparison of 5 sets of primers, the specificity was validated in the DNA of 13 recurrent species of powdery mildew present in the mentioned area. DNA was extracted from spore-hunting equipment tapes obtained weekly during spring to validate the ability of specific primers to detect the presence of *P. guttata* in three hazelnut orchards. Results showed that the PG 2 (f/r) primer set, with an amplification of 375 bp, is capable of specifically detecting *P. guttata* when extracting samples from spore hunting tapes, without detecting the presence of other powdery mildew species. This early detection allowed to start control treatment programs two weeks before to detect sign of the pathogen and improving the control of the disease.

P4.2-007

THERMAL TOLERANCES AND MOLECULAR PHYLOGENY OF THIELAVIOPSIS PARADOXA ISOLATES.

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Text

Many diseases of forest trees and agricultural crops have been linked to the ascomycete fungal pathogen *Thielaviopsis paradoxa*. This study compared the growth rate of 41 isolates

of *T. paradoxa* from different hosts and two countries (Nigeria and Papua New Guinea (PNG)) under six temperature levels (22°C, 25°C, 30°C, 32°C, 34°C and 35°C). Phylogenetic relationships were obtained from the analysis of their rDNA- internal transcribed sequence (ITS) data. All the PNG isolates and few from Nigeria grew optimally between 22°C and 32°C, the majority had their highest growth rate (2.9 cmd-1) between 25°C and 32°C. Growth performances were generally low between 34°C and 35°C; no sugar cane isolate grew at these high temperatures. The oil palm isolate DA029 was the most resilient with the highest growth rate (0.97cmd-1) at 35°C. Phylogenetic analysis delineated 3 clusters: a very large clade with the majority (31 Nigerian and 4 PNG oil palm isolates), a four-member and the smallest clade with two members. To a large extent, the clustering pattern failed to address the temperature-isolate growth relationship. However, only the smallest clade has members with perfectly matching temperature tolerances. A wider analyses with more diverse isolates and genetic markers could provide better insight on thermal resilience of *T. paradoxa*. The information provided will help in formulating effective management and control strategies against the pathogen especially in this era of climate change.

P4.2-008

RACE-SPECIFIC DETECTION AS THE FIRST STEP IN DISEASE MANAGEMENT OF FUSARIUM WILT IN LETTUCE

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Text

Fusarium oxysporum f. sp. *lactucae* (Fol), the cause of wilting and vascular browning in lettuce, has spread rapidly in Western Europe in recent years. In Belgium, two races have been identified: race 1 and race 4. Race 4 has been especially problematic, causing significant losses for the soil-grown lettuce industry. Race-specific and sensitive real-time PCR assays were developed for Fol race 1 and race 4. Based on Genotyping-by-sequencing, unique DNA loci in both races were identified and used to develop primers and hydrolysis probes. To ensure sensitive detection in soil and swabs from surfaces, we included an enrichment step based on incubation of the sample in a semi-selective Fusarium medium. By controlling the incubation conditions, it was possible to relate the real-time PCR signal to the number of spore equivalents in the original sample. The enrichment step results in the detection of exclusively living fungal propagules, allowing evaluation of control measures. Using this method, the epidemiology of Fol race 1 and race 4 was studied at lettuce farms and nurseries by sampling soil, irrigation water, surfaces of farming equipment, plant boxes, etc... The pathogen was found on several surfaces of farming equipment, indicating that hygienic measures remain important in stopping the spread. No detection occurred at plant nurseries, implying that these are currently not contributing in the spread of Fol. The novel assays are currently applied to assess potential control measures.

P4.2-009

METAFLORE 2.0: AN INNOVATIVE APPROACH TO DETECT PATHOGENIC FUNGAL AND BACTERIAL MICROORGANISMS IN SEEDS USING MINION SEQUENCING

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Text

Fungal, oomycete, and bacterial pathogens cause substantial losses in agriculture and forestry, but rapid and accurate identification can reduce disease impact and biocide costs. The Plant Health Laboratory (LSV) developed analysis methods based on detection and morphological characterization through conventional cultivation for determining the sanitary quality of seeds. However, these methods are time-consuming, prone to false negatives, only detects cultivable phytopathogens and therefore, they do not guarantee the detection of many others.

The Metaflore 2.0 project aims to revolutionize the field of seed health analysis by leveraging the power of the Oxford Nanopore MinION third-generation sequencer to detect pathogenic microorganisms in seeds. The project seeks to implement new and more efficient protocols, close to the metagenomic methods, for diagnosing plant pathogens. These analyzes make it possible to reduce the analysis time while being more exhaustive on pathogen detection.

By sequencing long DNA fragments from the hypervariable regions (16S/18S rDNA, Internal Transcribed Spacer (ITS)) of the microorganisms genome, the MinION sequencer provides easier identification and differentiation of species that are genetically very close, streamlining the diagnostic process and turning decision-making faster and more efficient.

P4.2-010

EVALUATION OF MOLECULAR TESTS FOR THE DETECTION 'CANDIDATUS LIBERIBACTER' SPECIES ASSOCIATED WITH HUANGLONGBING DISEASE IN CITRUS: RESULTS FROM AN INTERNATIONAL TEST PERFORMANCE STUDY AND A PROFICIENCY TEST

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Text

Huanglongbing (HLB), also known as Citrus greening disease, is a lethal disease of *Citrus* caused by three obligate biotrophic bacteria: '*Candidatus* Liberibacter africanus', '*Candidatus* Liberibacter americanus', and '*Candidatus* Liberibacter asiaticus'. These quarantine pests are considered priority pests by the European Union due to their potential economic, environmental, or social impact. Various tests have been developed for the detection of HLB-associated bacteria in host tissues. An international test performance study (TPS) organized by the Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP) in its role as

EU Reference Laboratory for pests of plants on bacteria (EURL) in 2020 evaluated three promising molecular tests for the detection of '*Candidatus* Liberibacter' species. These tests can be considered fit for purpose and are recommended for routine testing of survey samples of *Citrus* leaves for the detection of '*Candidatus* Liberibacter' species causing HLB.

A Proficiency Test (PT) on the molecular detection of HLB was organized in 2021 by NIVIP to assess the diagnostic competence of EU national reference laboratories (NRLs) to detect this bacterium in *Citrus*. Results revealed a high level of accuracy among participating laboratories. No false negatives were reported and only in a few cases HLB was confused with a sample containing '*Candidatus* Liberibacter' solanacearum, the zebra chip disease of potato.

P4.2-011

FUNGI SPECIES ASSOCIATED WITH POTATO APHIDS IN BAMENDA, NORTHWEST REGION OF CAMEROON.

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Text

Potato (Solanum tuberosum L.) is one of the world's most important cultivated tuber crop. There has been inaccurate cultural identification of fungi species associated with potato aphids. The aim of the study was to identify fungi associated with aphids of potato using a combined cultural and molecular method. Insect cadavers were periodically collected in the field (100 samples) and taken to the laboratory where they were cultured on potato dextrose agar. After a period of 7 days, they were subculture to obtain pure cultures. Cultural characters were measured and noted, and the mycelia of the pure cultures were harvested and stored in 10% glycerol. Molecular identification was done for the ITS and TEF regions. The species identity of the sequences was Identified using BLAST. Results of cultural identification gave three different groups of fungi, identified as Fusarium species, different species of Aspergillus including A. flavus, A. niger and A. fumigatus and Penicilium species. After performing the BLAST, 19 fungal species were identified belonging to Fusarium, Chaetomium, Trichoderma, Aspergillus, Cladosporium, Periconia, Claviceps, Curvularia, Microascus and Penicillium. These results portray a diverse group of fungi associated with potato aphids. There is great possibility that some of them may be entomopathogenic and hence, further research is ongoing to use them as biocontrol against potato aphids as a component of integrated pest management.

P4.2-012

A NEW DIAGNOSTIC TOOL FOR THE IDENTIFICATION OF FOUR BEET YELLOWS VIRUSES BY MULTIPLEX RT-QPCR

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Text
Virus yellows disease (VY) of sugar beet is caused in Europe by a complex of four aphid-transmissible virus species present as mono- or co-infections: beet yellows virus (BYV), beet chlorosis virus (BChV), beet mild yellowing virus (BMYV) and beet mosaic virus (BtMV). VY can impact yields by up to 50%. The losses were limited with neonicotinoids (NNI), that are now banned in UE.
To find alternatives to NNI, the National Plan of Research and Innovation has financed the Yellows Resistbeet project led by GEVES and involved in the evaluation of varietal resistance. As the ELISA method does not distinguish BChV from BMYV detection, BioGEVES with INRAE Colmar developed primer and probe pairs to detect and identify the four viruses simultaneously in a same sample.
To ensure viral diagnosis, GEVES validated a multiplex RT-qPCR method by evaluating performance criteria. Our first results showed that all primers and probes are specific to the four targeted viruses with 100% inclusivity and exclusivity and that RT-qPCR multiplex is 100 to 10,000 times more sensitive than ELISA.
Our new diagnostic tool is used for varietal resistance testing to control inocula and to provide in one step the viral composition as well as semi-quantitative data on the viral load in sugar beets tested. This detection method at a lower cost has been validated and offered as a service by GEVES. Later, it can be used in epidemiological surveillance to monitor the evolution of VY in the field depending on the year.

P4.2-013

MALDI-TOF MS AS AN ACCREDITED METHOD FOR RAPID AND ACCURATE IDENTIFICATION OF REGULATED PLANT PATHOGENIC BACTERIA

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Text
MALDI-TOF MS is an accurate technique for identification of bacteria on species level. Until recently, the technique was poorly implemented in phytobacteriology but it has shown recently it’s potential, even at a subspecies level. The lack of reference spectra for most plant pathogens in commercially available databases was solved relatively easy by creating the missing reference spectra in-house. Sets of reference spectra for regulated bacteria were validated according to international standards. Several performance characteristics were evaluated of which the analytical specifity is the most important as it reflects the accuracy of the technique. Experiments on inclusion (lack of false negatives) and exclusion (lack of false positives) were carried out, using strains of the target bacteria, close relatives and bacteria known to occur on relevant hosts of the target. Results confirm the accuracy on species level for the regulated bacteria Ralstonia solanacearum, R. syzygii, Clavibacter sepedonicus, C. michiganensis subsp. michiganensis, Curtobacterium flaccumfaciens, Pantoea stewartii, Erwinia amylovora and Acidovorax citrulli. For Ralstonia pseudosolanacearum the technique discriminates even below the species level, in phylotypes. Although the technique shows

poor seperation of pathovars or subspecies within a species, it still serves as a complementary technique for identification purposes, which is currently recommended in international diagnostic protocols.

P4.2-014

PRACTICAL APPLICATIONS IN PATHOGEN DETECTION: STRATEGIES AND PROGRESS OF A SENSITIVE SURVEILLANCE TOOL FOR DETECTING THE LAUREL WILT PATHOGEN

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Text
Laurel wilt (LW), caused by the ambrosia fungus *Harringtonia lauricola*, is a lethal vascular disease affecting numerous hosts in the Lauraceae, including important forest species and avocado trees. The disease has caused the death of 300,000 avocado trees and as many as half-a-billion native trees. Laurel wilt has experienced a rapid geographic expansion through host and vector jumping, which in turn generated a critical need for the development of early detection and monitoring tools. Monitoring *H. lauricola* is challenging due to the lack of selective media, the low titer of the pathogen often carried by alternative vectors, the abundance of secondary or transient fungal associates, and the recalcitrant nature of the target samples. Recent advances in molecular techniques and the development of specific markers have facilitated the detection of the pathogen directly from host and insect's DNA extracts. Screening susceptible hosts and potential alternative vectors for the presence of the LW pathogen represents a critical tool to prevent the spread and monitor the arrival and establishment of this devastating disease into larger avocado production areas. This presentation shares current molecular protocols available for the direct detection of the LW causal agent, and updates on progress in optimizing detection accuracy and sensitivity. These technologies are directly adoptable for use in the high-throughput screening of beetle trap samples from regional surveillance programs.

P4.2-015

DEVELOPMENT OF RAPID DNA-BASED DETECTION ASSAYS FOR COLLETOTRICHUM SPECIES CAUSING APPLE BITTER ROT IN THE MID-ATLANTIC U.S.A.

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Text
Bitter rot of apple is caused by many *Colletotrichum* species in the Mid-Atlantic U.S.A., and this can confound management strategies due to differences in life cycle, ecology and

pathogenicity among *Colletotrichum* species, in addition to differences in susceptibility of apple cultivars. A fast, inexpensive method for identifying *Colletotrichum* spp. would facilitate earlier classification of the causal species of bitter rot and future epidemiological studies. Alignments of 1,344 GenBank accessions were surveyed visually for areas of high DNA polymorphism in 12 gene regions in order to develop species-specific hydrolysis-probe qPCRs for the key players in the Mid-Atlantic USA: *C. fioriniae* and *C. nymphaeae* in the *Colletotrichum acutatum* species complex, and *C. chrysophilum, C. fructicola, C. gloeosporioides* s.s., *C. henanense, C. noveboracense, C. siamense,* and *C. theobromicola* in the *Colletotrichum gloeosporioides* species complex. Preliminary results indicate that the newly designed primer-probe sets for *C. fioriniae* (CAL), *C. gloeosporioides* s.s. (GAPDH), *C. henanense* (APN2), and *C. theobromicola* (TUB2) were highly species-specific. Further optimization is ongoing and will be necessary for other species but early tests were encouraging.

P4.2-016

DIAGNOSIS OF BACTERIAL AND NEMATODE INFECTION IN RYEGRASS SEED THROUGH METABOLITE VARIATIONS USING DIRECT IMMERSION SOLID-PHASE MICROEXTRACTION (DI-SPME) WITH GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

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Text

Annual ryegrass toxicity (ARGT) is an often-fatal poisoning of livestock that consume annual ryegrass infected by the bacterium Rathayibacter toxicus. This bacterium is carried into the ryegrass by a nematode, Anguina funesta, and produces toxins within seed galls. The actual mechanism of production of this toxin remains unclear and no clear-cut information is available on what type of volatile organic compounds accumulate in the infected galls. Therefore, to fill this research gap, the study was designed to analyze the chemical differences among nematode galls, bacterial galls and healthy seeds of annual ryegrass by using direct immersion solid-phase microextraction (DI-SPME) coupled with GC-MS. Overall, 48 compounds were identified in all three groups. Five volatile organic compounds are the most frequent indicators of bacterial infection, whereas the presence of 15-methylnonacosane, 13-methylheptacosane, ethyl hexacosyl ether, heptacosyl acetate and heptacosyl trifluoroacetate indicates nematode infestation. Metabolites occurring in both bacterial and nematode galls included batilol (stearyl monoglyceride) and 9-octadecenoic acid (Z)-, tetradecyl ester. This study demonstrated that DI-SPME is a valid technique to study differentially expressed metabolites in infected and healthy ryegrass seed and it may help to understand the biochemical interactions between plant and pathogen to aid in management of ARGT.

P4.2-017

SIMPLIFIED PLANT PATHOGENS DETECTION WITH AUTOMATED NUCLEIC ACID EXTRACTION AND INHIBITOR RESISTANT QPCR MASTER MIXES

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Text

Plant pathogens that cause plant diseases can harm our agricultural ecosystems and have devastating impacts on our livelihoods. Researchers in agriculture and phytopathology routine testing labs play a key role in monitoring and managing plant diseases.
The key to managing the spread of plant pathogens is rapid and accurate detection. Molecular techniques can be very efficient in detecting plant viruses, viroids, bacteria, fungi and yeast, provided that robust nucleic acid extraction methods and amplification reagents are available.
In this poster, we describe optimized protocols for the molecular detection of bacterial and viral plant pathogens, respectively *Xylella fastidiosa* (Xf) and Rose Rosette Virus (RRV). Xf causes olive trees death within 1 or 2 years after infection. With no existing cure and a worldwide spread, it causes immense destruction to our environment and dramatically affects economy of olive oil industry. RRV is an RNA virus infecting rose and eventually causing death. Widely spread in North America, surveillance is currently reinforced in Europe to avoid outbreak.
We have developed automated extraction techniques based on paramagnetic beads allowing isolation of high-quality pathogenic DNA or RNA from different plant parts with minimal hands-on. We will also discuss the use of optimized amplification master mixes, to overcome potential inhibition, frequently observed with plant samples.

P4.2-018

HARNESSING THE POWER OF COMPARATIVE GENOMICS TO SUPPORT DISTINCTION OF CRYPTIC SPECIES WITHIN PHYLLOSTICTA AND DEVELOPMENT OF HIGHLY SPECIFIC DETECTION OF PHYLLOSTICTA CITRICARPA CAUSING CITRUS BLACK SPOT BY REAL-TIME PCR

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Text

Phyllosticta citricarpa is quarantine fungus in the European Union. If introduced in the EU, it would jeopardize the citrus growing sector. Strict controls are regularly performed to verify its absence in fruit imports. Its sister species *P. paracitricarpa* was described in 2017, and was only distinguished from *P. citricarpa* by the occurrence of few SNP over the sequences of two housekeeping genes. In Europe, this new taxon has been reported in Greece, on lemon leaf litter, but has not being found as a pathogen on fruits. Its presence was later confirmed in China, after re-examination of *P. citricarpa* strains, but it is still uncertain if this new species deserves consideration as a quarantine pathogen. After a request from the European Commission, we re-examined the taxonomy of these two species and aimed at the development of a specific detection assay targeting *P. citricarpa*. Genomes of representative strains of *P. citricarpa*, *P. paracitricarpa* and other *Phyllosticta* species were sequenced. Based on 64 genes we assessed the phylogeny of these pathogens. We showed that the *P. citricarpa* and *P. paracitricarpa* clustered in two different clades, supporting the description of *P. paracitricarpa* as a new species. This genomic dataset was used to select a *P. citricarpa* species-specific marker and new real-time PCR assay was developed. These results demonstrate the power of genomics to solve taxonomic issues, with great consequences in terms of phytosanitary regulations.

P4.2-019

DEVELOPMENT OF DETECTION TECHNOLOGY FOR TOMATO PITH NECROSIS CAUSED BY PSEUDOMONAS MEDITERRANEA

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Text

The phytopathogenic bacterium Pseudomonas mediterranea mainly infects Solanaceae crops and can be transmitted through soil. In recent years, the pathogen has caused pith necrosis and wilt symptoms on tomato plants in Taiwan. For rapid diagnosis of the disease, the bacteria-specific primers were designed to be both used in polymerase chain reaction (PCR) and recombinase polymerase amplification (RPA) detection of pathogen. The specific PCR products were amplified for the pathogen in electrophoresis analysis. The commercial strips were used to test RPA products. The positive reaction was seen in diseased tomato DNA extracts and negative in healthy tomato DNA extracts. The technology can support farmers in diagnosing crop diseases, seedling propagators to control product quality independently, and biotechnology companies to detect plant pathogens to improve the health and quality of crops and seedlings.

P4.2-020

FIRST REPORT OF WATERMELON CRINKLE LEAF-ASSOCIATED VIRUS 1 AND 2 (WCLAV-1 AND -2) INFECTING STRAIGHTNECK SQUASH IN THE UNITED STATES

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Text
In fall 2022, straightneck squash showing mild leaf crinkling, as well as leaf and fruit mosaic symptoms were observed in scattered areas of a ~15-ha field in Jackson County, Florida. Seventeen plants were sampled randomly for testing. Plants tested negative for zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV) and squash mosaic virus (SqMV), using ImmunoStrips (Agdia, USA, Cat No: 77700, 44501, 26400 respectively). A conventional one-step RT-PCR (NEB, USA) was used to test all plants for cucurbit chlorotic yellows virus (CCYV) (Jailani et al. 2021), and watermelon crinkle leaf-associated virus WCLaV-1 and WCLaV-2 (Hernandez et.al. 2021). All plants tested negative for CCYV, and 12 out of 17 plants tested positive for WCLaV-1 and WCLaV-2 using primers targeting RNA dependent RNA polymerase (RdRP), and movement protein (MP) genes of both viruses (Hernandez et al., 2021). The partial RdRP sequences for WCLaV-1 (OP389252) and WCLaV-2 (OP389254) shared 99% and 98% nt identity with isolates KY781184 and KY781187 from China; the partial MP sequences for WCLaV-1 (OP389253) and WCLaV-2 (OP389255) shared 98% and 95% nt identity with isolate from Brazil (LC636069) and China (MW751425). Previously, both viruses were first reported in Texas, (Hernandez et al., 2021), Florida (Hendricks et al., 2021), Oklahoma (Gilford and Ali., 2022), and Georgia (Adeleke et al., 2022) in watermelon. This is the first report of WCLaV-1 and WCLaV-2 in straightneck squash in the USA.

P4.2-021

DIAGNOSIS OF FUSARIUM OXYSPORUM F. SP. CICERIS CAUSING FUSARIUM WILT OF CHICKPEA USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AND CONVENTIONAL END-POINT PCR

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Text
Fusarium oxysporum (Fo) is ubiquitous in soil and forms a species complex of pathogenic and putatively non-pathogenic strains. Pathogenic strains cause disease in over 150 plant species. Fusarium oxysporum f. sp. ciceris (Foc) is a major fungal pathogen causing Fusarium wilt in chickpeas (Cicer arietinum). In some countries such as Australia, Foc is a high-priority pest of biosecurity concern. Specific, sensitive, robust and rapid diagnostic assays serve as an effective biosecurity control measure and are essential for effective disease management on the farm. We developed and validated a novel and highly specific PCR and a LAMP assay for detecting the Indian Foc race 1 based on a putative effector gene uniquely present in its genome. These assays were assessed against 39 Fo formae speciales and found to be specific, only amplifying the target species, in a portable real-time fluorometer (Genie III) and qPCR machine in under 13 minutes with an anneal derivative temperature ranging from 87.7 – 88.3°C. The LAMP assay is sensitive to low levels of target DNA (>0.009 ng/µl). The expected PCR product size is 143 bp. The LAMP assay developed

in this study was simple, fast, sensitive and specific and could be explored for other Foc races due to the uniqueness of this marker to the Foc genome.

P4.2-022

DEVELOPMENT OF MOLECULAR DIAGNOSTIC METHODS FOR AGROBACTERIM SP. CAUSING ROOT MAT DISEASES

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Text

In June 2019, root mat disease was observed in hydroponically cultivated tomatoes in Jinju, South Korea, which occurred in at least 30% of the plants in the greenhouse. To isolate the causal bacteria, 10 g of infested tomato root mat sample was ground with 50 ml of sterile water. A 100-µl aliquot of the homogenate was serially diluted and spread on mannitol-glutamate medium amended with 0.1% yeast extract (MGY) and incubated at 28°C for 48 h. To confirm the identity, four housekeeping genes of GNIY2 were sequenced (16S rRNA, trpE, rpoB, and recA). Multilocus sequence analysis performed as previously showed that GNIY2 strain was grouped in *Agrobacterium* genomospecies 4. This is the first report on mat root disease caused by *Agrobacterium* biovar 1 in hydroponic tomatoes in South Korea. And then, we developed detection kits using diverse molecular biological methods based on genome sequences for *Agrobacterium* sp. The detection kit we developed enables diagnosis based on the differences in gene sequences between pathogenic strains and non-pathogenic strains. We wil study supplement the detection kit that enables more precise diagnosis through sequencing of pathogenic strains and non-pathogenic strains.

P4.2-023

DIVERSITY OF COLLETOTRICHUM SPECIES COMPLEXES ASSOCIATED WITH FRUIT ANTHRACNOSE IN SOUTH KOREA AND THEIR SENSITIVITY TO DIFFERENT FUNGICIDES

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Text

Several Colletotrichum species responsible for the anthracnose of apple, persimmon, plum, peach, and grape were identified by the present study based on molecular analysis, six genes and morphological characteristics, as well as pathogenicity test. The in-vitro sensitivity of identified Colletotrichum species to the common fungicides using for the control of anthracnose in South Korea was also evaluated. Seven Colletotrichum species including C. gloeosporioides, C. siamense, C. fructicola, C. vinifera, C. aenigma from the C.

gloeosporioides species complex, and C. fioriniae, and C. nymphaeae from the C. acutatum species complex were identified as the causal agent of anthracnose of these common fruits. The result also indicated that several Colletotrichum species are associated with a single host. C. siamense, C. fructicola and C. nymphaeae were isolated from apple, peach plum and persimmon and C. gloeosporioides and C. fioriniae were from apple and plum. In jujube, C. gloeosporioides and C. nymphaeae were identified.The anthracnose of grape caused by C. siamense, C. fructicola and C. vinifera. There were significant differences among the virulence of these Colletotrichum species to the same host in the development of anthracnose. Fungicides sensitivity test showed that the EC50 value of specific Colletotrichum species varied greatly among the fungicides. Same Colletotrichum species isolated from different host showed different sensitivity against same fungicide

P4.2-024

DEVELOPMENT OF A REAL-TIME PCR FOR THE DETECTION OF BLUEBERRY RUST (THEKOPSORA MINIMA) AND ITS PERFORMANCE DURING A UK OUTBREAK.

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Text

Thekopsora minima (syn. *Pucciniastrum minimum*) is a rust species which infects the economically important host highbush blueberry (*Vaccinium corymbosum*). It was added to the EPPO A2 list in 2017 as it had spread from North America and Japan to numerous European countries. On blueberry, *T. minima* causes small, yellow necrotic spots on the upper surface of leaves which as they enlarge and coalesce can lead to extensive defoliation. The entry risk of *T. minima* into a new country is high and most likely to occur through global trade of *Vaccinium* plants for planting. Therefore, it is essential that effective phytosanitary control measures are in place to test imports. The morphological appearance of *T. minima* is similar to native rusts on blueberries, such as *Naohidemyces vaccinii*. Therefore, we developed a molecular test to facilitate fast and reliable detection of *T. minima* on symptomatic blueberry leaves. The test was specific to *T. minima* when tested against a range of non-target pathogens found on blueberry and the test also proved highly sensitive, with a limit of detection of 10 spores on a sample for DNA extraction. In September 2021 *T. minima* was detected in the UK in a nursery in Perthshire, Scotland. This initiated a survey of other sites in the UK with samples being sent to Fera diagnostic laboratories for analysis. This new test was shown to be an effective tool in the diagnostic process for the rapid and sensitive detection of *T. minima* on blueberry plants.

P4.2-025

A MULTIPLEX REAL-TIME PCR ASSAY FOR THE UNIVERSAL DETECTION OF ORCHID FLECK VIRUS AND DIFFERENTIATION AMONG ITS FOUR STRAINS INFECTING MULTIPLE HOSTS

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Text

In the regulatory environment, the one-tube reverse transcription quantitative polymerase chain reaction (RT-qPCR) is mostly used for RNA-virus detection due to its rapidity, sensitivity, reproducibility, and to reduce risk of carry-over contamination. Recent findings of Orchid fleck virus (OFV) in citrus in South Africa and Hawaii, highlighted the need for a sensitive and specific RT-qPCR method for OFV detection. There are two orchid and two citrus strains of OFV. To know the presence of OFV and its strain in citrus, orchid and ornamentals, a panel of duplex and multiplex TaqMan RT-qPCR assays were optimized using total RNA of all four OFV strains. For developing generic and strain-specific primers and probes of OFV, conserved region of L-gene and variable regions of P- and G -genes were targeted along with the plant internal control Nad5 gene. Comparison between the Ct value of duplex and multiplex RT-qPCR assays of each strain indicates that there is no significant interference on assay sensitivities caused by multiplexing. The limit of detection (LOD) range between ~10-3 to 10-4 dilution. Specificity test on 90 citrus leprosis samples, 40 orchid and ornamental host target samples and 20 non-target samples revealed that both duplex and multiplex RT-qPCR panel are 100% specific, and there is no cross amplification observed. Results of RT-qPCR were also verified with high throughput sequencing followed by electronic (e) probe detection method.

P4.2-026

DEVELOPMENT OF DIAGNOSTIC METHODS FOR HOP VIROIDS

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Text

Intensive monoculture production, climate change, global trade, and the evolutionary ability of pathogens to adapt rapidly are the main causes of current epidemics in man-made agroecosystems that have become more susceptible to pathogens. Incurable plant diseases caused by viroids can limit crop production and quality and result in significant losses. One such example is the destruction of nearly 500 ha of Slovenian hop yards by the outbreak of the citrus bark cracking viroid (CBCVd). An important factor in preventing its spread is the use of rapid and reliable diagnostic tools. Due to the nature of viroids, which are non-coding naked RNAs, limits their detection only to RNA level. We developed and validated a methodology for reliable and sensitive RT-qPCR assays for individual and combined detection of CBCVd, hop latent viroid (HLVd), and hop stunt viroid (HSVd). The developed assays were found to be specific, reliable and suitable for rapid screening of hop viroids on a large-scale to prevent further spread of disease. In addition, we are developing CRISPR/Cas-RT-RPA assay that has the advantage of being applicable in field-based scenarios, as the tests require minimal sample preparation and are performed at constant temperature without the use of sophisticated equipment. The method will be used for rapid

detection of new outbreaks, evaluation of cultivar resistance, and epidemiological studies. This will limit further spread of the disease and the economic damage to hops.

P4.2-027

CURTOBACTERIUM FLACCUMFACIENS PV. FLACCUMFACIENS, A CHOICE OF DETECTION TARGETS

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Text

Detection of a target pathogen is ideally based on a unique genetic region, likely a phylogenetically informative gene or a pathogenicity gene. PCR of such targets can be used to detect and quantify the pathogen in host tissues or environmental samples, and report on relative susceptibility or pathogen sources. For *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*), the cause of tan spot in beans, the target for detection is not necessarily a singular choice. Recently, the most used target for detecting *Cff* was reported to be a gene on a plasmid, with the presence of the plasmid linked to virulence on mung beans in Australia. Strains missing the plasmid were also described, suggesting the potential to miss detecting portions of the *Cff* population in mixed samples. To improve detection, a PCR assay was designed targeting a conserved region of the *gyrB* gene on the main chromosome of *Cff*. Assays for both regions were combined in a droplet digital PCR assay to confirm detection of both targets in the pathogen DNA. Interestingly, based on *gyrB* being a single copy gene, the plasmid target was detected as twice as many copies in at least six strains. Investigations of more strains are ongoing to determine if there is copy number variation among *Cff* strains, and what effect that may have on virulence. These relatively simple tools will enable more informed investigations of *Cff* populations and their dynamics within plant tissues, between different strains and in the environment.

P4.2-029

COMPARISON BETWEEN FLUORESCENCE IMAGING TECHNIQUES AND LAMP FOR EARLY DETECTION OF SEVERAL PLANT PATHOGENS

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Text

Chlorophyll Fluorescence (Chl F) Imaging: An extremely important technique for the non-invasive study of photosynthesis dynamics in intact plants, algae, and in cyanobacteria is the measurement of Chl F kinetics. Light is absorbed by plants to be used in photosynthesis, but not all energy from this light is used. Some of the energy is emitted as fluorescent light. It is this emitted light that we collect and use to diagnose the plant health, unhealthy plants are

less efficient at using absorbed light in photosynthesis and so will emit more as fluorescence. Herbicides can reduce the photosynthetic capacity to zero, as can a strong pulse of light that transiently congests the photosynthetic electron transport pathway. so, these techniques are very useful for crop monitoring in alleviating stress at an early stage and thus substantially reducing yield losses. To confirm the imaging result, molecular method should be followed, thus, Loop-mediated isothermal amplification (LAMP) technique has been widely used due to its high efficiency, specificity, simplicity and quickness. LAMP has three great advances, 1- it can be carried out at a constant temperature with a short reaction time, which makes LAMP ideal for point-of-care detection of pathogens in open fields. 2- LAMP is relatively cost effective, as it requires simple equipment. 3- It has very high amplification efficiency and sensitivity as it generates large amounts of PCR products with low amounts of DNA.

P4.2-030

PREDICTING SOILBORNE DISEASE RISK OF PULSE CROPS IN MONTANA, UNITED STATES

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Text

Soilborne diseases have been a constraint on performance and sustainability of pulse crops worldwide. The pathogens associated with disease can be difficult to manage due to long-lived survival structures, limited fungicide options, and large host ranges. When environmental conditions such as moisture and temperature are conducive to pathogen growth, root rot pathogens can lead to yield loss and crop failure. In Montana pulse fields, numerous pulse crop pathogens have been identified through surveys, including *Aphanomyces euteiches*, *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., and more. To monitor soilborne pathogen presence and severity, 25 fields with a history of pulse crops were soil sampled in Northeast Montana. Selected fields had grown dry pea, lentil, or chickpea in 2022, the year of sampling. Soil was included in a greenhouse bioassay with chickpea, dry pea, and lentil seed planted. DNA was extracted from plant roots and soil, for analysis of samples using qPCR. Testing is ongoing; however, preliminary bioassay and morphology results show root browning and root lesions caused by a variety of pathogens in multiple field samples. Dry pea and lentil were particularly susceptible to diseases in the bioassay, compared to chickpea roots which were generally healthier. Results from this work will assist with development of detection protocols, assist growers with management strategies, and benefit breeding efforts for plant disease resistance.

P4.2-031

A SENSITIVE IMMUNO-DOT BLOT ASSAY FOR THE EARLY DETECTION OF CHONDROSTEREUM PURPUREUM USING ANTIBODY-CONJUGATED GOLD NANOPARTICLES

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Text

Chondrostereum purpureum is the causal agent of Silverleaf, a serious fungal trunk disease on fruit crops, that decreases fruit yield and orchard's longevity. Fungal spores infect wood injuries and mycelia colonize the xylem, producing necrosis and foliar silvering due to the accumulation of the endopolygalacturonase1 (endoPG1) enzyme. The disease doesn't have an efficient control method yet, so preventive measures, like an early diagnosis, are essential. A diagnostic kit with the potential to detect low concentrations of endoPG1, when foliar symptoms are not visible yet, was developed using a Dot-blot method. The endoPG1 was targeted by polyclonal antibodies conjugated to colloidal gold nanoparticles (GNPs) of 35,88 nm size. The pH 9 and 90 ug/mL of antibody were found the optimal conditions for conjugating GNPs (OD:5) achieved through salt-induced nanoparticle aggregation. The immune-dot blot method was assembled using nitrocellulose membranes, which were spotted with 2 µL of endoPG1 concentrations ranging from 0.039 to 0.49 ug, blocked for not specific binding with 10% skim milk. The membranes were revealed using conjugated GNPs dispersions. A goodness of fit of R2 >0.95 was achieved between spotted endoPG1 concentrations and dot intensities. A detection limit of 0.1 ug endoPG1 was observed. The results demonstrate the effectiveness of the dot-blot method, making it a promising tool for the rapid diagnosis of Silverleaf disease.

P4.2-033

ARADQ: AN AUTOMATED DISEASE QUANTIFICATION SOFTWARE FOR FLOOD-INOCULATED ROSETTE-TYPE SEEDLINGS

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Text

Assessment of plant disease severity has been so far dependent on a visual rating, pathogen growth assay, and quantification stress-responsive gene expression. Despite widespread adoption in numerous studies, these methods have been limited to a small set of samples due to labor-intensive and costly processes. Digital plant phenotyping is emerging as an alternative approach. Image-based analysis enables the measurement of plant size and color in a fast and non-destructive manner, allowing high-throughput evaluation of disease symptom development. In this study, we developed an image analysis tool, the Arabidopsis Disease Quantification (AraDQ), for examination of diseased rosette-type seedlings grown on agar plates. Its deep learning pipeline automatically calculates 10 different color and morphological parameters with a high accuracy of object detection and seedling segmentation in the given image. It provides a user-friendly graphical interface without any requirements for hardware/computing platforms or programming languages, and thus is easily accessible to all researchers. We demonstrated that through two case studies characterizing bacterial and plant mutants, AraDQ can be effectively used for the detection of subtle differences in plant disease severity between samples not distinguishable by naked

eyes. AraDQ has the potential to expand our understanding of plant-microbe interactions by providing a highly reliable and robust phenotyping methods for diseased plants.

P4.2-034

IMPROVED REVERSE TRANSCRIPTION–LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) TEST FOR RAPID AND SENSITIVE DETECTION OF YAM MOSAIC VIRUS IN SEED YAM SYSTEMS

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Text

Yam (Dioscorea spp.) productivity is constrained significantly by a lack of a formal seed system. Propagation through tuber setts and seed yams encourages the recycling of infected planting materials, contributing to high virus incidence and yield losses. Efforts are ongoing to increase the production of high-quality seed yams in a formal seed system to reduce virus-induced yield losses and enhance the crop's productivity and food security. Therefore, virus detection using sensitive diagnostic tests is imperative to prevent the multiplication of infected materials.

This research aimed to evaluate and optimise existing diagnostic tests for detecting Yam mosaic virus (YMV), the most destructive yam virus in West Africa. Five diagnostic tests, comprising DAS-ELISA, IC-RT-PCR, RT-PCR, RT-LAMP, and qRT-PCR, were evaluated for specificity and sensitivity in YMV detection.

An improved RT-LAMP assay was developed for the detection of YMV. Compared to other tests, it offers a rapid, sensitive and cost-effective approach for virus detection in the seed yam systems.

P4.2-035

LISIANTHUS DISEASES ASSOCIATED WITH FUSARIUM OXYSPORUM AND ITS POPULATION DYNAMICS IN FIELDS

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Text

Lisianthus is one of the most important cut-flowers in Taiwan for export to Japan. In 2020, lisianthus plants showed root rot, stem rot, and wilting symptoms in Changhua County, Taiwan. Based on morphological characteristics, pathogenic assay, and phylogenetic

analyses, the causal agent was identified as *F. oxysporum* f. sp. *eustomae* (*Foe*) with two groups (I and II) reported by Bertoldo et al. (2014). It is the first report that *Foe* causing lisianthus diseases in Taiwan. Wounds were necessary for *Foe* to infect lisianthus, and group I and II have diverse virulence. Furthermore, optimum temperatures for group I and II growth are 24 and 24-28 ºC, respectively. Because *F. oxysporum* has been considered as species complex (FOSC), the multi-locus genes (*cmdA*, *rpb2*, *tef1*, and *tub2*) were used to identify the FOSC isolates from lisianthus to species. The result indicated that Taiwanese *Foe* group I was classified into *F. nirenbergiae*; however, *Foe* group II was distinguished from other species in FOSC. For studying the population dynamics of *Foe* group I and II in the greenhouse, two specific primer pairs, *SIX6*-220628-F/R for group I and *SIX1*-220813-F/R for group II, were designed from *Secreted in Xylem* genes. Multiplex and touchdown PCR with the specific primer pairs could successfully detect *Foe* group I and group II. The PCR test demonstrated that *Foe* group I was the dominant pathogen to cause lisianthus diseases, which started from winter and kept appearing until the crop harvest.

P4.2-036

MULTI-PHASIC IDENTIFICATION OF FUNGI CAUSING FOLIAR AND POD DISEASES OF AFRICAN YAM BEAN (SPHENOSTYLIS STENOCARPA HOCHST. EX A. RICH.) IN NIGERIA

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Text

African yam bean (AYB; Sphenostylis stenocarp) is an underutilized tuberous legume indigenous to Africa. It is a security crop with lofty nutraceutical benefits but the low grain yield caused by fungal diseases deters farmers from large-scale cultivation. The causal agents of pod and tip dieback diseases associated with AYB are largely uncharacterized. To investigate major AYB fungal diseases leading to low grain yield in this crop, a survey was conducted in 2018 in major AYB-growing areas in Nigeria. Morphological and molecular assays were conducted to identify causal agents of these diseases. Fungi from four genera were isolated from AYB leaves and pods showing disease symptoms. However, Koch's postulates were satisfied for C. truncatum and C. gleosporiodes (Cg) complex. The other three genera produced no disease symptoms in healthy AYB tissues. The morphological characteristics of the isolates in the Cg complex were similar and difficult to delineate to the species level. Therefore, a representative panel of isolates was characterized by sequencing the ITS, glyceraldehyde-3-phosphate dehydrogenase, calmodulin, and Apmat loci. A combined phylogenetic analysis revealed four Colletotrichum species: C. jasmini-sambac, C. theobromicola, C. fructicola, and C. truncatum. The results from this study provide information useful for developing integrated management strategies that may stimulate greater cultivation of AYB, which in turn can contribute to diet diversification.

P4.2-037

HIGH-RESOLUTION MELTING CURVE ANALYSIS TO DETECT SEVERAL CERATOCYSTIS SPECIES IN THE LATIN AMERICAN CLADE

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Text

The Latin American Clade (LAC) of the genus *Ceratocystis* encompasses 16 species, many of which are aggressive tree pathogens. Due to the rising importance of these pathogens, the need for a rapid and cost-effective screening protocol to detect these fungi has arisen. The aim of this study was to develop a high-resolution melting curve analysis (HRMA) based on the cerato-platanin (CP) gene region, to detect and differentiate *Ceratocystis* spp. in the LAC, and bypass the need for laborious isolation and post-PCR procedures. Primers targeting a 172bp region of the CP gene were designed to amplify all species in the LAC. The accuracy of HRMA to detect and differentiate LAC species using these CP primers, was assessed on DNA from 12 cultured LAC representatives. The 12 LAC representatives resolved into seven HRMA clusters. Cluster one contained *C. curvata*, *C. mangivora*, *C. manginecans* (Type 2), *C. fimbriatomima*, *C. platani* and *C. adelpha*. Cluster two grouped *C. manginecans* (Type 1) and *C. eucalypticola* together. The remaining clusters represented single LAC species, including *C. fimbriata*. The efficacy of the CP-based HRMA analyses was also tested and validated on DNA extracted directly from *Ceratocystis* infected wood samples obtained in field. Combined with regional historic data, this HRMA diagnostic method allows for rapid screening and semi-specific identification of *Ceratocystis* pathogens in the LAC.

P4.2-038

DEVELOPMENT OF RECOMBINASE POLYMERASE AMPLIFICATION ASSAYS FOR SPECIFIC DETECTION OF XANTHOMONAS ORYZAE PV. ORYZAE AND XANTHOMONAS ORYZAE PV. ORYZICOLA

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Text

Xanthomonas oryzae pv. *oryzae* (*Xoo*) and *Xanthomononas oryzae* pv. *oryzicola* (*Xoc*) are serious pathogens of rice and listed as Select Agents in the United States. Both are widely distributed in Asia, Africa and Australia but have not been detected in the United States. A field-deployable sensitive, and robust tool is required for monitoring, eradication, and surveillance of these high consequence pathogens. Unique genomic regions were identified for *Xoo* and *Xoc* through comparative genomics, and specific primers and probes were designed—in silico analyses showed 100% similarity with target pathogens. An RPA assay targeting the rice genome was also developed and used as an internal control. The specificity of each assay was confirmed with 114 bacterial strains (n=45 *Xoo*, n=10 *Xoc*) representing different geographical locations and time period, closely related Xanthomonads

and other related species, endophytes, healthy rice, soil and wheat. No false positives or negatives were observed. Limit of detection (LOD) of *Xoo*-specific RPA with 10-fold serially diluted pure genomic DNA and spiked (rice sap was added in each dilution) assays were 1 pg and 10 pg, respectively. The LOD for *Xoc*-specific RPA was 1 pg for both pure genomic DNA and spiked assays. Both RPA assays have capabilities to detect and discriminate the target pathogens in the presence of rice matrix. These assays have potential applications in routine diagnostics, disease management, and agricultural biosecurity.

P4.2-041

RING TEST PROVIDES THE BASIS FOR HARMONIZATION OF TOBRFV DIAGNOSTIC PROTOCOLS FOR SEEDS IN THE NAPPO REGION

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Text

Global seed trade is subjected to various national, regional and international regulations to prevent the spread of harmful seed-borne and seed-transmitted pathogens including requirements for freedom of pest certification of seed lots. When trade partners use different detection protocols, different test results may be produced requiring additional testing that can result in trade delays. Establishing comparability of the protocols used by trade partners can harmonize test results, thus benefiting trade. NAPPO conducted a pilot project on harmonizing diagnostic protocols for a seed-transmitted virus, Tomato Brown Rugose Fruit Virus (ToBRFV), an emerging pathogen that has hampered tomato and pepper world seed production and trade. The project partnered academia, industry, trade organizations and regulatory plant health agencies from North America. Five RT-PCR protocols (three endpoint and two real-time) were selected for comparability studies via a ring test consisting of analytical, diagnostic and calibrator samples. Nine laboratories from the three countries participated in the ring test, generating 3,680 data points. Four out of five methods were found transferrable between the laboratories, and three of those demonstrated optimal performance for accurate, reproducible and user-friendly detection.

P4.2-042

UNDERSTANDING PLANT RESPONSES TO PATHOGEN STARTS WITH A GOOD PROTOCOL

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Text

Fraxinus excelsior, European ash, is a commercially and ecologically important tree species in the UK, that is host for a devastating fungal pathogen as well as an endemic bacterial disease. Developing knowledge of tree responses to pathogens is helpful for understanding how plants can resist pathogens as well as develop biomarkers for disease surveillance. The quantification of secondary metabolites could prove particularly useful in understanding the processes of disease progression. We used an untargeted metabolomics approach to do this and examined different protocols that are least biased towards one group of compounds. A fully factorial experiment was conducted, using healthy tree samples, to test the best metabolite extraction method using the following variables: three different temperatures (4, 20 and 50°C), number of extraction cycles (1 and 3), and three different solvents (10% and 80% methanol, and methanol:chloroform(MCH)). All variables had significant effects of on the overall variation of the metabolites extracted, with the solvent being the most important, explaining ~70% of the total variation. The more non-polar the solvent, and the higher the temperature, resulted in the largest amount of metabolite diversity in extracts, while the number of extraction cycles significantly affected the amount of yield. The best protocol to use for ash is 50°C, 80% methanol or MCH and 3 cycles.

P4.2-043

MOLECULAR DIAGNOSTICS OF ALLORHIZOBIUM VITIS, THE MAIN CAUSATIVE AGENT OF GRAPEVINE CROWN GALL IN CALIFORNIA NURSERIES

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Text

Grapevine crown gall is a devastating disease, particularly in regions with a cooler climate. Its causative agent *Allorhizobium vitis* (syn. *Agrobacterium vitis*) is not part of the California grapevine registration and certification program ensuring that nursery plant materials are free of this pathogen. A molecular diagnostic TaqMan assay was developed targeting the VirA gene on the *A. vitis* tumor-inducing (Ti) plasmid and the chromosomal gene pehA which differentiates *A. vitis* from *Agrobacterium tumefaciens*, because *A. tumefaciens* is found at low abundance in grapevine galls and harbors the Ti plasmid. The assay was deployed on Chardonnay and Cabernet Sauvignon clones grafted on 1103P rootstocks collected across several nurseries at different steps of the propagation pipeline (mother field, callusing, green vines, dormant vines) for two consecutive years. For each vine, samples were taken from three trunk compartments comprising the root-rootstock, below the graft union, and above the graft union. Initial results from the VirA target show drastic differences in incidence both between different nurseries, at different stage of the propagation pipeline as well as within the same nurseries in different years. Our results highlight the dynamic nature of *A. vitis* epidemiology, potentially being explained by differences in infection incidence of the nursery

mother vines and/or environmental substrata in which cuttings are being immersed during propagation.

P4.2-044

USE OF METABOLOMICS TO DIAGNOSE PLANT DISEASES AND TO EVALUATE POTENTIAL THERAPEUTICS OR PREVENTIONS: A CITRUS HLB CASE STUDY

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Text

Metabolomics affords superior plant disease detection when gold standard diagnostics fall short, and can be used to evaluate potential therapeutics or preventions. We demonstrate these capabilities with Citrus greening disease, or Huanglongbing (HLB), which has spread globally and caused severe crop destruction. The causal bacterium, Candidatus Liberibacter asiaticus, does not spread evenly in the tree canopy and moves seasonally between branches and roots, causing a high frequency of false negative readings per gold standard qPCR. With metabolomics, we measure the plant host response to infection and identify a HLB signature from citrus metabolites in leaf tissue, diagnosing HLB without the need for CLas to be present in the sample. In this presentation, we compare six metabolomics assays and determine a liquid chromatography-mass spectrometry (LC-MS) method as most accurate. Multivariate models were further optimized to identify HLB from hundreds of orchard-grown orange and grapefruit trees in Florida and Texas, which resulted in a sensitivity and specificity of >99%. Finally, we apply our HLB model to trees receiving one of several treatments that attempt to prevent or cure HLB infection in a field trial. We showcase the capabilities of metabolomics to accurately diagnose a plant pathogen, to screen therapeutics for the efficacy against disease, and to reveal biochemical pathways impacted by disease and by applied treatments.

P4.2-045

DIVERSITY OF PATHOGENS OF PURPLE SEED STAIN DISEASE ON SOYBEAN BY CERCOSPORA SPP. IN KOREA

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Text

Purple seed stain disease of soybean, which occurs in Korea, is one of the diseases that appear in the late stage of soybean growth stages and after harvest. As a result of an analysis of the disease generation ratio with 48 varieties cultivated by the National Institute of Crop Science in 2021, 3 varieties including Nuriol had more than 10% and 10 varieties including Cham-ol had less than 10%. The results of the analysis of the seed germination ratio between infected seed and ordinary seed, infected seeds reduced germination rate of 0

to 8% depending on the variety. More than 200 PSS pathogen candidates were isolated from soybean seeds collected from different regions and it was analyzed phenotype. And, to select representative pathogens from all isolates, it was analyzed cercosporin toxin contents related to virulence. Also, it was sequenced of ITS, TEF, HIS, and ACT genes and analyzed phylogenetic tree. As a result, 12 strains out of 18 analyzed individuals showed the closest genetic relationship with *C. flagellalis*, and 6 other strains showed the closest genetic relationship with *C. sigesbeckiae*. To confirm the virulence of *Cercospora* spp on the soybean in the natural condition, 5 different soybean cultivars were inoculated with *Cercospora* spp complexes. After harvest, Jangol(31.5%) and Nuriol(61.9%) showed a high infection ratio. This report is the first report to reveal the occurrence of purple seed stain disease caused by *C. flagellalis* and *C. sigesbeckiae* in Korea.

P4.2-047

A NOVEL METHOD FOR ACCURATE DETECTION AND QUANTIFICATION OF ASCOCHYTA RABIEI IN CHICKPEA (CICER ARIETENUM)

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Text

Ascochyta Blight (AB) disease, caused by the fungal pathogen Ascochyta rabiei, is a serious disease in many chickpea-growing regions globally, leading to significant reductions in grain yield and seed quality. Currently, host genetic resistance offers limited AB control. Increases in the pathogenicity of A. rabiei, even in clonal populations such as those in Australia, can further limit the efficacy of resistance sources. The rapid detection and quantification of A. rabiei are vital to screen chickpea germplasm for resistance selection, characterisation of A. rabiei pathogenicity and timely management interventions in the field. At present, this relies on the visual assessment by an expert pathologist. We developed a specific, sensitive, single-copy marker for the detection and quantification of A. rabiei using a digital droplet PCR (ddPCR) assay. Comparisons of this marker and ddPCR assay with conventional PCR, quantitative PCR and plant-based techniques confirmed it is highly specific for the detection and quantification of A. rabiei. The sensitivity of detection and quantification is ≤5×10-2 pg DNA from field samples. Therefore, we propose that this method has the potential for early diagnosis and precise quantification of AB in chickpeas with application to breeding, pathogen studies and integrated disease management strategies to support the increased production of plant-based protein.

P4.2-048

ONSITE DETECTION OF CUCUMBER MOSAIC VIRUS BY LATERAL FLOW STRIP-BASED REVERSE TRANSCRIPTION RECOMBINASE POLYMERASE AMPLIFICATION IN PEPPER

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Text

Cucumber mosaic virus (CMV) is a prevalent virus affecting the great loss of quality and yield of pepper in Korea. In this study, a reverse transcription recombinase polymerase amplification (RT-RPA) assay combined with a lateral flow (LF) strip assay was developed for the detection of CMV in pepper plants. A pair of primers that amplifies highly specifically a part of the coat protein gene of CMV was determined for RT-RPA assay. The RT-RPA assay involved incubation at an isothermal temperature of 32-42 degrees and could be performed rapidly within 30 min. In addition, CMV was detected in 1 pg of total RNA using RT-RPA combined LF strip (LF-RT-RPA) assay, and no cross-reactivity was observed to occur with five viruses caused in pepper crops. We demonstrated that LF-RT-RPA assay is the simple and accurate method for CMV detection since the assay did not require any equipment, comparing results with those of conventional RT-PCR. Furthermore, on-site application of LF-RT-RPA assay for CMV detection was validated in field-collected pepper. The additional results and further analysis will be discussed.

P4.2-049

DEVELOPMENT OF LATERAL FLOW RECOMBINASE POLYMERASE AMPLIFICATION ASSAY FOR THE SIMULTANEOUS DETECTION OF SOYBEAN MOSAIC VIRUS AND SOYBEAN YELLOW MOTTLE MOSAIC VIRUS

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Text

Soybean (*Glycine max* L.) is one of the world's most widely planted and used legumes for food, animal feed products, and industrial production. More than 14 viruses have been found in the soybean grown in the fields of Korea. Among them, soybean mosaic virus (SMV) and soybean yellow mottle mosaic virus (SYMMV) are the most prevalent viruses infecting soybean. This study aimed to develop duplex reverse transcription-recombinase polymerase amplification (RT-RPA) technique applied to rapid, sensitive, and simultaneous detection of SMV and SYMMV. A pair of specific primer sets for detecting SMV and SYMMV, respectively, were selected from over 10 designed primers and RT-RPA conditions were optimized to amplify a part of the coat protein gene of SMV and SYMMV. The optimized reaction temperature for duplex RT-RPA reaction was at 38 degrees for 20 min with two primer sets specific to SMV and SYMMV, but it could be detected at a temperature of 34-42 degrees within a minimum reaction time of 10 minutes. The duplex RT-RPA assay has no cross-reactivity with other soybean-infecting viruses. The limit of detection and reproducibility for detection were evaluated in the soybean seeds. Our result shows that the duplex RT-RPA assay could feasibly be used for rapid and reliable on-site detection of both SMV and SYMMV.

P4.2-050

SIMULTANEOUS DETECTION OF CYMBIDIUM MOSAIC VIRUS AND ODONTOGLOSSUM RINGSPOT VIRUS USING LATERAL FLOW STRIP REVERSE TRANSCRIPTION-RECOMBINASE POLYMERASE AMPLIFICATION ASSAYS

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Text

Orchid, one of the most prominent families of flowering plants, is commercially important cultivated in the world. Among 41 orchid-infecting viruses that occurred in Korea, cymbidium mosaic virus (CymMV) and odontoglossum ringspot virus (ORSV) are the most prevalent and economically important. Previously, we developed reverse transcription-recombinase polymerase amplification (RT-RPA) to detect CymMV on *Phalaenopsis* and *Cymbidium* sp. Here, we report that duplex one-step RT-RPA assays were developed to detect CymMV and ORSV simultaneously, and a three-segment lateral flow strip (LFS) has been established. Amplification of duplex RT-RPA assay was optimized with a specific primer set corresponding to CymMV and ORSV, respectively. The duplex RT-RPA reaction could be detected at a temperature of 34-42 degrees within a minimum reaction time of 10 minutes without cross-reactivity with other viruses. Further sensitivity and specificity analysis of duplex RT-RPA assay will be discussed. Taken together, a duplex RT-RPA assay for the simultaneous detection of CymMV and ORSV was established, and this RPA assay can be offered as a valuable tool for routine diagnosis and epidemiological studies of these viruses, as well as the production of virus-free orchids.

P4.2-051

GRAPEVINE RED BLOTCH DISEASE DIAGNOSIS: OPPORTUNITIES AND CHALLENGES

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Text

Grapevine red blotch disease (GRBD) diagnosis is often challenging due to the nature of symptoms in grapevines. We compared four GRBD diagnosis methods using samples collected from a commercial vineyard in southern Oregon. Tissue samples were collected at fruit set, veraison, harvest, and dormancy from basal, middle, and apical canopy of twenty GRBV-positive and negative vines. GRBD symptoms on grapevines were recorded at the time of collection, and leaf samples were tested for GRBV using LAMP, endpoint PCR, and qPCR. The detectability of GRBV-positive vines by the assays differed significantly among node positions depending on phenology. At fruit set and veraison, the sensitivity of qPCR and endpoint PCR assays was 98%, whereas the sensitivity of LAMP was 49% and 78%, respectively, from basal leaf samples. At harvest and dormancy, the sensitivity of all assays was 100% in basal and middle samples. None of the GRBV-positive grapevine samples expressed symptoms at fruit set and 31% of the basal canopy samples expressed symptoms at veraison. At harvest, 90% of these vines expressed symptoms which was not significantly different than other methods. At fruit set, the specificity of LAMP was less than 75%, whereas at veraison and harvest it increased to 100%. The result of this study shows that PCR-based

assays are the most accurate option if early diagnosis is needed, and less expensive methods such as LAMP and basal canopy symptoms are reliable at later phenological stages.

P4.2-052

CRISPR-BASED APPROACHES FOR RAPID AND SENSITIVE DETECTION OF POSPIVIROIDS

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Text
Pospiviroids continue to be a major concern as a production constraint as well as for quarantine purposes, including new germplasm arriving at the United States. USDA APHIS issued a federal order requiring all imported tomato and pepper seed be certified free of six pospiviroids of quarantine significance or produce in a country where these are not known to occur. The pospiviroids of interest are Potato spindle tuber viroid, Tomato chlorotic dwarf viroid, Tomato planta macho viroid, Pepper chat fruit viroid, Columnea latent viroid, and Tomato apical stunt viroid. The current detection of these six pospiviroids are based on RT-qPCR tests and requires a real-time PCR machine. Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK), is a CRSPR-based detection method highly specific and sensitive with the potential to detect and differentiate closely related pathogens and does not require expensive equipment and can be used for on-site detection. Here, we report a rapid and highly sensitive SHERLOCK platform via recombinase polymerase amplification (RPA) with CRISPR and CRISPR-associated (CRISPR-Cas) RNA-guided endoribonuclease, Cas13 for pospiviroids detection. Specific RPA primers and crRNAs were designed based on the pospiviroids sequences. This method is portable and quantitative, in which fluorescence can be detected by handheld fluorometers or a fluorescence plate reader and can be done in half hour.

P4.2-053

TESTING COMPOST AND CASING SOILS FOR VARIOUS MUSHROOM PATHOGENS

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Text
The Dutch production of the white button mushroom (*Agaricus bisporus*) is one of the biggest in the world. Mushroom diseases can cause substantial losses, and the presence of pathogens associated with substrates (compost and casing soil) used for mushroom cultivation therefore pose a serious threat to this sector. Especially since chemicals permitted to control these pathogens become limited. There is a need for methods to test (bulk)

substrates and their constituents before being used, but sampling methods are lacking while for various mushroom pathogens also suitable molecular diagnostic methods are not available.
We developed sensitive and specific detection methods based on enrichment TaqMan assays for detection of the causative agent of ginger blotch (*Pseudomonas gingeri*) in casing soil and for *Trichoderma aggressivum*, causative agent of green mold. For detection of *Agaricus bisporus* virus 16 (AbV16, former name Brown cap mushroom virus (BCMV)) and AbV6, two viruses from the so-called Mushroom virus X-complex, reverse transcriptase TaqMan assays were developed and successfully applied to diagnose recent outbreaks.
This work was financially supported by the Foundation TKI Horticulture and Starting Materials (grant nr. TU18025), Kekkilä BVB, CNC, Legro, Sterckx, Amycel, Walkro, Lambert Spawn and Sylvan.

P4.2-054

APPLICATION OF RAPID NUCLEIC ACID EXTRACTION METHOD TO SIMPLIFY MOLECULAR DETECTION OF PLANT PATHOGENS

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Text
Simplifying the extraction method of plant nucleic acid is one of the strategies to achieve the goal of simple and rapid detection of plant pathogen. Purification of the plant total RNA or DNA can be done with commercial nucleic acid extraction kits, but the required materials and operating time still needs a certain amount of cost when used in large sample numbers. A nucleic acid extraction solution (named CF fast extraction solution) was developed in this study, which can be used to extract nucleic acids quickly from plants and fungi for detection purposes. Plant species that have been tested including passion fruit, orchid, tomato, papaya, cabbage, cucurbit, Solanaceae seeds, potato, dragon fruit branches, quinoa and tobacco; plant pathogens including Phytophthora sp., Neoscytalidium dimidiatum and viruses, among those viruses can be effectively detected include cucumber mosaic virus CMV), tobacco mosaic virus (TMV), Cymbidium mosaic virus (CymMV), Odontoglossum ringspot virus (ORSV), Euphorbia leaf curl virus (EuLV) and Papaya leaf curl Guangdong virus (PaLCuGDV). For extraction of nucleic acids takes about 20-25 minutes by our technology, which can reduce the extraction time for rapid and large-scale sample detection. In addition to detection accuracy, this technology has the advantages of simplifying the processing flow, eliminating the need for organic solvents, saving detection consumables, and shortening operating time.

P4.2-055

PILOT INITIATIVE FOR SHARING OF POST ENTRY PLANT QUARANTINE AND DIAGNOSTIC SERVICES BETWEEN AUSTRALIA AND NEW ZEALAND.

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Text

Australia and New Zealand each have unique ecosystems and strong reliance on primary industries, thus both countries deploy stringent biosecurity policies and measures to afford protection from high impact pests and pathogens. Both countries have strict quarantine measures for the importation of new plant germplasm, with an overlap of regulated pests and diseases, and requirements for plants to undergo post entry quarantine in high containment facilities. Given each country has complementary strengths, a pilot initiative was carried out to test the concept of a shared PEQ arrangement, whereby plants undergo quarantine in one facility and are subsequently released to both countries simultaneously at the end of testing and the quarantine period. This paper details the harmonization of biosecurity outcomes, import and export regulations, diagnostic testing protocols and target lists, and the logistics of successfully releasing a consignment of strawberry (Fragaria x ananassa) to both countries in November 2022.

P4.2-056

EARLY DETECTION AS A TOOL TO STOP THE SPREAD OF DISEASES: THE CASE OF CERATOCYSTIS PLATANI IN EUROPE

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Text

In Europe, both Oriental plane and London plane trees are seriously threatened by the invasive fungal pathogen *Ceratocystis platani*, the causal agent of the canker stain disease (CSD) of plane trees, a lethal disease able to kill a mature tree in one-two years. The fungus is considered to be indigenous to North America and was unintentionally introduced into Europe during World War II. So far, the disease is reported in Italy, France, Switzerland, mostly in urban or peri-urban environments and in Greece, Albania and Turkey in both anthropized and natural environments. The impact of CSD in Europe can be compared with notorious tree diseases such as Dutch elm disease, chestnut blight, and, more recently, ash dieback, which have all caused devastating losses to natural woody ecosystems and ornamental trees. Once the disease is introduced, if not promptly detected, it is hard to be eradicated, since it is highly infective and easily transported by occasional vectors, although humans, through the use of infected pruning tools and terracing machinery, are the main agents of spread.
To prevent further eastward or northward spread and to study the biology of the pathogen, several early and sensitive detection tools, both biochemical and molecular based, have been developed and validated. Here we describe the different tools and their possible use to prevent the further spread of such a destructive disease.

P4.2-057

DNA-BASED SOIL ANALYSIS OF APHANOMYCES EUTEICHES INCREASES SUSTAINABLE PRODUCTION OF LEGUME-BASED FOODS

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Text

Swedish peas are among the most climate-smart legumes we can eat. Pea root rot, caused by the plant pathogenic oomycete Aphanomyces euteiches, is the major problem in pea cultivation in Sweden. Since the dormant oospores survive for up to 20 years in the soil, it is important for the grower to cultivate peas in fields with healthy soil. The aim of this study is to provide growers with a sensitive DNA-based method for analysing A. euteiches in soil. Soil samples were collected from different fields with suspected occurrence of pea root rot and they were tested in a bioassay with a susceptible pea cultivar “Linnea”. After three weeks the plants were uprooted, carefully washed and assigned a disease severity index (DSI). The shoots were dried at 60°C over night and then weighed. DNA was extracted from the roots and analysed by PCR and gel electrophoresis. DNA was also extracted from soil to assess the occurrence of A. euteiches. The DSI varied across the different samples and the correlation between the DSI and shoot dry weight was significant (R2=0.66). DNA of A. euteiches was detected in the roots of some of the plants. Even though the roots of other pea plants were heavily discoloured the occurrence of A. euteiches DNA could not be confirmed. This indicates either lower amount of DNA or that the symptoms were caused by other plant pathogens. Soil analysis by PCR-methods is under progress.

P4.2-058

IDENTIFICATION OF BLACKLEG PATHOGENS IN SWEDISH WINTER OIL SEED RAPE

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Text

Blackleg is a serious disease of Brassica napus var. oleifera and is caused mainly by the fungus Leptosphaeria maculans (Lm), but also to some extent by the species L. biglobosa (Lb). The disease is of major economic importance worldwide and causes severe yield losses, especially in winter oil seed rape (OSR). It is, however, not fully investigated which Leptosphaeria species that causes the symptoms in Swedish OSR. The aim of this study was to investigate the occurrence of these fungi using the previously developed Loop-mediated Isothermal Amplification (LAMP) assays. Plant samples were collected from winter OSR fields in three regions in Sweden during 2019-2021. Pooled leaf samples with visual

spots were collected in autumn and infected stems were collected from the same fields in the following summer. DNA was extracted with a commercial kit and real-time LAMP was performed with Genie® II platform. The results showed that both Lm and Lb were prevalent in the sampled fields. The most prevalent species on the leaves was Lm, whereas Lb was prevalent in the stems during summer. Both species occurred in the stem base and upper part of the stem. There were both regional and seasonal differences. DNA of Lm and Lb identified in 93 % and 87 % of leaf samples collected in 2019, respectively. In autumn 2020, 86 % of the samples were Lm positive whereas 67 % of samples were Lb positive. In general, the occurrence of both species was low in autumn 2021 compared to 2019 and 2020.

P4.2-059

DNA BARCODE IDENTIFICATION BEYOND SIMILARITY INDICES: INFORMATIVE NUCLEOTIDES OF XANTHOMONAS BACTERIA ON COMMON BEAN (PHASEOLUS VULGARIS)

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Text

Xanthomonas phaseoli pv. phaseoli and Xanthomonas citri pv. fuscans (Xpp-Xcf) cause common bacterial blight (CBB) on bean (Phaseolus vulgaris). Their detection in seeds relies on isolation on semi-selective media and subsequent identification of purified colonies. For many laboratories, the DNA barcoding is a method of choice for identification relying on sequence similarity between the isolates and the reference sequences in EPPO-Q-Bank. In this study, we have identified informative nucleotides in partial gyrB and avrBs2 barcodes through analysis of reference EPPO-Q-bank sequences. Differentiation of Xpp-Xcf from other Xanthomonas spp. relies on a single nucleotide difference and 6 nucleotides/684 (< 1 %) in partial gyrB and avrsBs2 sequences, respectively. Unequivocal differentiation of Xpp and Xcf is not possible for all reference isolates possibly reflecting their pathological convergence. The approach was validated on 45 target and non-target isolates with Xanthomonas-like morphology from 17 seed samples. Of these, 29 were identified as Xpp and 11 as Xpp-Xcf. Non-target bacteria (5) could be clearly differentiated from Xpp-Xcf. Overall, the analysis approach taking into account informative nucleotides improves the reliability of typing, enables its automation and a more high-throughput approach. Further analysis is underway to identify other informative nucleotides/sequences, which would allow differentiation between the Xpp and Xcf.

P4.2-060

DEVELOPMENT OF A NEW LATERAL FLOW IMMUNOASSAY FOR DETECTION OF BANANA BUNCHY TOP VIRUS

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Text
Enzyme-linked immunosorbent assay (ELISA) has previously been a valued method for detection of banana bunchy top virus (BBTV) in many countries due to its reliability and use of simple laboratory equipment. However, detection of plant pathogens using ELISA can be time-consuming. We used newly developed BBTV antibodies to produce a lateral flow immunoassay that can be used for rapid detection of BBTV in the field. Lateral flow test strips were constructed with capture antibodies as test and control lines on a nitrocellulose membrane. Two monoclonal antibodies specific to BBTV were conjugated to gold nanoparticles and loaded onto a conjugate release matrix. Functional testing of the lateral flow assay has shown that BBTV can be detected in crude leaf extracts from infected banana plants diluted up to 1:320 and in as little as 10 minutes. Optimisation of the lateral flow assay has produced a rapid test that is more sensitive than ELISA and is a simple method for detection of BBTV directly in the field.

P4.2-061

PATHOTRACER: A TOOL FOR MONITORING RICE PATHOGENS AND RESISTANCE GENES IN ASIA

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Text
In South and Southeast Asia, bacterial blight and leaf blast are two major rice diseases caused by Xanthomonas oryzae pv. oryzae and Magnapother oryzae, respectively. Climate change affects the distribution and severity of these diseases, making it essential to monitor the diversity and distribution of the pathogens to predict outbreaks. While more than 47 BLB and 100 blast-resistance genes have been discovered, their effectiveness varies depending on pathogen variants. Thus, having information on their effectiveness is crucial to deploy resistance genes effectively in a given region.

To monitor the bacterial blight pathogen population, a network of 18 institutions across 10 countries in Southeast Asia and South Asia was established for pathogen surveillance and monitoring. Using 40 SNP markers, the Xoo populations were monitored and their distribution was mapped. This information is available on PathoTracer, a GIS-based platform that provides detailed information on the genetic diversity of rice pathogens and the effectiveness of resistance genes and released rice cultivars in a given region or country. Plant pathologists can use this information to develop targeted and effective disease management strategies. PathoTracer provides a better understanding of the diversity and distribution of rice pathogens, which is crucial for developing sustainable and effective approaches to control these diseases.

P4.2-062

DETECTION AND STABILITY OF CITRUS BARK CRACKING VIROID (CBCVD) IN WATER

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Text

Citrus bark cracking viroid (CBCVd) is un-encapsidated, circular, single-stranded plant pathogenic RNA ranged 282–286 nucleotides that was discovered in 1988 from grapefruit and causes aggressive symptoms on hop. CBCVd spreads mainly by mechanical means such as residues of plant sap of infected plants on tools and plant residues on hop fields. There are no data available on viroid CBCVd survival in aqueous environments. Plant pathogens occur in water at low concentrations, so identification is based on successful extraction from sample. The purpose of the research was to develop detection method for CBCVd extraction from water samples and to study stability of CBCVd in water. We optimized detection of CBCVd in spiked water samples by using different RNA isolation commercial kits and with real time RT-PCR. For stability study we weekly sampled and tested artificially contaminated water which was stored in a growth chamber. Infectivity of samples was performed on hop plants by using mechanical inoculation. Our results showed that CBCVd could be detected with QIAamp Viral RNA Mini kit up to dilution 10-3 and with RNeasy Power Water kit up to dilution 10-1, whereas successful artificial infections were obtained up to dilution 10-2. In stability study we confirmed presence of CBCVd till 56th day, but infectivity of samples was confirmed up to three weeks. In our further work we will investigate the transmission of viroid CBCVd with water in hydroponic systems.

P4.2-063

COMPARISON OF RELIABLE AND EFFECTIVE PCR-BASED DETECTION METHODS FOR PCN IN POTATO FIELDS

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Text

The potato cyst nematodes (PCN) Globodera pallida and G. rostochiensis are a major constraint to the potato industry in the Netherlands and even worldwide. These pests can negatively impact yield and lead to economic losses due to limitations of cultivation on infested fields. Since PCN can survive for a very long time without a host, effective control and eradication measures have been implemented. Therefore, it is important to have a reliable, robust and efficient assay for the detection of (viable) PCN. In recent years, several new PCR methods have been developed for the detection of PCN. In this study, multiple PCR-based detection assays were compared that are included in the EPPO standard PM 7/40. This includes both TaqMan and conventional PCR tests targeting either DNA or RNA regions. Comparisons of the assays using the same samples revealed each of the assays applied have their own discrepancies that warrant further investigations. Therefore, care should be taken prior to selecting a reliable and effective PCR based assay for the detection on PCN.

P4.2-064

A MULTI-PRONGED AND GENOME-INFORMED REAL-TIME PCR DETECTION OF XYLOPHILUS AMPELINUS, A CAUSATIVE AGENT OF BACTERIAL BLIGHT OF GRAPEVINE

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Text

Xylophilus ampelinus (Xamp) is a plant pathogenic bacterium that causes bacterial blight of grapevine, previously reported from the European Mediterranean, South Africa, Japan and Russia. It is regulated in many countries and can restrict international trade with planting material. Because of its fastidious growth its detection relies on molecular methods. The real-time PCR for its detection in symptomatic plant material (Dreo et al., 2007) is one of the first real-time PCR tests included in the EPPO guidelines (PM7/96(1)). Recently, conflicting results of various detection tests were reported when testing grapevine cuttings for latent infections with Xamp. With the aim to improve reliability of detection for novel type of samples we have developed a multi-pronged detection approach consisting of a number of real-time PCR tests targeting genome-informed specific regions of Xamp core genome identified with RUCS (Thomsen et al., 2017). In total, 26 novel qPCR tests were designed with Primer Express 2 (Applied Biosystems) employing TaqMan and MGB probes. Their amplification efficiency ranged from 0.62 to 1.00 and none amplified DNA from a closely related *X. rhododendri* (KACC 21265). Further validation of the sensitivity and specificity of the newly developed approach for detection of Xamp in latent samples is underway. The preliminary results show high potential of the multi-pronged detection to facilitate international trade.

P4.2-065

PREVALANCE AND DISTRIBUTION OF AREOLATE MILDEW IN MISSISSIPPI COTTON

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Text

Areolate mildew is a fungal disease of cotton caused by *Ramulariopsis* spp. The disease is characterized by white, powdery fungal growth on the underside of the leaf. Although it was first reported in the United States in Alabama in 1890, the disease was not commonly observed in the southeastern U.S., until recently. In 2022, the disease was observed in 19 counties in Mississippi, between 08/15 and 10/03. Disease severity ranged from low (n=10), moderate (n=8) and high (n=1). Symptomatic leaves were collected from infected fields and were transported to the lab for microscopy observation, pathogen isolation and molecular identification. The pathogen was isolated on V8 medium amended with antibiotics. Conidia arising in short chains, fusiform, 0 to 3 septate, mostly 20–30 × 4 µm were observed from the symptomatic leaves. Surface raised, lumpy, pale grey, with margins undulate, and fimbriate colonies developed after 14 days incubation at 25°C. DNA was extracted from 11 isolates

and PCR was conducted using ITS universal primers. In addition, a preliminary screening of the cytochrome *b* gene (*cytb*) was conducted using Sanger sequencing. ITS sequences confirmed all isolates as *Ramulariopsis* spp. However, additional molecular testing is needed to confirm the species. No common substitutions (F129L, G137R or G143A) were observed in the *cytb* region. Screening of additional isolates is critical to monitor for QoI fungicide resistance development within the pathogen population.

P4.2-066

SUCCESSFUL ISOLATION OF XYLELLA FASTIDIOSA SUBSP. MULTIPLEX PORTUGUESE STRAIN, IN AXENIC CULTURE, FROM LAVANDULA DENTATA

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Text
Xylella fastidiosa (Xf) is one of the top 20 regulated quarantine pests in the European Union. The species is divided into several subspecies. The subspecies differ in their host range and determine the phytosanitary measures taken. All Xf are fastidious, slow growing and difficult to isolate in pure culture, even from highly contaminated plants. In this study we have attempted to isolate Xf in axenic culture from three samples of symptomatic *Lavandula dentata* plants, from Portugal (NLR-INIAV). Sub-samples of these plants were previously tested for Xf, in INIAV, confirming the infection. For the isolation procedure, extracts from each sample were prepared from: (i) individual leaves and (ii) two bulk samples of veins (approximately 1 g) as described in EPPO PM 7/24(4) with some modifications. Bulk samples were macerated and some additionally sonicated. Extracts were plated on PWG and BCYE medium. Additionally, DNA was extracted using the QuickPick™ Plant DNA Kit (BioNobile) and the presence of Xf was determined by real-time PCR (Harper *et al.*, 2010, erratum 2013). As expected, the isolation of Xf proved to be challenging. Preliminary results show that isolation was successful from one out of three samples and from a single leaf. No correlation was observed between Cq values obtained by molecular methods and isolation on media. The isolate was identified as subsp. multiplex, following Hodgetts *et al.* (2020), confirmed previously finding based on MLST on plant extract.

P4.2-067

NEPODETECT - DIRECT DETECTION OF PLANT VIRUSES FROM VECTOR NEMATODES

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Text
The Longidoridae and Trichodoridae are two families within the phylum Nematoda, of which

some species are classified as plant virus vectors. They can cause economic damage to agricultural and horticultural crops by feeding directly on the roots, and by transmitting viruses such as nepoviruses and tobraviruses into host plants, generating wilting diseases. Non-European populations of *Xiphinema americanum* group are quarantine regulated for Europe as certain species have been proven to transmit nepoviruses such as Tobacco ringspot virus (TRSV) and Tomato ringspot virus (ToRSV). Virus detection inside the nematode offers a good strategy to directly identify the possible presence of such plant viruses and determine if phytosanitary measures are necessary for nematode populations intercepted in trade. The aim of this project was to investigate the main molecular methods already available for identification of viruses vectored by nematodes, and to develop a new diagnostic approach for rapid detection and identification using HTS technology. Virulent nematodes were collected from different locations and RNA extraction directly from nematodes was performed using MagMAX™ Plant RNA Isolation kit for KingFisher method. Viruses such as Grapevine fanleaf virus (GFLV), ToRSV and TRSV were successfully detected by TaqMan real-time PCR. Efficacy of Illumina MySeq System will also be investigated, although considering the limitation of low RNA concentrations, this may pose a challenge.

P4.2-068

CRISPR/CAS-BASED BIOSENSING TOOL FOR POINT-OF-CARE DETECTION OF ERWINIA AMYLOVORA

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Text

Fire blight is a destructive disease of apples and pears that threatens agricultural production. Caused by *Erwinia amylovora*, it is classified as a quarantine disease due to its aggressiveness, contagiousness, and difficulty in containment. Despite efforts to prevent its transmission, fire blight is still spreading across borders and causing severe outbreaks. Moreover, controlling the disease is challenging as symptoms are difficult to distinguish from those caused by other pathogens such as *Erwinia pyrifoliae*. Here, we attempt to detect *E. amylovora* using a sequence-specific system originating from bacterial and archaeal immune systems involving clustered regularly interspaced short palindromic repeats (CRISPR) and the Cas protein. The system is highlighted as a valuable tool in genome editing and disease diagnosis. The *trans*-cleavage activity of the Cas protein can be coupled with a fluorescent readout by employing a reporter, thus producing a fluorescent signal only in the presence of the target nucleotide sequence. Specific sequences for *E. amylovora* were derived by comparative analyses of the genomes of *E. amylovora* and those of its close relatives. Cas and the crRNA complementary to the target sequences successfully detected *E. amylovora* strains including those responsible for the Korean outbreak. Our work provides a useful resource for developing a rapid as well as highly specific and sensitive tool via the CRISPR/Cas system for point-of-care diagnosis of fire blight.

P4.2-069

THE DIVERSITY AND PATHOGENICITY OF RAHNELLA SPECIES ISOLATED FROM DISEASED ONION BULBS IN THE UNITED STATES AND SOUTH AFRICA

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Text

The genus Rahnella contains widely distributed, facultative, Gram negative, anaerobic bacteria in the Yersiniaceae. Rahnella species have been isolated from water, human wounds, oak trees, beetle guts and, recently, symptomatic onion bulbs and foliage. There can be significant losses in onion crops from pre- and/or post-harvest diseases caused by bacterial pathogens. To develop management strategies, it is important to understand the diversity and pathogenicity of Rahnella species on onions. In 2020-2021, Rahnella was isolated from diseased onion bulbs in the USA and South Africa. The 60 isolates formed cream, round, convex colonies on nutrient agar, and were identified as Rahnella based on 16S rRNA sequences. A multilocus sequence analysis (MLSA) with atpD, gyrB, infB, and rpoB was used to define Rahnella strains to species. Pathogenicity trials were completed with onion bulb, foliage, and a red scale necrosis (RSN) assays. A concatenated maximum likelihood phylogenetic tree of the four genes revealed multiple Rahnella species, with large clusters of R. perminowiae, R. aceris, and R. aquatilis. Other species from the USA included R. varrigena and R. victoriana. Among South African isolates, R. perminowiae, R. aceris, and R. aquatilis were dominant. Mild to moderate internal bulb decay was observed with all the species but no isolates were pathogenic with the RSN and foliar assays. The mechanisms by which Rahnella species cause onion bulb rot should be investigated.

P4.2-070

DIAPASON : DIAGNOSTIC OF GRAY LEAF SPOT BY DIGITAL PCR

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Text

Fungal pathogens are a major threat to plants, whether they are cultivated for food or for recreational areas. Pyricularia oryzae is a fungal pathogen infecting more than 50 grasses and is particularly known on major food species such as rice and wheat. This fungus is also known on turf grass as the causal agent of gray leaf spot. As of 2016, in France, the disease is present on the lawns of professional Football stadiums. Gray leaf spot is a cyclic disease, difficult to eradicate once establish on the field. Indeed, phytosanitary treatments are often

ineffective if the application is too late. To manage efficiently the gray leaf spot disease on their sport fields, turf managers require an early and quick diagnostic during the first cycle of the disease. The objective of the Diapason project (partnership UMR PHIM / IAGE company) is thus to develop an early diagnostic method based on digital PCR. The diagnostic was first validated in vitro on pure strains of fungal pathogens and in vivo on samples produced under controlled conditions by artificial inoculations. The application of the diagnostic on sport fields was then done on grass clippings, sampled on a Football stadium presenting symptoms of the disease. An improvement of the diagnostic is under progress (i) to discriminate the P. oryzae lineage affecting turfgrass, rice and wheat and (ii) to extend the diagnostic to other turfgrass diseases identified in stadium, golf courses, and race courses.

P4.2-071

FROM ORCHARD TO STORAGE: DIAGNOSE YOUR APPLES.

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Text

Apple diseases can cause heavy losses, difficult to control for the farmers. Many of them develop during storage. Currently, phytosanitary products are used on the harvested apples to control these diseases. To limit such treatments and to assist farmers in reducing their economic losses, four main diseases must be considered: the emerging ramularia disease (caused by Ramularia mali/eucalypti), the already established and problematic Bitter Rot (caused by different species of Colletotrichum), or gleosporium rot (caused by the plant pathogen Neofabrae vagabunda), as well as the mildew (caused by phytophthora syringae/cactorum), a disease transmissible from apple to apple once harvested. Sampling is a key step to ensure a reliable diagnosis of the whole orchad because each one occurs at a different time. However, they are all detectable on apples a few days before harvesting the fruits.

IAGE guides the farmers on apples' sampling in the orchard and then uses an innovative diagnosis based on digital PCR to simultaneously target the pathogens causing the four diseases of interest. Then, IAGE's expertise allows advice to be given to the farmers about the apple's storage. The bottom line of this diagnosis is to help reducing the use of phytosanitary products from the orchard to the storage.

P4.2-072

PRESENCE OF CURTOBACTERIUM FLACCUMFACIENS IN BELGIAN AND DUTCH GREENHOUSE POINSETTIA PRODUCTION – RUINING THE CHRISTMAS SPIRIT?

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Text

Curtobacterium flaccumfaciens pv. *poinsettiae* (Cfp) is known to cause prominent symptoms on Christmas star or poinsettia (*Euphorbia pulcherrima*), ranging from leaf spots and stem cankers to blight, defoliation and vascular discolouration. The disease was first reported in 1942 in New Jersey, USA[1] and still occurs in American horticulture. Trade of (latently) infected plant material is the main pathway to disseminate Cfp. Latent infections are common and disease often appears on plants close to finishing, making it easy to overlook. Intermittent records in Germany since 2014 have alerted the EPPO region of the insidious threat Cfp poses to glasshouse poinsettia production. To elucidate the pathogen's phytosanitary status on EPPO territory, a survey was done in poinsettia production and wholesale/retail in Flanders (Belgium) and the Netherlands. Yellowish-pigmented *Curtobacterium*-like colonies were isolated from symptomatic plants from over half of the sampled sites. Majority of these were identified as *C. flaccumfaciens* based on MALDI-TOF MS profiles and partial *gyrB/recA* gene sequences. Pathogenicity tests confirmed the isolates as pv. *poinsettiae*. Comparative genomics clustered the 'yellowish-coloured European strains' into three closely related groups, clearly separated from the pink strains isolated from poinsettias in the 1950s in the USA. Early and specific detection in cuttings and mother plants by LAMP is explored.
[1]Starr & Pirone, 1942. Phytopathology, 32, 1076-81.

P4.2-073

KRISP: A COMPUTATIONAL PIPELINE FOR RAPID DEVELOPMENT OF CRISPR-BASED MOLECULAR DIAGNOSTICS FROM RAW READS

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Text

CRISPR-based molecular diagnostic assays like SHERLOCK and DETECTR are affordable, field-deployable, and easy to use. However, these require specialized primers and guide-RNAs that must be inferred from sequence data and tested in the lab. Designing such assays from genomic data is time consuming, potentially delaying effective pathogen monitoring and mitigation. We are developing a framework for rapid development of CRISPR-based diagnostic tests from raw reads using a computational pipeline called KRISP. KRISP outputs primer and guide RNA sequences needed to uniquely distinguish any number of user-defined groups of organisms. Both human-readable HTML reports and files optimized for downstream analysis are created. KRISP leverages many existing open source tools and introduces others that can be used independently, such as the KRISP python package that finds diagnostic sites from assembled genomes or variants stored in VCF files. A pipeline orchestration language called Nextflow allows for seamless use on personal computers, high performance computing clusters, and commercial cloud services. Results are fully reproducible due to the use of docker containers for all tools. Using KRISP we were able to find a crRNA locus that can differentiate Phytophthora ramorum from other Phytophthora species and validated it in the lab. We hope that this will allow for rapid development of diagnostics essential to track and manage emerging plant pathogens.

P4.2-074

RISK ASSESSMENT FOR TRUNK DISEASES IN SWEET CHERRY ORCHARDS BY USING SPORE TRAPS AND QPCR

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Text

Trunk diseases affect the longevity and productivity of sweet cherry trees in Chile. They are caused by numerous species of fungi that infect and damage the wood, causing chronic infections. The most frequent fungal canker pathogens are Calosphaeria pulchella, Chondrostereum purpureum, Cytospora leucostoma and Eutypa lata. There are not eradicative methods yet available for their control, hence,
to predict the risk of primary infections by these pathogens and subsequent outbreaks of canker, it is crucial to understand their occurrence in cherry orchards. The aim of this study was to analyze the occurrence of Calosphaeria, Chondrostereum, Cytospora and Eutypa airborne inoculum in cherry orchards in relation to weather conditions. For this purpose, three commercial orchards were monitored weekly during two years using microscope slide traps, and pathogens were detected and quantified by qPCR and specific pathogen-primers. The qPCR method was efficient to identify the DNA of each pathogen in spore traps, in both years and with a marked seasonal distribution, from mid-fall (mid-May) throughout winter and early spring (June–October). Spore dispersal patterns were correlated with rainfall events, with maximum detection levels of 5, 4.5-4 and 3.8 fg of DNA for Eutypa, Calosphaeria, Cytospora and Chondrostereum, respectively. The qPCR method demonstrated to be rapid and sensitive in detecting fungal inoculum and may contribute to the implementation of management strategies.

P4.2-075

ENHANCING PCR DETECTION OF XYLELLA TAIWANENSIS USING WHOLE GENOME SEQUENCE INFORMATION

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Text

Xylella taiwanensis (Xt) is a nutritionally fastidious bacterial pathogen causing pear leaf scorch disease (PLSD) in Taiwan. Xt detection plays a key role in PLSD management. Multiple PCR systems based on single- or two-copy genes have been developed for Xt

diagnosis. Xt detection could be further improved utilizing multi-copy genes. A total of 32 Xt whole genome sequences are now available in the GenBank, providing a sound resource for the development of robust PCR detection systems. By self-aligning the genome sequences of Xt Type strain PLS229, seven 714-bp sequences with similarity > 97% each other were identified. The sequences were annotated as part of the hemagglutinin-like protein gene. Based on this sequence, two primer sets, Xt7cp-378-F/R and Xt7cp-573-F/R, were designed. In silico experiment using GenBank database showed the high Xf-specificity of the two primer sets. SYBR green qPCR experiments using pure culture DNA from strains of Xt, X. fastidiosa and Xanthomonas campestris and PLSD plant DNA samples collected in Taiwan further confirmed the Xt-specificity. To evaluate PCR detection sensitivity, five previously developed Xt-specific primer sets, two from single-copy locus and three from two-copy locus, were simultaneously compared with Xt7cp-378-F/R and Xt7cp-573-F/R against the same set of PLSD samples. A reduction of 1-3 Ct values from the two 7-copy gene-based PCR systems were observed. Further evaluation of the two PCR systems is underway.

P4.2-076

DIAGNOSIS OF TAR SPOT OF CORN CAUSED BY PHYLLACHORA MAYDIS USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AND CONVENTIONAL PCR

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Text

The diagnosis of tar spot of corn currently relies on visual assessment. PCR is used for plant pathogen detection but requires specialized equipment and is not suitable for field applications. The loop-mediated isothermal amplification (LAMP) assay is a new method that is emerging as a simple and sensitive diagnostic tool. Here conventional PCR and LAMP tests for diagnosis of Phyllachora maydis in diseased corn leaves are described. The calmodulin gene of P. maydis was used as a target to design PCR and LAMP primers in the Open Reading Frame (ORF) and in a region extended 1 kb on both ends of the ORF to identify a unique region of the gene. Six primer pairs for PCR and five primer sets for LAMP were designed and synthesized. We tested the PCR primers on 13 P. maydis samples from the United States, five P. maydis samples from Ecuador, and 43 isolates from other corn pathogens. One PCR primer pair was specific for all P. maydis isolates. The LAMP assay is performed at 65 °C for 40 min and the amplification is observed by the addition of SYBR Green to the reaction. A green color reaction, that is visible to the naked eye, indicates amplification in the presence of P. maydis DNA, otherwise, the reaction turns orange. The LAMP assay is a promising technique for the rapid detection of P. maydis in diseased corn leaves in contrast with conventional PCR which requires a well-equipped laboratory.

P4.2-077

IMPLEMENTATION OF REAL-TIME PCR FOR QUANTITATIVE DIAGNOSIS OF CASSAVA COMMON MOSAIC VIRUS IN CASSAVA GERMPLASM (MANIHOT ESCULENTA CRANTZ)

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Text

Cassava is considered the sixth most important crop, with a production of more than 300 million tons with an annual yield of 11.03 tons/ha, with economic and main importance for food security. Currently, the Bioversity International Alliance and CIAT have a cassava germplasm bank made up of 5,965 accessions under in vitro conditions, which require the indexing of quarantine pathogens. To carry out continuous improvement in diagnostic processes, the objective was to establish a quantitative protocol based on real-time PCR (qPCR) for the detection of *Cassava common mosaic virus* (CsCMV). The establishment of the methodology required the design of primers and a probe, the standardization of concentrations, reaction volume, and amplification time. In addition to this, the sensitivity and specificity of the reaction were determined, and a comparison of methodologies was made between DAS-ELISA, PCR, and qPCR, using 140 accessions from the cassava bonsai collection to evaluate the level of sensitivity and specificity of each technique. The established qPCR protocol allowed the detection and quantification of the CsCMV virus up to 77.97 copies/µL, demonstrating a sensitivity 10 times greater than PCR. The optimization of the qPCR for the diagnosis of CsCMV, allowed us to obtain a sensitive, fast, and specific protocol for the diagnosis, contributing to the sanitary certification of the cassava materials for safe distribution.

P4.2-078

DEVELOPMENT AND VALIDATION OF A RAPID MOLECULAR DIAGNOSTIC TOOL FOR THE DETECTION OF PHYTOPHTHORA RAMORUM BASED ON RECOMBINASE POLYMERASE AMPLIFICATION (RPA)

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Text

The quarantine pathogen *Phytophthora ramorum* has at least 75 hosts and over 100 associated hosts. Outbreaks have been linked to the introduction and movement of ornamental landscape plants near susceptible forests. Uniform and reliable detection of *P. ramorum* from a diverse host range is complex. Traditional ELISA techniques are not specific enough to confidently identify pathogen presence and TaqMan assays require rigorous measures to handle the expansive host range. An RPA assay was developed and optimized for *P. ramorum* to improve diagnostic specificity and provide confirmatory molecular diagnostics for end users. The developed assay shows sensitive and specific detection of *P. ramorum* detecting down to 1 fg/µl of DNA from each lineage while not cross reacting to over 100 different oomycetes in at least 30 hosts. The RPA assay was put through three tiers of

validation. The first validation, a single lab multi-operator validation, produced diagnostic specificity and sensitivity scores of 100% and 92% respectively with 100% reproducibility and repeatability among operators. Tier two validation resulted in 100% diagnostic specificity and sensitivity on a blind sample set performed at two independent labs. The capstone validation is a blind conglomerate test performance study involving five independent labs representing academia, government, and industry. The developed RPA assay provides a rapid and simplified approach for molecular detection and identification of *P. ramorum*.

P4.2-079

DIGITAL DROPLET PCR PROVIDES REFERENCE-FREE HIGH THROUGHPUT QUANTIFICATION OF CEREAL PATHOGENS IN HOST SAMPLES

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Text

Fungal diseases of wheat are among the most damaging plant pathogens, causing significant losses of total grain yield if left untreated. Parastagonospora nodorum (Pn) and Pyrenophora tritici-repentis (Ptr) cause septoria nodorum blotch (SNB) and tan spot (TS) of wheat, respectively. Pn and Ptr can co-exist in the same lesion of infected wheat and are non trivial to distinguish from one another based on physical disease symptoms. Digital droplet PCR (ddPCR) is a high throughput and highly sensitive polymerase-based assay that allows for the quantification of SNP-level specific DNA sequences. We have developed a fluorescent probe-based assay capable of single well, reference-free simultaneous quantification of SNB and TS during host infection. The assay shows no off-target effects across a wide range of closely related cereal necrotrophs and can resolve pathogen titre at up to 5pg/µl of purified fungal gDNA, or 1ng/µl of infected plant tissue. Based on a hypervariable region within the highly conserved α-tubulin gene, the assay can be easily adapted for studying other cryptic plant pathosystems, and will provide another tool for dissecting the relationship between pathogens as well as with their hosts. We have also exploited ddPCR to examine competition between pathogen isolates of the same species during infection on wheat. The implications of this study will be discussed.

P4.2-080

MULTILOCUS SEQUENCE TYPING (MLST) AND PHYLOGENETIC STUDY REVEAL SPECIES DELIMITATION OF COLLETOTRICHUM SPP. ASSOCIATED WITH THE DIEBACK OF GUAVA

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Text

Colletotrichum species complex is an important group of fungal pathogens infecting many host plants worldwide. We investigated the population structure, genetic variation, and species delimitation in the *Colletotrichum* community associated with dieback-affected guava plants using integrative phylogenomics analyses. Five housekeeping genes, actin (*act*), calmodulin (*cal*), Glyceraldehyde 3-phosphate dehydrogenase (*gapdh*), tubulin (tub_2), and internal transcribed spacer region of rDNA (*ITS*), were used in this study. The isolate genotyping was based on allelic profiles. The typing efficiency of the GAPDH locus was shown higher in MLST analysis, which suggests it is a potential DNA barcode marker to delimit species boundaries for distinguishing *Colletotrichum* species in phylogenetic studies. A multilocus phylogenetic tree was generated for species delimitation through Bayesian inference analysis congruent with the coalescent theory-based species tree. The phylogenetic tree resolved these isolates as independent evolutionary lineages of the *Colletotrichum siamense* species complex. The Coalescent theory-based species tree also supported these lineages as independent *Colletotrichum siamense* species of its complex. Hence, based on morpho-genetics analyses, we reported two new *Colletotrichum* species (*C. parkukiae* and *C. psidium*) associated with guava dieback.

P4.2-081

TWO NEW QPCR ASSAYS FOR DETECTION AND QUANTIFICATION OF ASPERGILLUS FLAVUS CLADE AND ASPERGILLUS PARASITICUS CLADE IN MAIZE KERNELS

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Text

Aspergillus section *Flavi* mainly produce carcinogenic mycotoxins known as aflatoxins (AFs) divided into two types, B and G types (AFB and AFG). They are highly hazardous for human and animal health, so AFs are extremely regulated in food production with low accepted limits. In France, global warming has led to some AFs detection in maize harvests since 2015. Thanks to mycoflora analysis, the species *A. flavus* (AFB producer) and *A. parasiticus* (both AFB and AFG producer) were identified as responsible of AFs contaminations. However, mycoflora analysis is a time-consuming method which limits the characterization of many samples. Thus, we propose here a clade specific and functional TaqMan® qPCR method based on the calmodulin gene to discriminate the *flavus* clade (FC) and the *parasiticus* clade (PC). We applied these methods on more than 1100 maize samples, collected over seven different years (2016 to 2022). About 20% of all the samples were detected positive for FC, which is three times higher than PC. We found, as expected, significant positive correlations between AFB and FC DNA (R^2 = 0.665), and between AFG and PC DNA (R^2 = 0.844). Our methods will be useful to characterize maize grains contamination by *Aspergillus* section *Flavi* quickly, easily, and cheaply. Thus, these methods will be used to study the relationship between agroclimatic conditions, AFs content and species prevalence in order to anticipate AFs risks in France with global warming.

P4.2-083

PANTOEA STEWARTII SUBSP. STEWARTII A MOLECULAR DIAGNOSTIC METHOD AND BARCODING TO PREVENT THE SPREAD OF THE PATHOGEN THROUGH MAIZE TRADE

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Text

The EURL for Bacteria in plants activity is focused on the organisms listed in the EU Regulation 2019/2017 as union quarantine pests, among which *Pantoea stewarti*i subsp. *stewartii* (Pss). Pss is the causal agent of Stewart's vascular wilt of maize, responsible for serious crop losses, indigenous of North America and it spreads through maize seeds. For commercial seed certification and official analysis, several molecular and serological tests were developed to detect Pss but some of them cannot distinguish Pss from *P. stewartii* subsp. *indologenes* (Psi) non-pathogenic on maize. Italian isolates recovered in 2015 and 2018 were characterized (molecular, biochemical, pathogenicity and sequencing) and genomes were assembled through MinION and Illumina. Exploiting these results, a new primer combination was defined to specifically detect Pss by real-time PCR up to 10^3 CFU/mL in spiked maize seeds. Critical reagents were also assessed by a TPS involving Italian official laboratories to verify the possible influence on the robustness of the test. Another approach is ongoing to properly detect and identify Pss in maize seeds. It is based on the implementation of barcoding procedure described on Brady et al., 2008 and developing an amplicon sequencing approach by Nanopore. This study addresses the critical issue related to the import of maize seeds from regions where the disease is endemic and, in general, can prove to be a reliable tool in detecting different seed-borne pathogens.

P4.2-084

VALIDATION AND DEVELOPMENT OF DIAGNOSTIC METHODS FOR BANANA WILT ASSOCIATED PHYTOPLASMAS – A BIOSECURITY PERSPECTIVE

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Text

Phytoplasmas are uncultured pathogenic bacteria that colonise the phloem and are transmitted by sap-sucking insects. In Papua New Guinea, the centre of origin of banana plants, banana wilt associated phytoplasmas (BWAP) are causing devastating losses in banana and coconut, whose associated disease is called Bogia Coconut Syndrome (BCS).

BCS is localised to PNG's Madang Province, while BWAP on banana has spread within PNG and to Solomon Islands. Identification of infected plants and follow-up containment and eradication campaigns are reliant on accurate diagnostics. Detection tests are also crucial to prevent new disease incursions via worldwide movement of banana germplasm for genetic improvement. This is especially relevant as the ongoing global spread of Fusarium wilt TR4 increases the need for novel resistant banana varieties.
Although published methods to identify phytoplasmas are available, related bacteria are often detected in banana and lead to false positives. We developed a rigorous approach to validate novel and published nested PCR and real time PCR based diagnostic assays for phytoplasmas on a range of DNA samples from phytoplasma-infected plants and from plants that contain phytoplasma-related endophytic bacteria. We designed novel molecular assays using BWAP draft genomes and identified the most reliable method to detect phytoplasmas in banana. Our results enable a scientifically informed assay choice for phytoplasma indexing of banana germplasm worldwide.

P4.2-085

ELUCIDATING THE SOURCE OF RECURRING FINDINGS OF PANTOEA STEWARTII SUBSP. STEWARTII IN EUROPE

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Text
Pantoea stewartii subsp. *stewartii* (*Ps*) is a plant pathogen causing wilting in corn (*Zea mays*). It is native to the Americas where the disease is spread mainly via its beetle vector (*Chaetocnema pulicaria*). It is a quarantine pathogen in the European Union where it has been detected with some regularity despite the presumed absence of the vector. In recent years it has been reported mainly from Italy and Slovenia. From 2018 to 2022, the official survey for *Ps* in Slovenia confirmed its presence in 11 samples of corn in the Mediterranean region bordering on Italy. The infected plants exhibited leaf wilting in the summer and autumn and were of different varieties. The status of *Ps* in Slovenia following official phytosanitary measures is 'transient'; it remains under eradication and under surveillance. With the aim to identify the origin of infections whole genome sequencing of the *Ps* isolates was done with Illumina and Nanopore technologies. Following hybrid genome assembly, the ANI analysis confirmed the Slovenian corn isolates as *Ps*. However, phylogeny-inferring analysis grouped these isolates somewhat distant to the American *Ps*. At this stage, it is not clear whether this positioning is caused by the lack of more recent genomic data on *Ps* from the Americas or a historical divergence of the European *Ps* strains. Additional comparative genomics analysis is ongoing to shed more light on the origin of the findings in Europe.

P4.2-086

INDICANTS PROJECT: INNOVATIVE DIAGNOSTICS FOR BANANA PATHOGENS SURVEILLANCE

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Text

The aim of the INDICANTS project is to develop innovative diagnostics for four banana wilt pathogens, including *Fusarium oxysporum* f. sp. *cubense* (Foc) TR4 (Fusarium wilt), *Ralstonia solanacearum* (Moko disease), R. *syzygii* subsp. *celebesensis* (Blood disease), and *Xanthomonas vasicola* pv. *musacearum* (Xanthomonas wilt). The main objectives are to: (I) develop low-cost LAMP (loop-mediated isothermal amplification) assays (II) compare simplified DNA extraction methods for field application; (III) validate the LAMP protocols via inter-laboratory and field tests. LAMP primer sets were designed for the bacterial pathogens, using *in silico* comparative genomic analysis of target and non-target genomes, and showed 100% specificity when tested with a wide range of target and non-target strains. A limit of detection of 10^4 CFU/ml was obtained for the LAMP assays. A simplified DNA extraction method from banana tissue was developed and successfully validated in a banana plantation infested with Foc TR4, using several candidate LAMP primer sets. Ready-to-use diagnostic kits, based on these protocols, are currently being developed by a private company. These point-of-care diagnostic tools will allow rapid identification of the different pathogens in the field for disease management.

P4.2-088

CURRENT STATUS OF PRUNUS NECROTIC RING SPOT VIRUS IN MONTENEGRO

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Text

Prunus necrotic ring spot virus (PNRSV) is one of the most economically important viruses of stone fruit trees. It is distributed on peach and nectarine in Podgorica district but information about its incidence and genetic diversity on other Prunus sp. in other parts of the country is

limited. We determined the incidence of PNRSV in 11 municipal districts of Montenegro and studied its genetic diversity from different Prunus sp. Out of 65 samples analysed by RT-PCR, 12.3% were infected with PNRSV from 2 out of 11 districts. CP gene (675 bp) sequences from one peach, two nectarine and two cherry samples showed that Montenegrin isolates 139/21 (peach) and 55/22 (cherry) had 98.4% identity with a Spanish nectarine isolate (AJ133208), while isolate 46/22 (cherry) was 98.4% identical with an Italian peach isolate (AJ133205). Two nectarine isolates 137/21 and 138/21 showed 99.3% identity with previously described Montenegrin peach isolate (JX569826). The phylogeny reconstruction (minimum-evolution method) allowed clustering of Montenegrin isolates in two groups. Three isolates (139/21, 46/22 and 55/22) clustered within PV-96 group, while two isolates (137/21 and 138/21) clustered within PE-5 group. Five PE-5 specific amino acid residues (K59, N121, R139, N142 and I181) were also identified in two Montenegrin isolates. This study highlights the need for strict phytosanitary measures implementation and the production of certified propagative material in the country.

P4.2-089

DISPERSAL PATTERNS OF PETRI DISEASE ASSOCIATED PATHOGENS IN ROOTSTOCK MOTHER VINES AND IMPACT OF PATHOGENS ERADICATION IN THE PRODUCTION OF HEALTHY PROPAGATION MATERIAL

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Text

Petri disease caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* included in the complex of grapevine trunk diseases (GTDs), is a major threat to grapevine cultivation. The pathogens' presence in grapevine nurseries has been widely reported, affecting the produced propagation material, and leading to huge losses. The aim of this study was to investigate the pathogens distribution along canes of nine different clonal rootstocks and the effect of diseased mother vine eradication in pathogens inoculum dispersal in a three-year survey. Detection of the pathogens was carried out by using a Nested PCR approach. PCR reactions showed that the detection percentage followed a sequential decrease during the three growing seasons, where in some cases the pathogens were not detected. Due to the low concentration levels of the pathogens, quantification of the prevailing fungus *Pa. chlamydospora*, was carried out by utilizing a newly developed Nested qPCR methodology based on pre-amplification of the regions flanking the dual-labeled probe that was subsequently used in the qPCR assays. Quantification experiments revealed the pathogens biomass was decreasing up to 95% in the basal end of the canes not exceeding the levels of fg/ng of plant DNA. The data presented indicate that uprooting of mother vines showing typical GTD symptoms, could be an effective control measure to reduce pathogens inoculum in the field, and to produce pathogen-free grafted vines with added value.

P4.2-092

SNP4ORPHANSPECIES: A BIOINFORMATICS PIPELINE TO ISOLATE MOLECULAR MARKERS FOR STUDYING GENETIC DIVERSITY OF ORPHAN SPECIES

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Text

For several decades, an increase in disease or pest emergences due to anthropogenic introduction or environmental changes has been recorded. Many of these events involve species with poor or no genomic resources (called here "orphan species"). This lack of resources is a serious limitation to our understanding of the origin of emergent populations, their ability to adapt to new environments and to predict future consequences to biodiversity. We developed a generic bioinformatics pipeline to rapidly isolate such markers with the goal to be applied in studies of invasive taxa from different taxonomic groups, with a special focus on forest fungal pathogens and insect pests. This pipeline is based on: 1) an automated de novo genome assembly obtained from shotgun whole genome sequencing using paired-end Illumina technology; 2) the isolation of single-copy genes conserved in species related to the studied emergent organisms; 3) primer development for multiplexed short sequences obtained from these conserved genes. The pipeline's functionality was evaluated with sequenced genomes of five invasive or expanding pathogen and pest species in Europe (Armillaria ostoyae, Bursaphelenchus xylophilus, Sphaeropsis sapinea, Erysiphe alphitoides, Thaumetopoea pityocampa). We successfully isolated several pools of one hundred short gene regions for each assembled genome, which can be amplified in multiplex. The bioinformatics pipeline is user-friendly and requires little computational resources.

P4.2-093

SPECIES-SPECIFIC PCR REVEALS THE OCCURRENCE OF THE ALIEN FUNGUS ERYSIPHE CORYLACEARUM INFECTING HAZEL IN HUNGARY

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Text

The market demand for common hazel (*Corylus avellana*) fruit is steadily increasing. The powdery mildew (PM) on hazel in Hungary, and also in whole Europe, was caused earlier by *Phyllactinia guttata*. However, since 2017, an other fungus of Asian origin, *Erysiphe corylacearum*, has been infecting hazels in Europe. The aim of the study was to assess the presence of the alien fungus in Hungary. We collected ~40 samples from across Hungary in 2022. Morphology of the PM fungi was observed, and the internal transcribed spacer (ITS) of

the ribosomal DNA was sequenced. To aid differentiation of *P. guttata* and *E. corylacearum*, a species-specific PCR was developed. In approx. half of the samples, *E. corylacearum* was present; on the other half, both fungi could be found, and only few samples carried solely *P. guttata*. Mostly, but not exclusively, *P. guttata* was found on the lower, while *E. corylacearum* on the upper side of the leaves. *E. corylacearum* also infected nuts, and it was found not only on *C. avellana*, but also on *C. colurna* (Turkish hazel). As *E. corylacearum* spreads rapidly, it can be considered as an invasive pathogen. Its practical importance lies in its ability to infect nuts, potentially causing economic losses. Our species-specific PCR method supports the correct identification of the fungus, which is a prerequisite for successful plant protection.

P4.2-094

IDENTIFICATION AND QUANTIFICATION OF GRAPEVINE TRUNK AND BLACK-FOOT DISEASES PATHOGENS IN THE SOIL, USING REAL-TIME PCR COUPLED WITH HRM.

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Text

Identification of plant pathogens and inoculum quantification in soil samples using conventional methods is rather labor-intensive and time-consuming. Therefore, the development of rapid, and simple to perform PCR-based identification methods that use pathogen-specific primers is necessary. Herein, a real-time quantitative PCR approach coupled with high-resolution melting (HRM) analysis was developed with one primer set to identify and distinguish several fungal species associated with grapevine trunk and black-foot diseases. In detail, the developed method targeted several Cylindrocarpon-like asexual morphs belonging to the genera *Ilyonectria* or *Dactylonectria* and the fungal species *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Diplodia seriata*. The technique's reliability was first assessed on DNA extracted from pure cultures. The melting curve analysis of the amplicons allowed for the distinction of all target species with confidence levels >99%. For each targeted genera/species HRM curve profiles were generated. The identification of the target pathogenic species in the fortified soil samples was achieved in a range confidence between 60-75%. The quantification of the detected pathogen DNA in the soil material was assessed with quantitative PCR and the sensitivity was evaluated using standard curve. This study provides the development of a new molecular tool to detect and quantify several GTD or Black foot pathogens in soil samples of grapevine nurseries.

P4.2-095

BACTERIAL LEAF SPOT OF HYDRANGEA: ON A “NEW OLD” DISEASE AND THE IMPORTANCE OF GETTING IT RIGHT IN PHYTODIAGNOSTICS

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Text

The causal organism of bacterial leaf spot of *Hydrangea*, *Xanthomonas hydrangeae*, was the subject of a disease report and a new bacterial species description in 2021. These publications, including the developed *X. hydrangeae*-specific isothermal diagnostics assay, the raising of awareness about this pathogen thereof, and the favorable wet weather conditions during the summer of 2021 in Europe uncovered a wider historical and contemporary prevalence of this disease. In 2022, the pathogen was also reported for the first time on hydrangea plants in Tuscany, Italy. Global trade of plants appears to play an important role in the dissemination of this pathogen. Furthermore, scouring the literature revealed multiple instances, mainly in the USA, of bacterial leaf spot of *Hydrangea*, attributed to various *Xanthomonas* species (e.g., *Xanthomonas campestris*, *Xanthomonas hortorum*). The first known mention of bacterial leaf spot of *Hydrangea* dates to 1996 in Georgia, USA. However, those 1996 isolates were not available and thus their identity as *X. hydrangeae* cannot be confirmed. This work outlines the challenges encountered when studying *X. hydrangeae*, as related to 1) the development of a diagnostics assay targeting *X. hydrangeae*, especially given its phylogenetic similarity to *X. hortorum* and 2) the information discontinuity regarding the historical and contemporary occurrence of bacterial leaf spot of *Hydrangea*.

P4.2-096

DEVELOPMENT OF RAPID AND AFFORDABLE VIRUS-MIMICKING ARTIFICIAL POSITIVE CONTROLS AND THEIR APPLICATION IN DIAGNOSTICS

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Text

Existence of positive controls is the tailback in the development of detection assays. Failure to acquire positive controls, majority of diagnostics labs use an in-vitro synthesis approach e.g. the target sequence integrated into a plasmid. Even though plasmids yield positive results, they have disadvantages as it takes time between design and delivery, they are costly and increase the chances of contamination which risk the integrity of the assays. The high concentration of DNA in plasmids does not represent the natural titer of a pathogen in plants nor their tropism in tissues. Here, we present a new approach that is cheaper than the present alternatives i.e., plasmids, RNA transcripts or synthetic oligonucleotides and are ready to use within a week including the time for designing, ordering and detection. It is feasible to work with both DNA and RNA viruses and provides an actual representation of virus titer and tropism. They can be used in routine diagnostics as well as in outbreaks, where an immediate response is of utmost importance.

P4.2-097

DETECTION OF LATENT INFECTIONS OF APPLES CAUSED BY NEOFABRAEA SPP AND MONILINIA SPP FUNGI USING LAMP METHOD

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Text

Bull's eye rot of apple (BER; caused by fungi of the genus Neofabraea: N. vagabunda, N. kienholzii, N. perennans and N. malicorticis) is considered the most important storage disease of apples, due to significant yield losses. Brown rot of apples (caused by fungi from the genus Monilinia: M. fructicola, M, fructigena and M polystroma) also occurs on stored apples, however, less frequently. It is considered that the fungi present at the time of harvesting hidden in apple lenticels and arrested in grow, are mainly responsible for the BER development on stored fruits. On the other hand, the causal agents of brown rot infect the apple not only via lenticels but also via microcracks in the cuticle and mechanical damage. In terms of risk assessment and prediction of the severity of mentioned diseases, it is important to assess fruit health status prior to placing them in cold storage and during storage, which is enabled by fast, sensitive, and thorough diagnostic tests based on LAMP technique.
The study aimed to develop the detection methods of the fungi causing BER and brown rot on apples in infected but yet-asymptomatic apple fruits. Presented here diagnostic protocols, based on the LAMP technique, enabled sensitive and specific detection of fungal pathogens responsible for the development of bull's eye rot as well as brown rot in apples at an early stage of disease development. The performance of the whole diagnostic protocol: from the step of preparation of the apple peel to the step of final fungal DNA detection in mixed plant-fungal material lasted two days. At the same time the LAMP reaction itself allows for the detection in a time of 35 minutes. The whole proposed procedure allowed for the evaluation of the health status of the fruit before the disease symptoms development. The sensitivity of LAMP detection in reaction with DNA from axenic Neofabraea cultures was about 4 pg/µl, while with DNA from axenic Monilinia cultures was 10 pg/µl. The sensitivity of detection of fungal DNA in the mixture with apple DNA ranged from 1,5 to 10 pg/µl for Neofabraea – specific primers sets, while 2 pg/µl for Monilinia – specific primer set. In general, preamplification followed by LAMP increased the sensitivity of target detection about 10 times. The specificity of the tested protocol in the detection of DNA of three targeted Neofabraea or Monilinia species among the DNA of various fungi occurring on apple peel was confirmed.

P4.2-098

SPECIFIC AND SENSITIVE DETECTION TOOLS FOR XANTHOMONAS ARBORICOLA PV. CORYLINA, THE CAUSAL AGENT OF BACTERIAL BLIGHT OF HAZELNUT, DEVELOPED WITH COMPARATIVE GENOMICS

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Text
Xanthomonas arboricola pv. corylina (formerly *Xanthomonas campestris* pv. corylina; Vauterin *et al*. 1995) is the causal agent of the bacterial blight of hazelnuts, a devastating disease of trees in plant nurseries and young orchards. Currently, there are no PCR assays to distinguish *X. arboricola* pv. corylina from all other pathovars of *X. arboricola*. A comparative genomics approach with publicly available genomes of *X. arboricola* pv. corylina was used to identify unique sequences, conserved across the genomes of the pathogen. We identified a 2,440 bp genomic region that was unique to *X. arboricola* pv. corylina and designed identification and detection systems with conventional PCR, qPCR (SYBR Green and TaqMan), and loop-mediated isothermal amplification (LAMP). All PCR assays performed on genomic DNA isolated from all eight *X. arboricola* pathovars and closely-related bacterial species confirmed the specificity of selected primers. Moreover, these assays enabled accurate and sensitive detection of *X. arboricola* pv. corylina in pure cultures and plant tissues. These new multi-platform molecular diagnostic tools may be used by plant clinics and researchers to rapidly and accurately detect and identify *X. arboricola* pv. corylina in pure cultures and in hazelnut tissues.

P4.2-099

CURRENT ETIOLOGY OF ASPERGILLUS VINE CANKER AND SOUR ROT OF TABLE GRAPES IN CALIFORNIA

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Text
Fungal taxonomy is in constant flux and the advent of reliable DNA barcodes has allowed to improve the accuracy of identification of cryptic species. In California, Aspergillus Vine Canker (AVC) and Sour Rot (SR) are two diseases that affect the wood and the fruit of grapevines, respectively, and their causal agents have been previously studied using morphological characters. During the last decade, the taxonomy of *Aspergillus* section *Nigri* has been revised and modified. In this study, we aimed to reassess the etiology of AVC and SR using a combination of morphological and phylogenetic analyses. Thirty-two isolates were selected based on morphological characters from 266 isolates grown on malt extract agar. Upon DNA extraction, a fragment of the calmodulin (*CaM*) gene was amplified through PCR and sequenced using the primer pair CL1/CL2A. Results revealed that isolates associated with AVC recovered from recent detections correspond to *A. tubingensis*,

whereas isolates from previous studies that were initially identified as *A. niger* and *A. carbonarius* were re-identified as *A. welwitschiae* and *A. carbonarius*. The isolates from table grapes with SR corresponded to *A. tubingensis*, *A. welwitschiae*, and *A. carbonarius*. Overall, our results indicate that *A. tubingensis* was the dominant species causing both AVC and SR, and representative isolates were able to cause disease in both wood and fruits of Red Globe grapevines.

P4.2-100

MAJOR CASSAVA DISEASES IN THE TROPICS: THREATHS TO FOOD SECURITY AND LIVELIHOODS FROM ASIA AND THE AMERICAS

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Text

Cassava in the tropics is recognized as a prominent food security crop for poor and vulnerable communities. But its efficient growth under harsh environmental conditions is limited by re-emerging diseases occurring in distinct geographies. Cassava witches broom disease (CWBD) is spreading again in Southeast Asia (SEA), where it now has to coexist with the geminivirus Sri Lankan cassava mosaic virus (SLCMV), a recently introduction to the region. In the Americas, the center of origin of this crop, Cassava Frogskin Disease (CFSD) remains as a major constrain to cassava production in Colombia and neighbouring countries. Recent field and laboratory experiments, including high throughput DNA and siRNA sequencing, show that CFSD is associated with a unique virome, while CWBD is associated with a fungal infection, and not with phytoplasma as previously suspected. None of these diseases has been reported in Africa, where major efforts are focused on managing Cassava Brown Streak Disease caused by ipomoviruses, and Cassava Mosaic Disease, caused by a different set of geminiviruses. Neither CWBD nor CFSD re-emergence is completely unexpected, they are known to occur for over 15 and 30 years, respectively. However there is still limited knowledge on its biology which affects its local management and increase the risk of its distribution to new areas. Strenghtening existing regional research networks to tackle re-emerging disease threats is needed to implement pre-emptive responses

P4.2-101

PREVENTING A WORLD WITHOUT ROSES: RT-LAMP TARGETING GENE-FRAGMENTS OF ROSE ROSETTE VIRUS

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Text

Rose rosette virus (RRV) is a negative-sense, ss-RNA virus in the genus *Emaravirus* (*Fimoviridae*) and the causal agent of rose rosette disease (RRD). RRV is

windborne and transmitted by two mite species. RRD devastated rose gardens in the USA, is reported in Canada and India, and threatens rose industries worldwide. Loop-mediated isothermal amplification (LAMP) for RRV can be implemented in quarantine labs and nurseries. Although symptoms are characteristics, early diagnosis is misleading and may appear like herbicide damage. RRD takes a long incubation time for symptoms visualization. RRV gene sequences P3 and P4 were analyzed and two sets of four LAMP primers were designed. Direct virus-capture into polypropylene-PCR tubes was used to circumvent kit-based RNA extraction. RT-LAMPs take 1 hour at 64°C (RRV-P3) and 66.5ºC (RRV-P4) using either a thermocycler or portable dry bath. A synthetic artificial positive control (APC) conceived de novo concatenating sense and anti-sense primers created a plasmid positive control for use with most RRV and eriophyid mites reported primers. RRV was detected in symptomatic and non-symptomatic RRD tissue from Oklahoma. The limit of detection (LoD) was 1pg/µL and 1 fg/µL using Bst 2.0 LAMP and GspSSD LD qLAMP. The LoD was 10 pg/µL and 0.1 pg/µL using hydroxy naphthol blue (120 µM) and SYBR green I (1:10 dilution), respectively. No cross-reactivity was detected in the RT-LAMP reactions.

P4.2-102

DEVELOPMENT OF A REAL-TIME PCR FOR THE DETECTION AND QUANTIFICATION OF FUSARIUM EQUISETI INOCULUM IN SOIL FROM LETTUCE FIELDS

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Text
In this study, an emerging foliar disease observed in commercial lettuce farms has been associated to the pathogen *Fusarium equiseti*, a member of the *Fusarium incarnatum-equiseti* species complex (FIESC). Thirty *F. equiseti* isolates obtained from symptomatic lettuce plants were identified based on morphology and evaluated for their pathogenicity. The isolates were further characterized using amplification and sequence analysis of the internal transcribed region (ITS-rDNA), and of the translation elongation factor 1-alpha (*TEF1-a*), calmodulin (*CAM*), beta-tubulin (*Bt*), and small subunit (*SSU*) genes. Moreover, a novel RT-qPCR assay was developed, designing a primer pair and a probe based on the *TEF1-a* sequences. This assay showed high specificity, amplifying *F. equiseti* DNA samples, while no amplification product was observed from samples of other common soilborne fungi. The generated RT-qPCR assay could be a useful tool for the detection and quantification of *F. equiseti* in soil samples deriving from fields cultivated with lettuce and other leafy vegetables, hosts of this specific pathogen. The emergence of the specific fungus on new plant hosts could be associated with environmental changes. In addition, *F. equiseti* could be transmitted by seeds of several leafy vegetable hosts, such as wild rocket. This epidemiological aspect along with the climate change scenario, could constitute a potential cause for the outbreak of *F. equiseti* on leafy vegetables.

P4.2-103

A TAQMAN-BASED MULTIPLEX REAL-TIME RT-QPCR FOR THE SIMULTANEOUS DETECTION OF BOTRYOSPHAERIACEAE SPECIES IN WOODY CROPS

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Text

Botryosphaeria dieback is a fungal disease that is increasingly threatening woody crops worldwide. To develop a simple, rapid, accurate, and high-throughput detection method for diagnosis and quantification of Botryosphaeriaceae species, specific primers and probes were designed based on the translation elongation factor 1 alpha (tef) and B tubulin (tub2) genes. A TaqMan-probe-based multiplex real-time RT-qPCR assay was developed, optimized and validated to simultaneously detect Neofusicoccum parvum, Botryosphaeria dothidea and any species of the Botryosphaeriaceae family. Performance of multiplex and singleplex qPCR were compared. The results showed that the limit of detection can reach as low as 10 fg of genomic fungal DNA in simplex and multiplex real-time RT-qPCR assay, with high correlation coefficients (R2) and amplification efficiencies between 90 and 120%. This multiplex real-time RT-qPCR assay demonstrated high sensitivity, specificity, and repeatability and provides a rapid, accurate and easy-to-use tool for detection and quantification of Botryosphaeriaceae fungi. The high sensitivity of this qPCR assay allows the detection of Botryosphaeriaceae fungi in plant material even before the appearance of symptoms. Therefore, this powerful diagnostic tool could be applied for the preventive detection of Botryosphaeriaceae fungi in nursery plant material or recently planted young trees, avoiding he introduction and dispersion of these pathogens in production fields

Emerging Phytophthora's: Tackling Global Outbreaks that Impact Food security

C8.2-1

PREDICTING FUTURE PHYTOPHTHORA OUTBREAKS: NEW TOOLS TO IDENTIFY EMERGING LINEAGES AND TRACK SPREAD

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Text

The risk of introduction of pathogens into the US with trade requires continued surveillance and improved diagnostic capabilities at our borders. *Phytophthora infestans*, the causal agent of potato late blight was responsible for the Irish potato famine and is still a threat to

food security globally. A disease surveillance and mapping system called USAblight.org has been operative since 2011 to report disease and alert stakeholders; SSR genotyping and sequence based approaches have been used to track spread of modern and historic lineages (FAM-1) of the pathogen and now targeted amplicon sequencing is under development to monitor emergence of new lineages. We have also developed LAMP assays deployed on smart phones and a phone APP for rapid in field detection of emerging species and lineages of *Phytophthora*. A a tree based complete phylogeny of *Phytophthora* has been released as a community tool for identification and phylogenetic tracking of SSR lineages of *P. infestans* and emergent new *Phytophthora* species. All these tools will help us respond to and mitigate outbreaks, improve deployment of host resistance and inform policy.

C8.2-2

THE RANGE AND CONTRIBUTION OF BARCODING IN PHYTOPHTHORA AND OTHER OOMYCETES

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Text

Biosecurity protocols are based on listed threats to plant health but monitoring is increasing our awareness of a potential reservoir of novel undescribed *Phytophthora* taxa. We need to understand the range and spread of known and unknown *Phytophthora* taxa to protect both food production and plant health in natural ecosystems. We have combined in situ filtration, a generic rDNA ITS1-based PCR test and high-throughput sequencing technology to explore the diversity of plant pathogenic oomycetes in environmental DNA (eDNA) samples. We discuss current applications of this metabarcoding to the study of *Phytophthora* and downy mildew diversity in natural and plant production systems. The method is proving valuable but technical challenges and questions remain. Validation of the downstream computational biology pipeline to process the data while accessing a robust database of contemporary reference sequences is critical. We also report on the crucial role that internal synthetic sequence controls play in the interpretation of metabarcode data and explore the options for other barcodes to improve those based on rDNA ITS.

C8.2-3

MULTIVARIATE BAYESIAN ANALYSIS TO PREDICT INVASIVENESS OF PHYTOPHTHORA PATHOGENS

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Text
Global concerns are many for the invasive impacts of Phytophthora pathogens on native vegetation, agriculture, nurseries and urban parks and gardens. We compiled a database of 32 traits on 204 species of Phytophthora including data on each species' taxonomy (clade and subclade), historical knowledge (years since first described), impacted ecosystems, micro-environments inhabited, dispersal mode, physiology, and morphology. Drawing from approximately 11 394 unique host, pathogen, and country plant disease records from Genbank and other sources, we calculated potential invasiveness of 103 better-studied species from cluster relationships. We used the species data to create a Bayesian network model predicting the degree and probability of invasiveness of individual Phytophthora species. Model calibration testing resulted in <1% error rate in classifying invasiveness categories of well-known species. We applied the model to predicting potential invasiveness of 101 other species with unknown invasiveness dynamics. The model can also be used to predict the invasive risk of other poorly-studied and newly-identified Phytophthora species, and the general modeling approach can be used for other pests and pathogens, to advise land and resource managers to thwart potential invasions before they occur or intensify.

C8.2-4

IMPLICATIONS OF HOST RANGE AND THERMAL RESPONSE EVOLUTION FOR EMERGENCE OF PLANT PATHOGENIC PHYTOPHTHORA SPECIES

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Text
We investigated the evolution of temperature response and host range in the genus Phytophthora to determine which, if either, niche axis is under stronger phylogenetic constraint and thus more likely to control geographical distributions. We found a small but statistically significant phylogenetic signal in temperature relations for Phytophthora species. While closely related Phytophthora species had more similar thermal physiology than random pairs, in all but one case differences were greater than that expected under a Brownian motion evolutionary model. Apparent latitudinal range shifts of plant pathogens in response to global warming suggest niche conservatism rather than adaptation to new climates in situ. Our analysis suggests limited phylogenetic constraint in temperature niche evolution in Phytophthora. Host jumps (to a host phylogenetically distant host) and transitions from specialist to generalist or vice versa are known in plant pathogens, suggesting that host range could be more evolutionarily labile than temperature physiology. We found a small but statistically significant co-phylogenetic association between the topologies of three Phytophthora phylogenies and the phylogeny of their plant hosts using Procrustean super-imposition. Taken together, our results show that both the thermal niche and host ranges are evolutionarily labile within genus Phytophthora, but retain weak phylogenetic signal.

C8.2-5

INTEGRATING TRAITS, PHYLOGENY AND HUMAN DRIVERS INTO RISK ASSESSMENT FRAMEWORKS FOR EMERGING PHYTOPHTHORA THREATS

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Text

Plant diseases emerge ever more frequently as global trade connections diversify and climate and land-use changes alter the environmental context for plant-pathogen interactions. Phytophthora are oomycete pathogens impacting diverse hosts in ornamental, agricultural and forestry sectors and threatening urban and natural, highly valued, ecosystems. We examine the use of biological traits and phylogeny to inform global horizon-scanning for future threats from Phytophthora. We use a species-level trait database and phylogeny for 203 Phytophthora species to develop risk frameworks for pathogens' successful transport, presence in nurseries, establishment in the wider environment and interactions with hosts to promote targeted surveillance of higher risk pathogens, hosts and locations. Phytophthoras with a broader thermal tolerance range are more likely to be introduced globally. Species with multiple dormant survival structures, (potentially facilitating asymptomatic infections) may also spread more easily through global trade networks. Species with similar traits and/or phylogenetic proximity to known high-impact species may be expected to behave similarly. These risk frameworks are currently limited by incomplete and biased recording globally, but fostering international and cross-sectoral collaborations has significant potential to capture more complete data on the host ranges and niche breadths of pathogens and improve the transferability of predictions to novel pathogens.

C8.2-6

INVESTIGATING THE RISK FROM PHYTOPHTHORA IN PLANT NURSERY GREEN WASTE

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Text

Phytophthora is a genus of destructive plant pathogens. Novel introduced species are increasingly causing damage to native ecosystems, forestry and horticulture sectors. There is evidence that some species have arrived in the UK through the trade in live plants. Central nodes of the plant trade are horticultural nurseries and it was hypothesised that green waste and spent growing media disposal sites in nurseries could act as a reservoir and conduit facilitating *Phytophthora* spread into nursery stock as well as the wider environment. This

project identified *Phytophthora* species associated with waste disposal sites at three Scottish plant nurseries by sampling waste piles, water run-off from waste piles and roots from discarded plants. *Phytophthora* species were identified using a traditional baiting method where the organism is baited into live culture, as well as metabarcode sequencing of sample DNA. Eighteen *Phytophthora* species were found, including the highly damaging species *P. ramorum* and *P. austrocedri*. Plant nursery green waste was shown to harbour highly diverse and varied *Phytophthora* species assemblages, and differences between the three nursery sites appeared linked to their different approaches to biosecurity. Results suggest that the improved management of waste from the horticultural sector, for example through effective on-site composting, is essential to reduce the risk of *Phytophthora* pathogens spreading from nurseries into the wider environment.

P8.2-002

IMPACT OF CLIMATIC FACTORS ON GROWTH AND DEVELOPMENT OF PHYTOPHTHORA INFESTANS CAUSING POTATO LATE BLIGHT IN MAURITIUS

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Text

Potatoes are considered as one of the most economically important non-sugar food crops in Mauritius but they are affected by many pathogens; Late Blight (LB) being a greater threat. The aim was to study the prevalence of LB based on weather factors and to identify the causative agent of LB. A disease surveillance of potato plantations was conducted in potato fields over a two-year study period. Disease Incidence (DI) of LB, was estimated in different potato fields. Infected leaf samples with LB symptoms were identified by microscopy, culturing and molecular methods. Equally, a predictive model was developed using multi-stepwise regression analysis based on DI of LB and weather data. To characterize the growth kinetics, the isolates were grown on Pea Sucrose Agar plates, incubated at five different temperatures and relative humidity conditions up to 10 days in climate-controlled chambers. Results revealed mean DI for LB was 26.95% and the causative agent was confirmed to be *Phytophthora infestans*. LB was significantly influenced by weather factors temperature, rainfall, wind speed and relative humidity ($R2 = 0.27$, $P < 0.05$). Controlled chamber studies indicated that a temperature of 20ºC (*Gr* = [6.30 ± 0.498] mm/day) and a relative humidity of 86% (*Gr* = [8.42 ± 1.770] mm/day) were most favorable for the growth of *P. infestans*. We can infer that LB incidence was strongly dependent on weather conditions in Mauritius that can compromise the yield of this important commodity.

P8.2-003

IMPRESSIVE TAXONOMIC VARIABILITY OF PHYTOPHTHORA SPP. IN COMMERCIAL NURSERY STOCK

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Text

The active management of *Phytophthora* (Oomycetes) in commercial nurseries represents a priority for the biosecurity of traded plants. Furthermore, the constant monitoring of the incidence of this important plant pathogen is a crucial prerequisite to prevent its spread in the nearby environment.
For this purpose, potted plants showing Phytophthora spp. symptoms were selected and sampled, together with irrigation and runoff water from one commercial nursery in Tuscany during autumn and spring season. The samples were processed to detect *Phytophthora* species using baiting technique and molecular identification of the isolated colonies.
The results showed a very high presence of the pathogen during the spring season, with eleven different *Phytophthora* species isolated from both potted plants and water, and where singular symptomatic ornamental potted plants were found to have up to four Phytophthora highly pathogenic species. The sample type ‘run-off water from the drainage canals’ showed the highest number of Phytophthora species, followed by ‘flow-through potted plants irrigation water’ and ‘puddles’ water’.
This study provided considerable evidence of the high incidence of Phytophthora in the ornamental nursery sector; highlighted how a substantial taxonomic variety of the pathogen could potentially spread in the urban environment; as well as the high likelihood of a hybridization event between two previously geographically isolated species.

P8.2-004

EVIDENCE FOR INCREASED SEXUAL REPRODUCTION OF PHYTOPHTHORA INFESTANS UNDER GLOBAL WARMING

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Text

The reproductive system of eukaryotic pathogens plays an important role in disease epidemiology, but how global warming may affect the reproductive system of plant pathogens is poorly understood. In our study, we analyzed oospore production of five *Phytophthora infestans* populations sampled from the same potato variety grown at different altitudes (1976-2677 m) along a single hill under five temperature regimes. We found that both the altitude, a proxy for adaptation of the pathogen to historical temperature, and the experimental temperatures affected the capacity of the pathogen to produce oospores, with the experimental temperature playing a more important role than the historical temperature. In addition, the sexual reproduction potential of *P. infestans* was positively associated with the estimated temperature breadth of the pathogen and reached the maximum at the experimental temperature of 21 ?, which is higher than the annual average temperature in many potato producing areas and the optimum temperature of asexual reproduction of the pathogen. These results suggest that sexual reproduction of *P. infestans* may occur

throughout the potato and tomato growing seasons, and that increased air temperatures associated with global warming may enhance sexual reproduction of the pathogen, which could result in an increased threat to agricultural production and highlights the need to implement new epidemiological strategies to ensure future food security.

Endophytes and diseases

C9.3-1

ENDOPHYTISM, AN EVOLUTIONARY GATE TO SYMBIOSES

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Text

An accumulating number of studies report fungi of ‘known’ ecological niche in bizarre, unexpected situations, especially thanks to molecular barcoding. This occurs in fungi forming mycorrhizal symbioses with the roots of Ericaceae, orchids or forest trees: they also colonize various other plant species that are not their mycorrhizal hosts as endophytes. (Endophytism is a symptomless, loose colonization without morphological differentiation nor tight dependence on the plant side). Based on our research, we provide direct and indirect evidence, both morphological and functional, for root endophytic abilities in fungi mycorrhizal on trees, including Tuber spp., but also Sebacinales or Russulales. Similarly, fungi that are characterized as plant parasites from symptomless endophytism in non-host species.
We propose here an evolutionary interpretation of such dual niche: the evolution of tight interactions with plant (symbiosis, parasitism) through a pathway called the ‘waiting room hypothesis’. Root endophytism may act as a symbiotic ‘waiting room’, where loose biotrophic coexistence predisposes evolution towards tighter interactions, with more complex morphologies and host adaptations. Some fungal taxa, now mycorrhizal or parasitic, also retain their ancestral endophytic habit. We will illustrate this on the evolution of the orchid mycorrhizal symbioses.
To conclude, fungal ecological niches are largely postulated by mycologist, and as such, not reliably realistic.

C9.3-2

APOPLASTIC SPACE OF TWO CULTIVARS PROVIDES HIGHLY DIFFERENT ENVIRONMENTS FOR PATHOGEN COLONIZATION: INSIGHTS FROM PROTEOME AND MICROBIOME PROFILING

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Text
Endophytes can affect the composition and function of the plant apoplast, for example, by triggering apoplastic immunity. The role of the plant immune system in detecting and controlling pathogens is well described; however, its effect on plant-associated endophytes is still poorly understood. Z. tritici is a fungal pathogen that colonized the apoplastic space of wheat plants. Wheat resistance and susceptibility can be mediated via gene-specific between wheat and Z. tritici. Here, we validated the apoplastic fluids as a proxy to understand the warfare between plants and microbes. Using proteomic analysis, we show that Z. tritici diminishes the photosynthetic functionality in the susceptible cultivar, while the resistant is significantly enriched in defense response-related proteins. This difference in plant immune response affects the apoplastic microbial composition. Next, we determined the tolerance of apoplastic microbes to plant-produced immune-related antimicrobial compounds. We also found several bacterial isolates showing susceptibility to antimicrobial compounds and negatively affecting the growth of Z. tritici. Finally, we analyzed the genome of antagonistic bacterial strains and found that a biosynthetic gene cluster associated with fungal growth inhibition is under positive selection. Our findings highlight the potential of a multi-omics approach targeting the outcomes of complex plant defense compounds and microbial-microbial interactions in apoplastic fluids.

C9.3-3

RELATIONSHIP BETWEEN FUNGAL ENDOPHYTES AND PLANT DISEASE

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Text
The endophytic community of plants includes diverse fungi ranging from true endophytes (not causing disease in a specific host species) to latent pathogens. In fact, a fungus can be pathogen in one species and endophyte in another species. Both endophytes and pathogens have developed ways to circumvent recognition to the extent that the plant immune system does not hinder colonisation. However, why some organisms turn pathogenic while others develop a mutualistic or commensal endophytic relationship is poorly understood.
We have studied endophytic fungal communities in tomato and wheat using amplicon sequencing and isolation of fungi primarily for functional characterisation of their interaction with plants. Communities in symptomless plants harboured high frequencies of known pathogens. Lifestyle testing of isolated fungi confirmed the presence of both beneficial endophytes and latent pathogens, suggesting complex interactions within the microbiome, which is in equilibrium with the plant, preventing pathogens from causing disease. Inoculating plants with endophytes revealed induction of plant defence-related genes and changes in specialised metabolite composition both locally and systemically. This may be key for keeping pathogenic organisms in check.

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C9.3-5

FROM FIELD TO MICROBIAL LANDSCAPES: IMPACT OF SUSTAINABLE PRACTICES IN MICROBIAL COMMUNITY IN A CORN-SOYBEAN ROTATION SYSTEM

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Text
Cover crop rotation is a sustainable practice to improve soil fertility and enhance soil health. Cover crops are associated with increasing the diversity of microbial communities, providing a range of symbiotic relationships with the plant host. We hypothesize that beneficial microbes will prevail in the soil and be recruited by crops to enhance plant growth and mediate interactions reducing disease. To investigate this, a long-term study focused on a corn-soybean cropping system in combination with a cover crop rotation was used to determine the influence of rotations on soil health, the severity of soilborne diseases, and plant health. Five cover crop treatments: fallow, cereal, legumes, soil health recommendation (60% cereal and 40% legume), and alternation between winter cereal (before soybean) and legume (before corn). Aerial images were used to establish NDVI and one “healthy” and one “unhealthy” point were located within each strip. A combination of amplicon-based approaches characterizing fungal and bacterial communities and bioassays have been used to understand the community composition and statistically classify communities that could drive plant health toward a tolerant host. There are standing questions on the complexity of the communities and the actual mechanisms by which cover crops enhance soil health promoting plant health. Using a three-pronged approach would provide a framework to understand the role of rhizospheric/endophytic microbes on plant health.

C9.3-6

PATHOGENICITY OF ENDOPHYTIC FUSARIUM SPECIES FROM CORN PLANTS (ZEA MAYS) IN PENINSULAR MALAYSIA

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Text
Endophytes are widely known as microbes that infect internal tissues of host plants for all or part of their life cycles, without causing any visible symptoms of disease. The present study was carried out to identify and investigate the pathogenicity of endophytic *Fusarium* spp.

resident of corn plants, grown on different fields in Peninsular Malaysia. Endophytic *Fusarium* spp. were identified using a combination of morphological characterization, and molecular analysis of β-tubulin and TEF-1α sequences. Endophytic *Fusarium* spp. isolated from corn plants were identified as *F. pseudocircinatum, F. verticillioides, F. andiyazi, F. sacchari, F. mangiferae, F. fujikuroi, F. proliferatum,* and *F. incarnatum.* Pathogenicity test showed that all tested endophytic fungi produced varying levels of disease symptoms on healthy corn plants. *Fusarium verticillioides* was the most pathogenic, followed by *F. fujikuroi, F. andiyazi, F. sacchari, F. proliferatum, F. mangiferae, F. pseudocircinatum* and *F. incarnatum*. The study revealed that endophytic *Fusarium* spp. in corn plants grown in Peninsular Malaysia, are able to cause serious effects on the host plants. Measures targeted at controlling corn infection by latent pathogens masking as symptomless endophytes, are required to improve plant health and preserve yield of corn plants in Malaysia and other corn-growing regions.

F9.3-1

EXTREMOPHILIC FUNGAL ENDOPHYTES SHOW HIGH EFFICACY AGAINST SOIL-BORNE OOMYCETE AND FUNGAL PATHOGENS

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Text

Soil-borne oomycete and fungal pathogens, mainly Pythium, Fusarium and Monosporascus are a limiting factor to vegetable production in different countries. They have been reported to result in high mortality and yield reduction in diffrenet crops, especially cucurbits and tomatoes. Deserts of the Arabian Peninsula are characterized by their dry conditions and high temperatures that can reach 50oC. A three year study reveled the isolation of more than 250 fungal endophytes from desert plants in Oman, in the southern part of the Arabian Peninsula. A number of fungal species belonging to Talaromyces, Aspergillus, Cladosporium and Trichoderma, some of which are novel species, were found to have high efficacy in inhibiting soil-borne pathogen growth in culture. Scanning electron microscopic studies indicated the effect of the antagonists on hyphae morphology and spore production and morphology. Culture filtrates from the antagonistic fungi induced electrolyte leakage from the mycelium of the pathogenic fungi and oomycetes. GC-MS analysis of metabolites showed the presence of different antimicrobial, antifungal and plant growth promoting volatile compounds such as fatty acids, alcohols, alkenes, ketones and triterpenes. The antagonists also suppressed damping-off and vine decline diseases and imporved growth and yield of cucurbits and tomatoes. The study shows that endophytic fungi from desert plants can act as biocontrol agents against fungal and oomycete pathogens.

F9.3-2

FUNGI ASSOCIATED WITH MACADAMIA PLANTS AT DIFFERENT GROWTH STAGES

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Text
Macadamia is an Australian native tree that is grown for its edible kernel in tropical and subtropical regions worldwide. Young plants infected by fungi causing stem and leaf pathogens in the nursery may cause disease problems in commercial macadamia orchards. To investigate, we used a culture dependent approach to examine the structure and diversity of the fungal community in macadamia nursery plants at four different growth stages. Fungi isolated from roots, stems, and leaves of germinated seeds,1-3 month-old seedlings, 12-month-old non-grafted plants and 18-month-old grafted plant stages were characterised. Twenty-two fungal genera, mostly in the phylum Ascomycota were identified from the various macadamia tissues. The fungal community structure was significantly ($P < 0.05$) influenced by the growth stages and plant organs. Grafted plants had the richest fungal composition and diversity (21 fungal genera). Neopestalotiopsis, Alternaria, Collectotrichum and Neofusicoccum populations were more frequent in leaf tissues than other organs. In contrast, Diaporthe, Lasiodiplodia and Pestalotiopsis populations were dominant in stem tissue. This study revealed that fungal community richness and diversity in macadamia plants are dependent on the growth stage. Ongoing investigations would determine the pathogenicity of these fungal species in mature macadamia plantations.
Keywords: Fungal diversity, Endophyte, Ascomycota, Tree nut.

P9.3-001

BIO-FUMIGANT PROPERTIES OF VOLATILE METABOLITES FROM ENDOPHYTES IN POST-HARVEST DISEASE MANAGEMENT

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Text
Volatiles from plant-beneficial endophytic fungi is considered promising alternatives to be used in the biological control of dreadful phytopathogens, as a sustainable approach in an agroecosystem. Here, a volatile emitting endophytic fungus Diaporthe sp. CEL3 with bio-fumigation activity was isolated from leaves of the ethnomedicinal plant Chloranthus elatior Sw., collected from North-East India. Camphor odor volatiles of CEL3 inhibited eight phytopathogens in-vitro and minimised the infections of Monilinia fructicola and Penicillium digitatum, causal agents of fruit rot of cherry and orange, in VOC-exposed fruits. Rhizoctonia solani, Botrytis cinerea, Pythium ultimum, M. fructicola, and P. digitatum were maximally inhibited upto 51.5%, 55.8%, 61.9%, 87.9%, and 78.5% respectively in comparison to control by the volatiles. Another isolate CEL7 identified as Curvularia sp. synthesised antifungal metabolites (mainly phenol and imidazole derivatives) in its cell-free extracts with a MIC of 250-2000 µg mL-1. Optimum VOC emitted in a modified PDA media with wheat husk (20 g L-1). CEL3 emitted different volatiles- Trans-verbenol (32.25%), Geraniol (30.32%), Trans-ocimenol (12.90%), Mentha-4,8-diene (5.16%). They cause lethal leakage of protein and necessary intracellular molecules from fungal pathogens. Thus, CEL3 could potentially be used as a bio-fumigant to control post-harvest infections and paves opportunities for the discovery of novel antifungals.

P9.3-002

HOW PLANT IMMUNITY SHAPES THE COEXISTENCE OF PATHOGENIC AND COMMENSAL STRAINS DURING EARLY LEAF INFECTION

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Text

Crop pathogens significantly reduce agricultural production. Yet, pathogen ecology during the infection remains elusive. In particular, the determinants underlying the pathogen-microbiota-immunity interactions during early infection steps, which are critical to determine if disease will occur, are not well understood. To successfully establish infection, bacterial pathogens use the type III secretion system to repress plant immune defenses, which in turn can facilitate the growth of co-localizing non-pathogenic strains in plant tissues. However, which properties of the commensal strains or the plant tissue influence the co-existence between pathogenic and non-pathogenic strains is unclear. Here, we use single-cell time-lapse microscopy in microfluidic chambers using the Xanthomonas campestris pv. campestris phytopathogen and combine it with an individual-based model to explore the conditions leading to pathogen establishment or exclusion. We consider a simple case where a bacterial mutant lacking the type III secretion system co-infects the plant along with a pathogenic wild-type strain and ask how different fractions of pathogen-to-mutant cells, growth rates and plant tissue constraints, affect pathogen establishment. While the work is still in progress, our project will help to better understand strategies of plant pathogens in their natural ecological context.

P9.3-004

COMPARISON OF THE SOYBEAN ENDOPHYTES BACTERIAL COMMUNITY OF HEALTHY AND UNHEALTHY TO IDENTIFIED FUNCTIONAL CORE MICROBE

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Text

Soybean is known for being an important source of protein and for a wide range of agricultural, food, and industrial applications. However, soybeans are being affected by *Xanthomonas axonopodis* pv. *glycines* cause bacterial pustule, which results in a reduction in crop yield and quality. According to the importance of soybean, research is more essential to increase yield under biotic stresses. As part of the study, it is known that the diverse microbial communities of plants are involved in various plant stresses. We designed to study the microbial community differential depending on the infection of *X. axonopodis* pv. *glycines* and the non-infected soybean. When microbial community abundance, diversity, and similarity analysis was

performed that showed a difference between infected and non-infected soybean. In analysis except for *X. axonopodis* pv. *glycines*, it turned out that an increase in Pseudomonadaceae was observed in the infected group. Additionally, *Streptomyces bacillaris* S8, an endophyte microbiota member, was nominated as a key microbe in the healthy soybeans. It will be composited for core microbiota and applied to soybeans for verification to control *X. axonopodis* pv. *glycines*. Composition research of the soybean-associated microbiota will serve as information on the core microbes in soybeans and enable biological control against *X. axonopodis* pv. *glycines*.

P9.3-005

STRUCTURAL AND FUNCTIONAL PROPERTIES OF ENDOSPHERE MICROBIOTA COMMUNITY AND CORE TAXA IN APPLE TREE

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Text

Fire blight has caused significant economic harm in over 50 countries since the 1870s. In response, many countries have used chemical control, such as copper-based bactericides, to manage the disease. However, these methods can lead to phytotoxicity and the development of pathogen resistance. Alternative methods, such as biological control, have been attempted, but the microbial structure analysis of the plant-microbe interactions in the ecological metabolites of apples is not well understood. In this study, we compared the bacterial community structure between healthy and unhealthy apples, identified the core taxa in healthy conditions, and analyzed twigs and leaves endosphere in healthy orchards in 9 regions in the Republic of Korea. Our findings indicate that orchards in different regions share many common taxa and functions that suppress *Erwinia amylovora*. Specifically, we observed high relative abundances of *Pseudomonas* and *Methylobacterium* in healthy orchard. Additionally, the analysis of microbiota community metabolites pathways showed 11 common pathways in healthy orchards, including fatty acid and lipid biosynthesis and amino acid biosynthesis. These results highlight the importance of understanding the ecology of pathogens for gaining a better understanding of the dissemination route of pathogens in the environment. In conclusion, metagenomics is a revolutionary field in microbial ecology that provides techniques for analysis without the need for culturing methods.

P9.3-006

METAGENOME ANALYSIS OF BACTERIA PRESENT IN STORAGE ONION BULBS IN THE USA

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Text

Storage diseases of onions have the potential to cause serious losses. Bacterial rot is caused by a number of genera and species, with many infections involving a cohort of co-occurring pathogens rather than a single agent. Aim of this study was to undertake a functional analysis to determine their potential role in bulb rot using a metagenomics approach. The bacterial communities present in asymptomatic and symptomatic mature bulbs harvested in Georgia and Washington States were determined. DNA extracted was sequenced using Illumina MiSeq and assembled into metagenomes, from which metagenome-assembled genomes (MAGs), were identified and analysed. Seven MAGs [*Burkholderia gladioli* pv. *gladioli, Enterobacter ludwigii, Gluconacetobacter diazotrophicus, Pantoea agglomerans* (2 MAGs) and *Pseudomonas simiae* (2)] were assembled from the Washington State symptomatic bulb dataset. In the dataset collected from the symptomatic bulbs in Georgia, *Acetobacter* (2), *B. gladioli* pv. *gladioli* (3), *B. cepacia (*2) and *Rahnella* sp. (1) were assembled. With the exception of *Acetobacter*, *G. diazotrophicus* and *P. simiae*, the assembled MAGs belonged to bacteria known to cause bulb rot. Functional analysis of both the metagenomic and MAG data revealed the presence of several genes involved in thiol and oxidative stresses, T3SS and T6SS, and other pathogenicity factors. The results suggest that bacterial rot of bulbs can be caused by a diverse microbial cohort of primary and opportunistic pathogens.

P9.3-007

THE POTENTIAL OF ENDOPHYTIC BACTERIA OF PASPALUM SPP. AND SILICA NANOPARTICLES FOR PLANT GROWTH PROMOTION AND BIOCONTROL

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Text

The study aimed to evaluate the antagonism of endophytic bacteria to the phytopathogenic fungi Claviceps purpurea and Fusarium oxysporum and the effect of bacteria associated with silica nanoparticles (SiNPs) on Paspalum notatum. For the in vitro antagonistic tests, Alcaligenes sp., Pseudomonas sp., Enterobacter sp., and Serratia sp. were plated with C. purpurea and F. oxysporum in a BDA medium. In the in vivo tests, seeds of P. notatum and Poa annua were inoculated with treatments composed of bacteria and bacteria plus F. oxysporum. Seeds submitted to the same treatments were plated in Petri dishes with 0.7% agarose, and the seedling roots were 3,3'-diaminobenzidine stained for visualization. In a different experiment, 85 nm SiNPs at 0.05 and 0.1 mg/mL concentrations were added to the bacteria culture media. Treatments were inoculated in seeds of P. notatum and subjected to the same conditions. Serratia sp. and Enterobacter sp. inhibited C. pupurea growth on the plates. In the soil experiment, Serratia sp. promoted P. notatum growth. F. oxysporum with Enterobacter sp. promoted the greatest plant growth. Seedlings inoculated with F.

oxysporum exhibited thin and fragile roots and shoots. Pseudomonas sp. promoted significant growth of P. annua. The three isolates promoted the growth of P. notatum. The highest germination and shoot dry weight were obtained with 0.1 SiNPs plus Serratia sp. and Enterobacter sp. SiNPs associated with the three bacteria increased root.

P9.3-008

CHARACTERIZATION OF ENDOPHYTIC FUNGI ON BANANA PLANTS GROWN IN SOIL FROM THE RHIZOSPHERE OF PLANTS WITH AND WITHOUT SYMPTOMS OF PANAMÁ DISEASE.

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Text
Endophytic fungi are microorganisms that inhabit the living internal tissues of host plants without causing any immediate and overt negative effects. Endophyte-host plant relationships have important implications for fungal biodiversity and plant health. In Canary Islands, banana crops are affected by *Fusarium oxysporum* f. sp. *cubense* (*Foc*-STR4). Therefore, knowing the diversity of endophytic fungi that share a niche with the pathogen can provide information on the microbiome of a diseased plant. The aim was to characterise the endophytic *Trichoderma* and *Fusarium* species in banana plants. Soil rhizosphere of banana plants with and without symptoms of *Foc*-STR4 were collected in different zones of Tenerife. These soil samples were used to cultivate banana seedlings (in 3 l pots) during six months under controlled conditions. After that, plant tissues (rhizome and pseudostem) were sampled. Samples surface were sterilised and cultured on PDA medium. Isolates were identified by phylogenetic analysis of tef1 gene. It was obtained 119 isolates: 75 of *Fusarium* and 44 of *Trichoderma*. Eleven endophytic *Fusarium* species were identified: *F. veterinarium, F. languescens, F. gossypinum, F. tardicrescens, F. solani, F. phialophorum, F. nirenbergiae, F. inflexum, F. contaminatum, F. foetens* and *F. annulatum*. In relation to *Trichoderma* endophyte, eight species were identified: *T. harzianum, T. virens, T. hamatum, T. gamsii, T. atrobrunneum, T. koningii, T. atroviride* and *T. cf. harzianum.*

P9.3-009

FIGHTING FUNGI WITH FUNGI: THE BIOCONTROL POTENTIAL OF TRICHODERMA AGAINST ARMILLARIA ROOT ROT

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Text
Armillaria root rot (ARR) is a major fungal plant pathogen which spreads through soil and

attacks the roots of plants. There are currently no chemical controls available and the possibility of utilizing biocontrol agents against ARR is lacking in the scientific literature. *Trichoderma* are commonly considered for the biocontrol of plant pathogens, as many species are endophytic, produce an array of lytic enzymes, and directly attack other fungi. We previously isolated a collection of 42 root endophytic *Trichoderma* isolates across 12 species and these have been tested in plate-based studies and evaluated for protective potential against ARR *in planta*. Two isolates of *Trichoderma atrobrunneum* have shown strong potential to prevent disease in strawberry and privet plants. My current work investigates how *Trichoderma* is able provide control against ARR through the production of extracellular enzymes and volatiles. This presentation will highlight how *Trichoderma* is altering the rhizosphere to the detriment of *Armillaria*, whether effective control of *Armillaria* is feasible, and further characterisation of the promising *T. atrobrunneum* isolates.

P9.3-010

DIVERSITY OF ENDOPHYTIC FUNGI ASSOCIATED WITH CITRUS SPECIES IN INDIA AND THEIR BIOCONTROL POTENTIAL AGAINST PHYTOPHTHORA ROOT ROT DISEASE

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Text

Endophytic fungi were isolated from tissues of various commercial citrus cultivars grown in India. A total of 152 endophytic fungal isolates were obtained from 527 segments (262 root, 159 bark and 106 leaf tissue segments). Based on their morphological characteristics, the isolates were preliminarily grouped to 60 morphotypes and finally identified by ITS sequence analysis into 52 species belonging to 14 orders of Ascomycota (Hypocreales, Microscales, Sordariales, Glomerellales, Diaportheales, Trichosphaeriales, Xylariales, Botryosphaeriales, Saccharomycetales, Pleosporales, Eurotiales, Capnodiales, Patillariales and Bezeromycetales) and 2 orders of Basidiomycota (Cantharellales and Agaricales). The species composition was highly diversified with Simpson's diversity index figuring 0.932. Phylogenetic analyses confirmed their species identity and evolutionary processes. The most frequent species found associated were belonging to the genera *Fusarium, Colletotrichum, Daldinia* and *Hypoxylon*. The fungal endophytes were tested for anti-oomycete activity against *Phytophthora nicotianae* by in vitro dual culture assay and in vivo pot experiments. Isolates CFE 109 (*Chaetomium globosum*) and CFE-142 (*Aspergillus terreus*) were found very effective. Overall these results suggest that *Citrus* spp. in India harbors diversified endophytic fungi, which could be exploited as sources of potential biocontrol agents against *P. nicotianae* causing root rot and decline of citrus plantation.

P9.3-011

PRELIMINARY ANALYSIS OF FUSARIUM OXYSPORUM F. SP. CUBENSE (FOC) IN BANANA PLANTS WITH SYMPTOMS OF PANAMÁ DISEASE IN MADEIRA (PORTUGAL)

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Text

Different diseases affect the banana crop in the subtropical areas, among which Panamá disease stands out for its severity. In Canary Islands (Spain) the subtropical race 4 (*Foc*-STR4) has been described, however, this information is not known in detail in Madeira. For this reason, a preliminary sampling was carried out in banana plantations affected by the disease. The aims of this work were to determine the *Foc* race that causes Panamá disease and to characterize the community of cultivable *Fusarium* endophytes. In each farm, pseudostem and rhizome samples were collected from symptomatic and healthy plants. Each sample was processed in two ways: a) DNA extraction from plant material, and b) PDA culture to isolate the fungal colonies with *Fusarium* morphology. From the plant tissue DNA extractions, we performed: a) conventional PCR using race-specific primers based on the secreted in xylem gene effectors, and b) real-time PCR based on the intergenic spacer. The results showed that all the plants with symptoms (15) were positive for the subtropical race 4 of *Foc* (STR4) and negative for the tropical race 4 (TR4), while the pathogen was not detected in healthy plants. Culture on PDA confirmed these results, since the predominant species in all symptomatic plants was identified as *F. phialophorum* (*Foc*-STR4) by sequencing the *tef1* gene. Likewise, five other endophytic *Fusarium* species were detected in the plants with symptoms, while no *Fusarium* was isolated from the healthy ones.

P9.3-012

INVESTIGATING THE MECHANISM OF CHLORELLA FUSCA CHK0059 IN RELATION TO THE STRAWBERRY MICROBIOTA COMMUNITY

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Text

Microalgae are well-known biofertilizers to various crops, known to contain high contents of fatty acids, protein, steroids, carotenoids, and other nutrients. Despite the observed plant growth-promoting activity of microalgae in crops, research on the mechanisms of this efficacy remains scarce. This study aimed to investigate the plant growth-promoting activity of microalgae from a microbiota level using *Chlorella fusca* CHK0059 to strawberry as a model strain. This type of microalgae is known to promote plant growth and exhibit resistance to *Fusarium oxysporum* f. sp. *fragariae*. This study results showed that Chlorella treatment led to increased leaves and shoot weights compared to untreated samples. Although the beta diversity was not significantly different between treated and untreated samples, the richness

of the treated root endosphere was decreased. In the rhizosphere, specific bacteria seemed to be correlated with Chlorella that had phosphate solubilizing activity to promote plant growth. The study also showed that the concentration of plant-available P (phosphate) in the strawberry pulp was higher in the Chlorella-treated samples. Overall, this study suggests that microalgae have the ability to change alpha diversity and stimulate specific microorganisms in the rhizosphere to promote plant growth.

P9.3-013

TERROIR, SEASON, AND VINTAGE EFFECTS ON THE GRAPEVINE PATHOBIOME

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Text

In viticulture and oenology, the *terroir* concept is widely used to explain differences among wines. The concept itself partly is based on spatial differences in edaphic and mesoclimatic factors. These environmental differences likely affect plant-associated microbes also, with implications for plant health. In this study, we compared the compositional dynamics of plant pathogenic fungi in three different microhabitats: soil, woody tissue, and bark of grapevine cv. *Furmint*, sampled in late winter and summer of 2020 and 2021 in three different *terroirs* in the Tokaj wine region. Sequence data of the ITS2 region of the ribosomal DNA repeat were generated by Illumina NovaSeq. Of the 123 plant pathogenic genera found, *Diplodia*, *Phaeomoniella*, and *Fusarium* showed the highest richness in bark, wood, and soil, respectively. Both richness and abundance differed significantly among microhabitats, with plant pathogenic fungi known to cause grapevine trunk diseases (GTDs) showing highest richness and abundance in wood and bark samples, and non-GTD pathogens dominating soil. We found significant compositional differences among *terroirs*, season, and vintage, with *terroir* explaining 14.5-24.7%, season 1.8-2.98%, and vintage 3.7-6.4% of the variance in community composition. Some of the observed differences likely are caused by environmental filtering both at microhabitat and *terroir* levels, while weather and fungicide applications may explain the observed temporal dynamics of fungi.

P9.3-014

TEMPORAL SUCCESSION OF PLANT PATHOGENIC FUNGAL COMMUNITIES IN GRAPEVINE LEAVES UNDER ORGANIC AND CONVENTIONAL MANAGEMENT

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Text

Plant health is highly dependent on plant-associated microbes and despite recent advances, we still lack a systematic overview of how the fungal grapevine microbiome is influenced by cultivation methods. In this study, we compared the diversity and composition of plant pathogenic fungal communities in grapevine leaves under organic and conventional management. We hypothesized that the differences in fungicides used in conventional and organic vineyards would have a significant impact on the diversity and composition of the leaf-associated fungal community. We generated DNA metabarcoding data of the cultivar *Bianca* collected throughout the growing season at the experimental vineyard of the Eszterházy Károly Catholic University, Hungary. The rarefied dataset contained 911 amplicon sequence variants (ASVs) of plant pathogenic fungi, representing 88 genera. *Phaemoniella* showed the highest richness, followed by *Alternaria*, *Epicoccum* and *Diplodia*. Differences in richness and composition did not differ significantly between organic and conventionally managed grapevines, but significant changes were apparent among months, explaining the greatest compositional variation. We found that this strong temporal turnover likely is caused partly by the application dates of different fungicides and possible differences in sensitivity among fungal species, particularly in mid-summer, and partly by seasonality, i.e. leaf maturation and the gradual onset of senescence by September.

P9.3-015

EXPLORING THE MICROBIAL CONNECTIONS BETWEEN GRAPEVINE AND NEARBY WILD AND CULTIVATED WOODY ROSACEAE SPECIES IN EGER WINE REGION IN HUNGARY

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Text

Grapevine (Vitis vinifera) is one of the major fruit crops worldwide and is naturally colonized by commensal, beneficial or pathogenic microorganisms that influence grapevine health. However, the composition of the microbial community in leaves and trunk parts has been less studied than in soil and roots. In addition, relationships between the microbiomes of different cultivated plant species and environmental microbial reservoirs in nearby vegetation are scarcely known.

In this study, we focus on shared endophytic fungi between grapevine and nearby cultivated or naturally occurring woody Rosaceae species at a landscape level. We generated and analyzed DNA metabarcoding data to assess the compositional overlap of leaf- and wood-associated fungi associated with grapevine, apricot (Prunus armeniaca), pear (Pyrus communis), dogrose (Rosa canina) and blackthorn (Prunus spinosus).

We found that both sampling source (leaf vs. wood) and host identity had strong influence on fungal community composition, explaining ca. 20% and 26.6% of compositional variance, respectively. The observed compositional overlap among grapevine and wild and cultivated Rosaceae fruit species living near vineyards suggests that a landscape-level approach is

needed to better understand the microbiomes of grapevine and fruit trees, with implications for integrated crop protection.

P9.3-016

GRAPEVINE CULTIVAR, PHYSIOLOGY, AND CHEMICAL PARAMETERS INFLUENCE LEAF AND BERRY MYCOBIOME

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Text

Improving our knowledge on biotic and abiotic factors that influence the composition of the grapevine mycobiome is of great agricultural significance, due to potential effects on plant health, productivity, and wine characteristics. We assessed the influence of scion cultivar on the mycobiome diversity and composition in berries and leaves by generating DNA metabarcoding data from three different cultivars and explore correlation with chemical and physiological parameters of the leaves sampled. Fungal communities in leaves and berries show contrasting patterns among cultivars. Richness and relative abundance of fungal functional groups statistically differed among berry and leaf samples, but less so among cultivars. Community composition of the dominant functional groups of fungi, i.e., plant pathogens in leaves and saprotrophs in berries, differed significantly among cultivars. We also detected cultivar-level differences in the macro- and microelement content of the leaves, and in acidity and sugar concentration of berries. We found significant correlation between mycobiome composition and measured differences in chemical composition and physiological traits of leaves which merits further research to explore causality. Our findings suggest that a relatively diverse set of fungi make up the grapevine mycobiome, spanning several cultivars at the sampled terroir, and that both berry and leaf mycobiomes are influenced by the chemical characteristics of berries and leaves.

P9.3-017

RELATIONSHIP BETWEEN FUNGAL ENDOPHYTES AND PLANT DISEASE?

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Text
The endophytic community of plants includes diverse fungi ranging from true endophytes (not causing disease in a specific host species) to latent pathogens. In fact, a fungus can be pathogen in one species and endophyte in another species. Both endophytes and pathogens have developed ways to circumvent recognition to the extent that the plant immune system does not hinder colonisation. However, why some organisms turn pathogenic while others develop a mutualistic or commensal endophytic relationship is poorly understood. We have studied endophytic fungal communities in tomato and wheat using amplicon sequencing and isolation of fungi primarily for functional characterisation of their interaction with plants. Communities in symptomless plants harboured high frequencies of known pathogens. Lifestyle testing of isolated fungi confirmed the presence of both beneficial endophytes and latent pathogens, suggesting complex interactions within the microbiome, which is in equilibrium with the plant, preventing pathogens from causing disease. Inoculating plants with endophytes revealed induction of plant defence-related genes and changes in specialised metabolite composition both locally and systemically. This may be key for keeping pathogenic organisms in check.

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P9.3-018

DNA METABARCODING STUDY REVEALS GREATER EFFECT OF MICROHABITAT AND VINTAGE ON GRAPEVINE MYCOBIOME THAN CULTIVAR, SEASON OR HEALTH STATE

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Text
Grapevine is an important plant cultivated in more than 80 countries worldwide. Our knowledge on to what extent environmental factors influence grapevine microbiome and how the microbiome of „healthy" (asymptomatic) and diseased plants differ is scarcely known. In this study, we characterized the mycobiome of grapevines affected by the Esca type of grapevine trunk disease (GTD) and that of asymptomatic individuals. We tested the influence of cultivar, season, vintage and microhabitats (isolation sources) on the mycobiome. DNA metabarcoding data were generated from bark, soil and wood from four different cultivars collected in four sampling times, February and August of 2020 and 2021 each. The strongest driver of mycobiome was microhabitat, explaining 4,2% of the variaton in community composition. Wood decomposers and plant pathogenic genera associated with GTDs mainly occurred in bark and wood, mycoparasites were mostly found in bark, while non-GTD pathogens, soil and litter saprotrophs dominated soil. Abundance and richness values differed across microhabitats but not among health states. Season and cultivar did not affect the mycobiome significantly. Richness values of 2021 were lower in case of plant pathogens, wood saprotrophs and mycoparasites, probably due to the drier vintage. This suggest that

environmental factors could be particularly important shaping the mycobiome of grapevine, and studies are needed to investigate the effect of abiotic conditions on mycobiome.

P9.3-019

ENDOPHYTIC FUNGI AND BACTERIA FROM NURSERIES AND ORCHARDS OF AVOCADO PLANTS RELATED WITH DISEASES CAUSED BY BOTRYOSPHAERIACEAE

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Text

Botryosphaeriaceae (Bot) fungi include pathogens of woody plants, that can cause cankers, diebacks and postharvest diseases, but they are also endophytes or latent pathogens. The diseases caused by fungi of this family are common to avocados in Canary Islands, where their production has reached relevance. The aims of this study were the isolation and identification of endophytes from seedlings in nurseries and from adult plants in orchards (symptomatic and asymptomatic plants), and to assess the role that they would play in the epidemiology of the diseases. Samples from 166 seedlings from nine nurseries and 18 plants from two diseased orchards were superficially disinfected and two isolation methods were use: small internal fragments plated on PDAS for fungi and extracts of internal tissue streaked on YPGA for bacteria. The ITS1-2 region and 16S gene were sequenced for the identification of fungi and bacteria, respectively. Endophytic isolates were obtained from 37.3% of the nursery plants (146 fungi and 41 bacteria) and from 50% of the field plants (125 fungi and 36 bacteria). Also tef1, tub2 and rpb2 genes were used for the molecular characterization of Bot fungi, that were isolated in 8.0% of the seedlings and 16.7% of the adult plants and identified as *Neofusicoccum cryptoaustrale*/*stellenboschiana*, *N. parvum* or *N. luteum*. Other pathogenic species of fungi and bacteria were also isolated as well as potential antagonist of the Bot pathogens of avocado plants.

P9.3-020

INVESTIGATING AN INTEGRATED APPROACH TO CONTROLLING POSTHARVEST PATHOGENS OF AVOCADO

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Text
Persea americana a super fruit and a worldwide commercial crop is mostly affected by diseases incited by plant parasitic fungi that commonly cause important reductions in yield and quality of its fruit. Fungal pathogens are a major cause of pre-harvest infections that cause both pre- and post-harvest diseases resulting in significant crop losses. Agrochemicals have been used to manage most avocado plant diseases. However, they are being lost to the avocado industry due to EU MRL levels being reduced in many countries, including those of the EU, a major market for South African fruit exports. Therefore, there is an urgent need to find alternative that will comply with the regulations in the management of the fungal pathogens. This study was initiated to successfully isolate endophytic strains of Trichoderma spp, and evaluate in-vitro and in-vivo their abilities of controlling key fungal pathogens. Result of isolation revealed successful isolation of both fungal pathogens and strains of Trichoderma spp. The screening test indicated their endophytic properties which were tested in-vitro and in-vivo using various techniques for their pathogenicity capabilities. Some of the Trichoderma isolates were able to control the fungal pathogens during in-vitro screening (at levels of between 70 and 100%). The best strains tested for in-vivo activity on avocado fruit confirmed the potential of endophytic strains of Trichoderma spp. to control key pre-harvest infections of avocado fruit.

P9.3-022

A PROTEOMIC STUDY OF THE TRIPARTITE INTERACTION OF WHEAT, FUSARIUM GRAMINEARUM AND AN ENDOPHYTIC STREPTOMYCETE DURING NORMAL AND DROUGHT CONDITIONS

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Text
Fusarium graminearum is one of the main pathogen of *Triticum aestivum* causing Fusarium head blight (FHB) and Fusarium crown rot (FCR), contaminating grains with mycotoxins. *Streptomyces sp*. DEF39 was previously described to be able to colonize systemically wheat plants after seed treatment and to reduce disease and deoxynivalenol contamination in grains, during field trials. Under drought stress, DEF39 induced a shortening of the life cycle in seed inoculated plants, without affecting the grain production. This work aims to study the bacteria-plant-fungus crosstalk during their tripartite interaction, deciphering the mechanism of action of the DEF39 strain. An in-vitro system was developed to assess the interactions, comparing the wheat root proteome of plants exposed to 4 treatments both in normal and drought conditions: the control plant, the DEF39 seed inoculated plant, the *F. graminearum* infected plant and the DEF39 seed inoculated infected by the fungus. The 1D GeLC-MS/MS approach allowed to quantify more than 300 proteins, of which about 90% belongs to wheat. The root proteome changed in response both to the fungus, DEF39, their interaction and water availability. The major effect was induced by the fungal infection, involving primary and redox metabolism, transport, and defence. Interestingly, the plant responses were also influenced by the seed inoculation, suggesting that Streptomyces sp. DEF39 has an effect on the plant defence mechanisms against different stress.

P9.3-023

GENOMIC INSIGHTS INTO LATENT FUNGAL PATHOGEN LIFE-STYLES IN THE BOTRYOSPHAERIACEAE

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Text

Species in the *Botryosphaeriaceae* establish asymptomatic infections that persist for extended periods in their plant hosts. Under certain conditions, typically when the hosts are subjected to stress, these fungi cause dieback and cankers, amongst other symptoms. A number of recent genome and transcriptome studies have provided unprecedented insights into this infection and disease development process. Here we reflect on the insights from comparative genomic analyses on the genetic basis of the interactions of these fungi with their plant hosts. Our studies have shown that *Botryosphaeriaceae* genomes are enriched in carbohydrate-active enzymes (CAZymes), proteases, lipases and secondary metabolic biosynthetic gene clusters (BGCs). Some genomes in *Botryosphaeriaceae* genera, such as *Botryosphaeria*, *Macrophomina*, *Lasiodiplodia* and *Neofusicoccum*, are notably expanded for elements of the secretome such as CAZymes involved in plant cell wall degradation. The composition of the genomes with respect to secreted hydrolytic enzymes and secondary metabolite BGCs in species of the *Botryosphaeriaceae* are similar to those in necrotrophic plant pathogens and some other endophytes of woody plants. The results of these studies provide useful hypotheses to explore the mechanisms underlying *Botryosphaeriaceae* host-plant interactions.

P9.3-024

ENDOPHYTIC FUNGI AND BACTERIAL DISEASES OF HAZELNUT

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Text

Among biotic adversities of hazelnut (*Corylus avellana* L.) bacterial canker and bacterial blight, respectively incited by *Pseudomonas avellanae* and *Xanthomonas arboricola* pv. *corylina*, represent a major threat for hazelnut farmers affecting crop yield in several production areas worldwide. Nowadays, their control is mainly based on the use of copper-based products, which have been announced to be phased out in the European Union because of undesired side effects on the environment. In the need of finding alternative

control measures following the paradigm of sustainable agriculture, the possible employment of plant-associated microorganisms is to be particularly considered. Endophytic fungi are renowned for their involvement in defensive mutualism with plants, and they are regarded as a possible tool to be exploited for disease management in semi-extensive crops. However, data so far collected with reference to their occurrence and ecological role in hazelnut are scanty. In the context of an accurate investigation concerning the endophytic mycobiome associated to both healthy and diseased hazelnut plants currently in progress in the major Italian cropping areas, the antibiotic properties of endophytic fungi isolated from secondary branches were assessed against the two bacterial species in vitro. A series of strains were selected to be further analyzed for their capacity to synthesize bioactive products, and ultimately perform an antagonistic role in planta.

P9.3-025

DIVERSITY OF WOOD INHABITING FUNGI IN OLIVE CULTIVARS SUSCEPTIBLE TO XYLELLA FASTIDIOSA SUBSP. PAUCA ST53 IN APULIA

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Text

Xylella fastidiosa subsp. *pauca* ST53 (Xfp) is the causal agent of Olive Quick Decline Syndrome (OQDS), which has resulted in a devastating impact in Apulia (Southern Italy). Recently, several reports from Greece, Spain, and South Africa suggested a direct pathogenic activity on olive of different *Pseudophaeomoniella* spp. Considering that olive resistant genotypes appear as the most promising management strategy of OQDS, the role of these wood inhabiting fungi, and their possible correlation with OQDS severity need to be further investigated. Wood fragments were collected from susceptible and symptomatic olive trees showing a different OQDS severity, from both Xfp-infected and uninfected areas. Isolations, performed on potato dextrose agar amended with streptomycin sulfate, showed that 72% of fungal strains from discoloured wood belonged to *Pseudophaeomoniella* and 28% to other genera. Representative isolates of *Pseudophaemoniella* (n=35) and other endophytes (n=13) were molecularly identified. The multilocus phylogenetic analyses performed on *Pseudophaemoniella* isolates using ITS, LSU, TEF and ACTIN genes showed that most strains belonged to *P. oleae* (40%) and *P. oleicola* (37%), and 23% formed a separate clade not defined yet. The other fungal endophytic strains belonged to *Pleurostomophora richardsiae* and *Paraconiothyrium brasiliense* species. Pathogenicity tests on susceptible and resistant cultivars are ongoing to determine the role of each species in the etiology of OQDS.

P9.3-026

ENDOPHYTIC BACTERIA FROM OLIVE DRUPES AS PLANT DEFENCE INDUCERS AGAINST COLLETOTRICHUM ACUTATUM IN OLIVE TREE

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Text
Olive anthracnose is one of the most important diseases affecting olives worldwide and is caused by fungi belonging to the genus *Colletotrichum*. The predominant strains are associated with *C. acutatum* and *C. gloeosporioides* species complexes. Due to the withdrawal of several fungicides and the risk of pathogen resistance, finding more sustainable control measures of the disease, such as using biological control agents and plant-resistance inducers is considered essential. Some biological agents can act as inducers of plant defense mechanisms, while they can also combine more than one mode of action, which makes them more suitable for their use in agriculture. This study aimed to evaluate antagonistic endophytic bacteria from olive drupes against *C. acutatum* for their ability to induce plant defense mechanisms. The experiments were conducted on olive trees treated with the bacterial antagonists and the pathogen. The expression of ten defense genes was evaluated by RT-qPCR. All four tested bacterial strains showed increased expression of genes associated with Pathogenesis-related proteins (*PR10, Mpol*) compared to controls, while the application of bacteria K13 (*Bacillus methylotrophicus*), B1 (*B. amyloliquefaciens*) and Π8 (*Serratia* sp.) caused increased expression of genes related to biosynthetic pathways of phenylpropanoids and salicylic acid. Finally, strain B1 also induced increased expression of the lipoxygenase (*LOX*) gene involved in the jasmonic acid biosynthetic pathway.

P9.3-027

ETIOLOGY OF BRANCH DIEBACK AND CANKER DISEASE OF APPLES IN CALIFORNIA

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Text
Apple production in California reaches 5,665 ha, being the fifth largest apple-producing state in the United States. During a routine survey in 2021, branch dieback and wood cankers symptoms were observed in several apple (‘Fuji’, ‘Gala’, ‘Granny Smith’, ‘Newtown’, ‘Red Delicious’) orchards in Lodi, San Joaquin, and Santa Cruz counties in California. Symptomatic plant samples from dying twigs, and cankered branches were collected from nine commercial orchards. The most common symptoms in cross-section were light brown to brown, irregular, and hard wood necrosis. Isolations from the symptomatic tissues revealed the occurrence of *Eutypa lata* as the most prevalent species, followed by *Diplodia* spp. (*D. seriata*, *D. mutuila*), *Diaporthe* spp. (*Di. australafricana*, *Di. eres*, *Di. chamaeropis*, *Di. foeniculina*) *Cytospora parasitica*, *Kalmusia variispora*, and *Phaeocremonium* sp. Isolates were identified by morphological characters and multi-locus phylogenetic analyses using the internal transcribed spacer (ITS) region of rDNA, partial sequences of beta-tubulin and translation elongation factor 1-alpha gene regions. Pathogenicity of all isolated species was demonstrated by successfully fulfilling Koch’s postulates on wounded 2-years-old branches of 12-years-old ‘Fuji’ trees at UC Davis field station. In conclusion, *E. lata*, *Diplodia* spp., and

Diaporthe spp., were the most prevalent fungal species causing branch dieback and canker of apples in California.

P9.3-028

SELECTION OF BACILLUS STRAINS AS POTENTIAL CANDIDATE AGAINST PATHOGENIC FUSARIUM

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Text
Fusarium species are important fungal pathogens of many crops of food interest, able to synthetize harmful mycotoxins and cause worldwide Fusarium Head Blight (FHB), the most devastating disease of cereals. The disease is caused by co-occurrence of several Fusarium species, mainly F. graminearum and F. culmorum. Also the ubiquitous, toxigenic F. proliferatum has been largely isolated from cereals. Nowadays, the increasing interest to reduce chemicals in agriculture, prompts researchers to select new eco-friendly strategies. The aim of this study was the identification of antagonistic bacteria potentially suitable as bio-control agent against Fusarium species. Thirty-eight Bacillus strains, isolated from wheat and maize kernels, and belonging to Bacillus velezensis, B. amiloliquefaciens, B. subtilis, B. licheniformis, B. mojavensis, B. simplex, B. megaterium, B. oleronius, B. pumilus and B. safensis were considered. The antagonistic activity against F. graminearum, F. culmorum and F. proliferatum species, by co-colture assay, and the antimicrobial effect of bacteria filtrates were evaluated. Thirty-seven strains, including all B. velezensis and B. amyloliquefaciens strains, showed a good antagonistic activity. Deeper investigations will elucidate the molecular mechanisms involved in the synthesis of bioactive molecules of the potential candidates for biological control

Food Security for Sustainable Food Systems

C2.7-1

CONNECTED: A COMMUNITY NETWORK FOR AFRICAN VECTOR-BORNE PLANT VIRUSES

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Text
The CONNECTED Network (Community Network for African Vector-Borne Plant Viruses) builds multidisciplinary research capacity to tackle crop diseases spread by insects in Sub-Saharan Africa, contributing to food security and reducing poverty. The Network brings together plant pathology and entomology research communities to tackle the complex problems of vector-borne crop diseases. The CONNECTED Network comprises 1500 members across 84 countries, this community represents a valuable resource of technical expertise and knowledge, and is a pool of potential international collaborators. CONNECTED has partnered with a range of international partners (including IITA in Nigeria and BecA-ILRI Hub in Kenya) to fund international research, provide innovative training, and run networking events primarily for early career researchers. The CONNECTED Network has funded a portfolio of 20 innovative international projects involving 14 countries, 11 different crops, and collaborations of 55 researchers in 34 institutions, each representing a new productive collaboration between UK and African institutions. The Network has delivered over 100 training opportunities awarded for multidisciplinary courses or educational visits for delegates from 18 different countries. The Network is free to join and members can access a programme of conferences, workshops, seminars and online resources, including training manuals and other educational materials.

C2.7-2

A QUANTITATIVE AND QUALITATIVE ANALYSIS OF RHIZOSPHERE MICROBIAL POPULATIONS OF MAIZE AND SOYBEAN AS INFLUENCED BY SOIL AND PLANT GENOTYPE

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Text
The importance of soil health in modern agriculture is gaining increased attention as more emphasis is placed on holistic, sustainable, and regenerative agricultural practices. The use of glyphosate tolerant (GT) crops in modern cropping systems has made a significant impact on yields and food security in modern agriculture, but the impact that these crops might have in terms of biosafety has become very concerning. The effect of GT maize and soybean on rhizosphere microbial populations was compared in soil having a high clay content (HCC) and low clay content (LCC), assessed across non-isogenic and conventional cultivars. Microbial activity in rhizosphere soil was determined by means of five biochemical techniques (active carbon, community level physiological profiling, ergosterol quantification and fluorescein diacetate hydrolysis) as well as molecular techniques (DGGE and tRFLP). No effect of the CP4-EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene on rhizosphere microbes, either qualitatively or quantitatively, was observed. Higher microbial activity was evident in HCC than in LCC soil. Results could not confirm GT maize and soybean as a potential biosafety hazard to rhizosphere microbial activity.

Keywords: DGGE, tRFLP, Rhizobiome, Glyphosate-tolerant soybean, Glyphosate-tolerant maize, clay content sl.

C2.7-3

UNDERSTANDING THE BENEFITS OF BREEDING MAJOR FOOD CROPS FOR DURABLE RESISTANCE: A CONCEPTUAL REVIEW AND META-ANALYSIS OF EMPIRICAL EVIDENCE

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Text

A critical challenge for food security is to protect crops from damage caused by microbial pathogens. Breeding crops for disease resistance is a sustainable approach to meeting this challenge. However, pathogen adaptation, leading to the breakdown of resistance, is common and can cause damaging outbreaks of disease. While the importance of genetic, evolutionary and epidemiological factors to managing resistance breakdown are reasonably well understood, there has been little effort to understand the parallel socio-economic dimension. Consequently, incentives for individual decision-makers to invest in managing pathogen evolution are often difficult to articulate or support with solid evidence. We will present research investigating how socio-economic factors influence the management of genetic resistance and pathogen evolution. We first develop a conceptual framework that illustrates the socio-economic challenges to proactively managing resistance ineffectiveness. We extend our conceptual model with a meta-analysis of the agronomic and economic impacts of the adoption of disease-resistant crops worldwide to consolidate empirical evidence. Our assessment highlights that resistance delivers considerable economic and agronomic benefits. However, such benefits will only be fully realized if a significant effort is put into the identification of effective incentives for the adoption and uptake of resistance deployment strategies to increase resistance durability.

C2.7-5

PEARL MILLET-FUTURE FOOD FOR ASIA AND AFRICA: THE IMPORTANCE, BIOTIC CONSTRAINTS AND THEIR MANAGEMENT

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Text

Millets are grown in 131 countries, and 60 million people in Asia and Africa consume millets as a traditional diet. Pearl millet (Pennisetum glaucum (L.) R. Br.) is one of the most widely grown millet and an important crop in India and Africa, extensively cultivated in arid and semi-arid regions after rice, wheat, Maize and sorghum. In terms of both production and area, India is the leading producer of pearl millet. The crop grows best in locations with poor soil fertility, droughts, extreme heat, low pH, or high salinity. The crop replaces other important cereals that are otherwise impractical to cultivate, which is crucial for the food and energy security of rural populations, especially in rain fed areas. The major biotic constraints are downy mildew and blast diseases. These diseases are one of the key challenges to boosting the grain yield potential of improved pearl millet cultivars in India and Africa. New aggressive strains of downy mildew and blast pathogens have emerged as a result of the commercialization of new hybrids in India over the past decades. For the first time, we report a de novo complete genome assembly and analysis of S.graminicola and M.grisea, one of the most virulent pathotypes from India. This research potentially contribute in deciphering

pathogen evolution and elucidating effector evolution in order to develop effective durable resistance breeding techniques in pearl millet.

C2.7-6

CHICKPEA SEED ENDOPHYTIC (BACILLUS SUBTILIS) AS EFFECTIVE PLANT GROWTH PROMOTING MICROBES TO IMPROVE CHICKPEA PRODUCTIVITY AND NUTRITIONAL QUALITY UNDER SUSTAINABLE AGRICULTURE

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Text
Chickpea used as a rich source of protein and energy both in human and livestock's diets. Moreover, the chickpea foliage can serve as an alternative fodder. Our present study evaluates the effect of chickpea seed endophytes inoculation on the nutritional values in the edible parts of chickpea and productivity. The competent chickpea seed endophyte Bacillus subtilis BHUJPCS-12 was selected on the basis of plant growth promoting biochemical properties and antagonistic against soil borne phytopathogen Fusarium sp. Pot experiment was conducted with the chickpea seed to check the plant growth and development. Chickpea seeds treated with the seed endophytes showed significant increase in plant growth (20%), biomass (19.76%) and nutrients quality in chickpea seed and plant. Additionally, nutritional quantity like total protein (5%, 7%), carbohydrate (14%, 10.90%), flavonoid (33.3%, 25%), micro and macro nutrient were also found significant enhancement in seed and foliage of the endophyte treated chickpea plants as compared to the control plant. Therefore, this potential seed endophyte strains Bacillus subtilis can be used as potential plant growth promoting inoculants for enhancing plant growth, nutritional quantity and productivity of chickpea crops under sustainable agriculture. This PGPM can be further used for field demonstration for chickpea productivity. This should be cost effective, economically viable and environment friendly.

P2.7-001

IMPROVED SHIITAKE MUSHROOM CULTIVATION IN THE NATURAL GROWING CONDITIONS FOR THE FOOD AND NUTRITIONAL SECURITY OF TRIBAL FARMERS OF INDIA

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Text
The shiitake mushroom is a source of protein and vitamin D for villagers and is collected in the jungle from December to April. It is consumed fresh, dried, and stored. The

collected Lentinus edodes cap varied from 3.65 to 4.87 cm, and the stalk was measured from 2.20 to 3.75 cm. We have cultured the shiitake mushroom on PDA, paddy extract, and wood extract media. The fastest growth of mycelium is seen on a wood extract of Meliosma simplicifolia. The highest mycelium growth was noticed in the Meliosma simplicifolia medium extract (30 mm), followed by Terminalia arjuna (28 mm), paddy extract medium (20 mm), and PDA medium (19 mm) in 28 days. We have produced the spawn on the paddy grain; it took 10 to 15 days for full growth. To standardize the natural and improved way of cultivation, we have used different species of logs measuring 60–90 x 5–15 cm (150–200 cubic feet) and inoculated the spawn artificially by trilling and plugging with cotton. The highest yield of shiitake mushrooms was recorded in Meliosma simplicifolia (1 kg/cm3). We have obtained the second-highest yield of 0.56 kg in Terminalia arjuna logs. The lowest yield was noticed in Terminalia bellerica, at 0.30 kg. The selling price is INR 800 per kilogram, which will provide a stable income to poor farmers and provide economic security worth $1000 per year

P2.7-002

INTEGRATED MANAGEMENT OF FINGER MILLET PRODUCTION SYSTEM IN NORTHEAST INDIA FOR FOOD SECURITY AND SUSTAINABILITY

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Text

Northeast India comprises of eight states accounts 7.9% of total geographical area in India,has a predominantly humid-subtropical climate that favours the cultivation of varieties of crops including finger millet but millet is grown only in certain pockets of the region.Although finger millet is largely resistant to plant diseases and pests, its yield is low, variable and unpredictable often because of damage caused by emerging fungal diseases (blast,banded blight,brown leaf spot,etc.) which were minor in the past, becoming major ones in the face of changing climatic conditions.As the crop is grown in marginal organic soil,farmers mitigate further spread of the fungal diseases using a range of traditional control options including habitat management.Finger millet is grown by subsistence farmers in the region and highly valued by traditional communities as it is nutritious in terms of micronutrients,drought tolerant,short duration and requires low input.Integrated management of the crop using trasitional knowledge system offers an efficient cost effective to increase small holder production,serve market needs and significantly improve food and nutritional security across marginal rural communities of the region.Considering the importance of millet,expansion of production system under various land use systems such as Jhum fallow land,terraced land, dry land,etc has been suggested to espoused vulnerable farmers increase food security and sustainability across northeast India.

P2.7-003

ASSESSMENT OF COMBINING MICROBIAL AGENTS AND SILICON DIOXIDE TO CONTROL STEM ROT AND SOUTHERN BLIGHT DISEASE ON KIDNEY BEAN IN TAIWAN

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Text
Si is the second most abundant element in the earth and has not been regarded as an essential growth nutrient. It shows the effectiveness of improving crop resistance and pest control. The possible mechanism of silicon dioxide to disease resistance and stress tolerance is reported through the formation of silicon complexes in cell walls to increase the rigidity of plant tissues. The rhizosphere microorganisms improve plant disease resistance through plant growth promotion and production of antagonistic substances. The feasibility of combining SiO2 and rhizosphere microorganisms for controlling the soil-borne diseases of kidney beans was conducted. Our preliminary results showed that the disease severities caused by Pythium myriotylum after applying conventional chemicals and Bacillus siamensis CB36 + SiO2 on kidney beans were 32.0% and 4.0%, respectively; while the disease severities caused by Sclerotium rolfsii after applying conventional chemicals and B. siamensis CB36 + SiO2 on kidney beans were 83.0% and 6.0%, respectively. Otherwise, the soil microbiomes were analyzed and the results showed that applying B. siamensis CB36 + SiO2 were different from other treatments. It is obviously that B. siamensis CB36 + SiO2 treatment showed the best control effects against both stem rot and southern blight of kindey bean. Therefore, the management strategy of combination of B. siamensis CB36 + SiO2 is a potential combination for controlling soil-borne diseases of kidney bean.

Forest pathogenic fungi interacting with insect pests: research fronts and perspectives

C9.5-1

A PHYLOGENETIC EPIDEMIOLOGY APPROACH TO PREDICTING THE ESTABLISHMENT OF A MULTIHOST PEST-PATHOGEN COMPLEX

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Text
Decision-makers require robust and broadly applicable analytical tools to manage emergent microbial and insect pests that attack multiple host species, but current approaches built on assumptions of narrow host ranges do not address their complexity. Here, we take an evolutionary ecology approach and develop and test a new approach to modeling the establishment and spread of Fusarium dieback-invasive shot hole borers (FD-ISHB), an emergent multihost beetle-pathogen complex introduced from Southeast Asia to Southern

California, where it now affects trees in urban-wildland forests and avocado groves. To determine which locations are most vulnerable to pest establishment and impacts, we established a network of 260 0.25-ha permanent monitoring plots across a range of host communities and environmental conditions in California. We estimated the probability of an infested site based on the interaction between phylogenetic structure and host density of a local plant community (community favorability) and microclimate. Site susceptibility was strongly associated with the interaction between community favorability and microclimate-unfavorable host communities were still susceptible where microclimate supported beetle development. Microclimate had a smaller influence on site susceptibility in locations with a highly favorable host community, but sites with unfavorable host communities could be susceptible where microclimate favored beetle development.

C9.5-2

A NEW AND UNUSUALLY WIDESPREAD INFESTATION OF THE PREVIOUSLY RARE STROMA-FORMING FUNGUS CURREYA PITYOPHILA IN ASSOCIATION WITH THE ADELGID SPECIES PINEUS PINI AFFECTING PINUS SYLVESTRIS ACROSS SCOTLAND

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Text
In late 2022, reports were received of canker and dieback symptoms on Scots pine (Pinus sylvestris) at various locations across Scotland. Investigations are still at an early stage, but a common factor observed at all sites is the presence of a previously rare stroma-forming fungus Curreya pityophila (syn.Cucurbidothis pityophila) infesting shoots and branches of Scots pine in an apparently symbiotic association with the adelgid species, Pineus pini. The fungus colonises the outer bark of Scots pine, frequently encircling young branches at shoot junctions but appears itself to remain superficial. Encased beneath the fungal stroma are colonies of P. pini nymphs which feed on the phloem causing necrosis of host tissues, in some cases to the cambium. Affected Scots pine also exhibits lower crown dieback and abundant older, blackened shoot and branch cankers from which the fungal pathogen Crumenulopsis sororia and various endophyte species have been isolated. The primary cause of these cankers may be P. pini in association with C. pityophila, with feeding sites subsequently colonised by canker-causing pathogens. Curreya pityophila and its association with adelgids has been described occasionally from the UK, continental Europe and north America on various conifer species since the 1800s, yet it remains obscure in the literature. We will present the results of our early research findings and discuss the possible reasons for this current, widespread outbreak in Scotland.

C9.5-3

XYLOSANDRUS SP. AND ASSOCIATED FUNGI: A HIGH-RISK SYMBIOSIS FOR NATURAL ENVIRONMENTS

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Text

Insects, like many other organisms, live in association with many fungal symbionts, which impact their host's fitness. Symbiotic fungi associated with Ambrosia beetles contribute to insect damage to the impact on invaded environments. Also providing food for the insect development stages, some of the Ambrosia beetle symbiotic fungi are severe pathogens of plant hosts. Moreover, like many insects, ambrosia beetles may carry commensalist fungi on their body. Thus, monitoring of fungi introduced through specific pathways (mostly trading of living plants), their identification and determination of pathogenicity behavior is essential to design and apply prevention and mitigation quarantine measures.
The EU LIFE project SAMFIX was launched having among the objectives to monitor the fungal population associated to three species of Xylosandrus (*X. compactus*, *X. crassiusculus* and *X. germanus*) after the invasion of the Mediterranean basin in natural areas and nurseries. The present work aims to describe and analyze the total fungal community associated and to determine their functional guild, ecology, and taxonomic position; to analyze 'new' and already recorded associations using taxonomy and lifestyle as references; finally to discuss the possible risk for an insurgence of invasive insect-fungus interactions.

C9.5-4

OPHIOSTOMATOID FUNGAL SPECIES ASSOCIATED WITH MEDITERRANEAN BARK BEETLE ORTHOTOMICUS EROSUS IN CROATIA

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Text

Trees in the urbanized landscape are highly influenced by the presence of harmful insects and diseases due to anthropogenic conditions and recent climate changes which, in some cases, can change the habitat of native pests that can become therefore more aggressive. Mediterranean bark beetle *O. erosus* is native to Croatia and so far it has been considered only as a minor pest but a surprising outbreak that occurred in Forest Park Marjan, Split in 2017 had serious consequences on this important forest in the following years. *O. erosus* is associated with ophiostomatoid species that can show increased virulence and it is assumed that they help their vectors, bark beetles, to break the defensive mechanisms of the host plant. The aim of this research was the identification of ophiostomatoid fungi transmitted by *O. erosus* in different locations of the Mediterranean part of Croatia, as well as investigating possible hosts other than Aleppo pine. Isolates were obtained from *O. erosus* adults and their blue-stained galleries and sapwood and identified according to the morphological characteristics and DNA sequencing. A total of six ophiostomatoid fungi (*Ophiostoma ips*, *O. piceae*, *O. rectangulosporium*, *O. floccosum*, *Sporothrix pseudoabietina,* and *Ceratocystiopsis minuta*) were identified in the study. This is the first research of ophiostomatoid fungi associated with *O. erosus* in Croatia that gave new insight into this bark beetle-fungal symbiotic relationship and opened up a new research area.

C9.5-5

EFFECTS OF LYMANTRIA MONACHA OUTBREAKS ON FOLIAR FUNGAL COMMUNITIES AND NON-TARGET INSECTS

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Text

Pinus sylvestris L. is comparably resistant to defoliation, caused by phytophagous insects. However, abiotic factors are expected to increase the frequency of many insect pests in the future due to climate change. Historically, *Lymantria monacha* L. has been one of the major forest pests in temperate Europe, regularly reaching outbreak levels, with increasing incidence in the last decade. This study was aiming to investigate complex changes in fungal communities and actively moving insects in the outbreaks of *L. monacha* in Scots pine forests. We hypothesized that pest outbreaks cause a significant effect on the composition of fungal communities and non-target insects. To determine this, we sequenced libraries of PCR products, using fungal-specific primers on a PacBio high-throughput sequencing platform, meanwhile, insect counts were analyzed using a binocular microscope and identification keys. A comparison was made between an outbreak and a stand treated with the biological insecticide Foray76B (before damage). Preliminary results show significant structural changes in fungal communities and non-target insect species that may disrupt the normal functioning of pine forests. It is expected that detailed data analysis will provide valuable information on species changes that may significantly influence the resilience of stands in the future and will improve the selection of *L. monacha* outbreak management measures.

C9.5-6

ELMS, BEETLES, OPHIOSTOMA NOVO-ULMI AND GEOSMITHIA SPP.: A COMPLEX INTERACTION BETWEEN MAIN PLAYERS OF DUTCH ELM DISEASE

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Text

Bark beetles of the genus Scolytus Geoffroy are the main vectors of the fungus *Ophiostoma novo-ulmi*, the pathogenic agent of Dutch elm disease (DED), a tracheomycosis that has devastated elm populations throughout Europe, North America and part of Asia. Recently it has been shown that in the DED pathosystem is also present *Geosmithia* spp., an anamorphic ascomycete genus mainly associated to phloem-feeding bark beetles. *O. novo-*

ulmi and *Geosmithia* spp., more than just occupying the same habitat and having the same vectors, are connected in a complicate association: it seems that *Geosmithia* acts as a mycoparasite towards *O. novo-ulmi.*
We used a specially developed duplex qPCR to specifically identify and quantify the presence of *O. novo-ulmi* and *Geosmithia* spp. on the insects' bodies. The beetles were collected with funnel traps, baited with a pheromone lure, at locations characterized by different stages of the DED epidemic, where Ulmus minor trees were naturally present.
The work allowed to determine the real quantity of both fungi DNA on male and female insect bodies all season round; and the ratio between the two fungi in different periods.
The results strengthen our knowledge on disease spread and the role of insect vectors, providing essential information on the life cycle of *Geosmithia* within the DED pathosystem, and provides relevant data to support the use of *Geosmithia* as a natural biocontrol agent of *O. novo-ulmi*.

P9.5-001

THE ROLE OF MICROHABITAT CONDITIONS AND SUBSTRATE QUALITY ON DIVERSITY AND COMPOSITION OF INVERTEBRATES INHABITING HETEROBASIDION SPP. FRUITBODIES ON DECAYED WOOD OF PICEA ABIES

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Text
Pathogenic fungi from the genus Heterobasidion cause root rot in conifers of temperate and boreal forests, particularly in Norway spruce (Picea abies). Heterobasidion produces conspicuous fruitbodies on infected P. abies wood, which may represent a habitat for a range of different organisms, including invertebrates. The aim of this study was to determine the invertebrate diversity and factors affecting their composition in Heterobasidion spp. fruitbodies on decayed P. abies wood. Sampling was carried out in the autumn of 2018 in three P. abies stands in Latvia. A total of 247 Heterobasidion fruitbodies and 247 decayed P. abies wood samples were collected from logging residuals of different dimensions and stages of decomposition. The collected samples of Heterobasidion fruitbodies were of different successional stages, thickness, and age. By placing these samples in Tullgren funnel traps, a total of 7198 invertebrate individuals were collected representing 13 orders. The results showed that the environmental factors significantly affecting the number of trapped invertebrates were wood diameter, fruitbody thickness, fruitbody wet weight, relative humidity. The number of trapped invertebrates increased with the increase of wood diameter, fruitbody thickness, fruitbody wet weight. Further studies are needed on interactions between invertebrates and Heterobasidion, including the study assessing the possible role of insects as vectors for Heterobasidion spores.

P9.5-003

FOREST PATHOGEN SPORES CARRIED BY INSECTS VERSUS AIRBORNE SPORES

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Text

Forest pathogens are infecting trees via airborne spores or carried by insect vectors, but the contribution of both pathways is not well studied. We deployed 240 black Lindgren 12-funnel traps baited with four semiochemical lures known to increase captures of several species of bark and wood boring beetles in the families Cerambycidae and Curculionidae (subfamily Scolytinae) to study spore dispersal in a forest environment. At each site, we deployed 60 baited and 60 unbaited traps (unbaited traps were also covered with a screen to block insect from entering). Total DNA was extracted from the preservative fluids collected in each collection cup, PCR amplified for fungal ITS gene and COI insect gene and sent for Illumina paired-end MyseQ sequencing. Insect traps are excellent aerial spore collectors, allowing identification of both airborne fungi and insects captured by analysis of collection cup preservative fluids. Analyses for the comparison of fungi in the preservative fluids from baited traps and screen protected traps are ongoing but will be completed and presented to determine the contribution of insect carried spores versus airborne spores.

P9.5-004

FUNGI ASSOCIATED WITH THE PINE TORTOISE SCALE TOUMEYELLA PARVICORNIS

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Text

The non-native pine tortoise scale, *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae), has emerged as a severe threat to pine trees in pinewoods and urban areas in Italy. Traditional chemical control measures have proven difficult due to the large size of the trees and the urban environment. Therefore, alternative management practices must be explored, with a focus on the ecological factors that influence pest population dynamics, such as manipulation of antagonistic and mutualistic microbial partners. Fungi have been found to impact the population dynamics of scale insects and other hemipterans in multiple ways. Entomopathogens may be responsible for epidemics in colonies of these insects, even if they are generally unable to stop outbreaks. Plant pathogenic fungi may weaken hosts, compromising their ability to react to pest attacks. Alternatively, scale insects may act as vehicles of pathogenic fungi or facilitate the conversion of endophytic associates into pathogens, promoting opportunistic host infection through damaged tissues. Moreover, recent studies have highlighted the potential role of fungi belonging to the Ophiocordycipitaceae as scale mutualists, stimulating further consideration about their true

impact and possible benefits of disrupting this association. In this study, we investigated *Toumeyella*-associated fungi in urban contexts of Campania and Lazio, Italy, based on both isolation on agar media and direct identification through DNA-marker sequencing.

P9.5-005

AN AMBROSIA BEETLE RECENTLY INTRODUCED IN EUROPE SHOWS SPECIFIC ASSOCIATION WITH A YEAST FUNGUS

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Text

Ambrosia beetles are wood boring insects which can become tree killers. They are also characterized by a high invasion success rate and by symbiotic and phoretic relationships with different fungi. Together, insects and their fungal communities cause important damages to forest and agricultural ecosystems worldwide. A better preparedness to this threat requires to identify the fungal communities associated with any newly introduced ambrosia beetle and a better knowledge of tree killing mechanisms operating in such complex associations.
We aimed to determine whether the fungi associated with the insects intercepted during a monitoring program were sufficiently stable to be characterized with a culture-independent method. The insect species used for this study was *Amasa* sp. near *truncata* (Erichson). Beetles were captured by using interception traps in 2018 and 2021 in different locations France. DNA extraction was performed from the 53 *Amasa* individuals. Fungal ITS1 and ITS2 regions were amplified and library pool was sequenced on the Illumina MiSeq platform. In all Amasa samples, fungal communities showed a low richness and diversity compared. In all samples, OTUs assigned to a *Millerozyma* sp. were dominant. *Millerozyma* species belong to a yeast family frequently associated with wood-boring insects but never reported as phytopathogens. The type of relationships between this fungus and Amasa sp. near truncata needs to be investigated.

From the deciphering of host pathogen interactions to disease management: the Leptosphaeria maculans /rapeseed case study

C8.6-1

A SET OF INTERNATIONAL ISOLATES OF THE BRASSICA NAPUS PATHOGEN LEPTOSPHAERIA MACULANS TOWARDS ELUCIDATING THE BASIS AND EVOLUTION OF PLANT DISEASE

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Text

A collection of isolates of the plant pathogenic fungi *Leptosphaeria maculans* and *L. biglobosa*, agents of disease on *Brassica napus* (canola) and other *Brassicaceae* species, were assembled to represent the global diversity of these pathogens and to establish an international research resource. The collection consists of 226 isolates, 205 *L. maculans* and 21 *L. biglobosa*, representing eleven countries. All 205 *L. maculans* isolates had their genomes sequenced and were characterized for the distribution of avirulence gene alleles using this information and phenotypically for reactions on *Brassica napus* lines with specific resistance genes. Analysis of the SNP diversity within the *L. maculans* isolates revealed geographical separation of the populations. This ‘open access’ resource provides a standardized set of isolates that can be used to define the basis for how these fungi cause disease, and as a tool for the discovery of new resistance traits in *Brassica* species.

C8.6-2

ADVANCES IN CHARACTERISATION OF QUANTITATIVE RESISTANCE TO LEPTOSPHAERIA MACULANS (BLACKLEG) IN RAPESEED (CANOLA)

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Text

Blackleg disease caused by the hemibiotrophic pathogen *Leptosphaeria maculans* is a major constraint to rapeseed (canola, *Brassica napus*) production worldwide. Host genetic resistance underpins blackleg control. Both major gene (complete) and quantitative (partial) resistance effectively limits yield losses but the large genetic diversity and evolutionary potential of *L. maculans* can rapidly render major genes ineffective. Thus, quantitative resistance (QR) is considered a more sustainable approach but breeding efforts are restricted by the absence of a biologically relevant screening method for this complex pathogen due partly to the lack of fundamental knowledge. We conducted experiments in controlled environment and field conditions quantifying blackleg disease using standard visual assessments and highly accurate digital droplet PCR. Contrary to the current understanding, we found that QR is expressed at the seedling and adult plant stages but there is no relationship between the presence of QR in cotyledons at the seedling stage and QR in mature plants for crown canker, and QR does not provide partial resistance to all isolates but instead reacts with individual isolates differently. QR expression was strongly influenced by the environment and potentially also by the major gene resistance. This research advances our understanding of QR expression in rapeseed, improving efforts to identify QTL's and the development of a rapid and reliable phenotyping method.

C8.6-3

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF EFFECTOR PROTEINS TO PROPOSE KNOWLEDGE-DRIVEN PLANT RESISTANCE MANAGEMENT

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Text

The main strategy to control the fungus *Leptosphaeria maculans*, the causal agent of stem canker in *Brassica napus*, is genetic control by plant varieties carrying resistance (R) proteins. However, the massive deployment of a single source of resistance in the fields exerts strong selection pressures towards pathogen populations, leading to the rapid breakdown of the resistance. Recently, advances in effector repertoire prediction, in effector protein heterologous production and structural prediction algorithms have resulted in the identification of several effector families in L*. maculans*, these families being also present in other plant pathogenic fungi, mainly from the Dotheomycetes and Sordariomycetes. Furthermore, some of the structural analogues identified both in *L. maculans* and other plant pathogenic fungi share conserved functions during plant infection, suggesting an interesting evolutionary model. In addition, using transcriptomic data collected along the *L. maculans* life-cycle highlighted expression waves of distinct structural families, suggesting a striking coordination of them during the infection process. Here, we will review the recent advances in structural prediction / structure determination among *L. maculans* secretome and their implication to gain insight into the putative functions of candidate effectors. We will also propose possible strategies to improve resistance management or increase R proteins recognition spectrum using structural effector families.

C8.6-4

A MULTIPLEX HIGH THOUGHT PUT SEQUENCING TOOL FOR THE STUDY OF AVIRULENCE ALLELIC DIVERSITY AND RACE STRUCTURE IN POPULATIONS OF LEPTOSPHAERIA MACULANS.

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Text

Leptosphaeria maculans is a pathogen of oilseed rape, mainly controlled by major resistance genes (Rlm) in varieties. Such genes may be rapidly overwhelmed due to the selection of virulent isolates displaying inactivation, deletion, or mutations in the corresponding avirulence genes (AvrLm). It is thus important to monitor the presence of virulent isolates in populations. First, we analysed the AvrLm gene diversity in 89 French isolates. We found a highly variable level of polymorphism, depending on the gene, ranging from one to 14 alleles per gene, with the occurrence, or not, of deletions or inactivating mutations. We developed a tool using multiplex PCR and Illumina sequencing to rapidly characterize allelic variants for eight avirulence genes in field populations.
We tested the multiplex PCR on DNA of pooled samples of 32 L. maculans leaf spots. After paired-end sequencing with Miseq technology, reads were mapped on an in-house AvrLm sequence database. The data were filtered using thresholds defined from specific control samples included in each run. Proportions of each allelic variant, including proportions of deletion events, were then calculated in each sample. The method was found to be highly reproducible and accurate. Finally, around 1400 symptoms from nine experimental fields were analyzed in a single run. The proportion of virulent isolates estimated by sequencing the pooled leaf spots perfectly matched their known proportions in local L. maculans populations.

C8.6-5

FUNCTION OF B. NAPUS CELL SURFACE RECEPTORS IN RESISTANCE AGAINST BLACKLEG DISEASE OF CANOLA.

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Text

Race-specific disease resistance (R) genes have been widely applied in canola/rapeseed (Brassica napus) breeding for resistance to blackleg disease, caused by the fungus Leptosphaeria maculans (Lm). Technological advances in genomics have led to the cloning of five race-specific resistance genes (Rlm or LepR) belonging to two classes of cell surface receptors, receptor-like proteins (RLP) and wall-associated kinase-like (WAKL) proteins. Cloning of the five Rlm and LepR genes, along with their corresponding Lm effectors has promoted the Brassica-Leptosphaeria interaction to a model pathosystem to study the function of cell surface receptors, especially WAKLs for which there are very few functional examples reported to date. Here we present our current knowledge of the molecular function

of the WAKL gene cluster on B. napus chromosome A07 (Rlm3-4-7-9), gained through allele sequence comparison and functional studies.

C8.6-6

SUCCESS IN R-GENE LABELING, MULTI GENES, KASP MARKERS AND QR: A GAME CHANGER IN THE CANOLA BLACKLEG PLAYBOOK IN CANADA

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Text
Blackleg caused by Leptosphaeria maculans is the most important disease in canola in Canada. Blackleg became a major issue since 2010 with an increase in prevalence, incidence, and severity in Canada. Studies identified that Rlm3 was the predominant gene in most Canadian canola cultivars. Rlm3 breakdown with the advent of avrLm3 was reported, and the increase in the disease was attributed to it. A systematic approach was undertaken to mitigate this occurrence with the seed industry, researchers, and government participating. R-genes in commercial cultivars were identified, and some of the seed companies decided to label their commercial cultivars with the known R-genes. Stacking of R-genes occurred. KASP markers were developed to identify the pathogen avirulent and virulent alleles in grower fields, so growers can select the right R-gene package for the following season. An R-gene rotation was introduced in 2018. Since then, we have monitored the nature of the disease in canola in a country that grows 22 million acres of canola each year to understand the nature of the disease and monitor the pathogen through profiling. The use of stacked R-genes and their performance including the nature of quantitative resistance in disease mitigation has been studied in grower fields. Disease mitigation through a combination of certain R-genes has been identified. The proposed talk will give the success behind these approaches, the challenges faced and what needs to be done in the future.

P8.6-001

PLENODOMUS (LEPTOSPHAERIA) SPECIES SIGNIFICANTLY DIFFER WITH EXTRACELLULAR POLYMERIC SUBSTANCES AND SIDEROPHORE PRODUCTION

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Text
Plenodomus lingam (*Leptosphaeria maculans*, LM) and *P. biglobosus* (*L. biglobosa*, LB)

cause phoma leaf spotting and stem canker of oilseed rape (*Brassica napus*). Extracellular polymeric substances (EPSs) play a key role in shaping the interactions within microbiome and between the microbiota and plants; enable adaptation to changing environmental conditions, affect the colonization of rhizosphere, rhizoplane and endosphere of plants, and provide a competitive advantage in the environment. EPSs were obtained from liquid cultures of 3 LM and 3 LB isolates grown on Czapek-Dox medium. The highest concentration of EPSs produced by LB were obtained on the 8th day and in LM on the 12th day of incubation. The average EPS concentration on the highest production day was 0.47±0.15 for LB and 1.3-fold higher (0.62±0.13) for LM. Strains belonging to both species secreted very strong complexing compounds capable of taking iron ions from the ternary Fe complex with HDTMA and CAS in the Schwyn and Neilands blue medium. There was a very clear difference in the efficiency and rate of siderophore production between species; the rate on the blue medium for LB averaged 28.5±3.61 and was twice as high as for LM (15.5±1.0). LB strains produced siderophores after 24 hours of incubation at 20oC and LM strains after 120-144h. The results indicate that EPSs and siderophores produced by *Plenodomus*/*Leptosphaeria* species differ in their effects on plants and other microorganisms.

P8.6-002

ADVANCES IN STUDYING LEPTOSPHAERIA MACULANS COMPLEX SPECIES CAUSING BLACKLEG DISEASE OF OILSEED RAPE (BRASSICA NAPUS L.) IN TUNISIA

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Text

The complex species *Leptosphaeria maculans* Desm. Ces and de Not. (*L. maculans*)-*Leptosphaeria biglobosa* (*L. biglobosa*) represent one of the most damaging phytopathogenic fungi threatening oilseed rape (*B. napus*) growing regions worldwide (Wang et al., 2020). Since their first occurrence in Tunisian oilseed rape production in 2018 (Maghrebi et al., 2023), the Phoma stem canker disease has been affecting mainly 7 regions and increasing in prevalence over the past few years.

Given the rapid expansion of oilseed rape cultivation in Tunisia, studying the *L. maculans* – *B.napus* pathosystem and research into these newly emergent disease has become of crucial importance. From 2018 to 2020, researches have specially focused on the pathogen's biology, race structure, epidemiology and genetic diversity. Lately, other studies have touched to some degree the disease management control. In this work, we state the principal results of research conducted to date on blackleg disease in Tunisia and outline the scope of the current progress made in the fight against this disease.

The outcomes provide essential information for the development of disease management strategies in the Tunisian and Mediterranean context.

P8.6-003

CHARACTERISATION AND MANAGEMENT OF LEPTOSPHAERIA SPP. CAUSING BLACKLEG OF CANOLA IN SOUTH AFRICA

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Text

Blackleg is a major constraint in global and local canola production. As part of a larger project on managing blackleg in the Western Cape, the prevalence, and species identity of *Leptosphaeria* species associated with canola in the Western Cape were investigated. Cultivar trials were also established at two locations in 2021 and four locations in 2022, across the two canola productions regions (Swartland and Overberg) in South Africa. All commercially available cultivars were included, with 12 cultivars planted in 2021 and 17 cultivars in 2022. *L. maculans* was predominantly isolated from canola in the Western Cape. In both years disease severity was significantly higher in the Swartland region. Significant differences in disease severity were found between cultivars at all locations, with cultivars performing similar within regions. Hyola 559TT were most susceptible at all locations in 2021, while Diamond and CHYB3688TT were most susceptible at all location in 2022. In both years, 45Y95 and 45Y93 performed best across all locations. Both cultivars have the resistance gene Rlm3, suggesting that there is pressure on the pathogen population to shift away from the corresponding avirulence gene (*AvrLm3*), since most locally planted cultivars lack the Rlm3 resistance gene. Determining the vulnerability of commercially available cultivars to blackleg in different locations will assist farmers in choosing the cultivars best suited for their region to prevent losses due to blackleg.

P8.6-004

CHILLING STRESS MODIFIES OILSEED RAPE RESISTANCE TO PLENODOMUS LINGAM

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Text

Global warming also brings new problems in terms of plant resistance to pathogens and pests. In addition to the emergence of new pathogens, which have so far mainly occurred in warm regions, plants have to face other abiotic stress factors. Besides high temperature stress, plants can also be damaged by more frequent temperature fluctuations. In this context, cold stress is becoming increasingly important. In our study, we investigated how chilling stress can modulate plant susceptibility or resistance to subsequent infection by a fungal pathogen. Winter (cv. Columbus) and spring (cv. Westar) cultivars of oilseed rape

(*Brassica napus*) were inoculated with *Plenodomus lingam* and both the extent of symptoms and the underlying mechanisms were investigated. Both cultivars were significantly more susceptible to *P. lingam* when inoculated immediately after short-term cold stress. However, the winter cultivar Columbus showed a higher degree of resistance when inoculated after a few days of recovery at normal temperatures. The mechanisms underlying these results were determined by studying the transcription of defence genes and genes involved in cold stress, and by proteome, metabolome, and hormonome analyses.

Genome evolution in filamentous plant pathogens

C4.3-1

WALTZ WITH PLANTS: GENOME EVOLUTION IN FILAMENTOUS PATHOGENS

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Text

Fungi and oomycetes include deep and diverse lineages of eukaryotic plant pathogens. Fundamental concepts have emerged from the sequencing of the genomes of a multitude of species of these filamentous plant pathogens. Filamentous plant pathogen genomes tend to harbor large repertoires of genes encoding virulence effectors that modulate host plant processes. Effector genes are not randomly distributed across the genomes but tend to be associated with compartments enriched in repetitive sequences and transposable elements. This particular distribution of effector genes is the result of coevolutionary dynamics with host plants. Here, I will discuss how plant pathogens are great model systems to study evolutionary adaptations at multiple time scales. I will also discuss how genome evolution can be applied to the study of pandemic plant pathogen lineages.

C4.3-2

A BROAD GENOMIC SURVEY OF MACROPHOMINA SPP. REVEALS HOST-GENOTYPE ASSOCIATIONS AND EVIDENCE OF ON-GOING RECOMBINATION

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Text

Macrophomina phaseolina has a broad host range, but individual isolates may be pathogenic to a limited number of hosts. Previous work demonstrated: 1) strawberry is not susceptible to all isolates of the fungus, and 2) host specialization may have occurred among highly aggressive, strawberry-pathogenic isolates. A survey of the genus was conducted to identify host-genotype associations, pangenomic structure and mechanisms of genetic exchange. Short-read sequence data were obtained for 422 *Macrophomina* spp. isolates collected from 94 host plant species in 27 countries (113 from strawberry, 54 from soybean, 255 from other hosts). High-quality short-reads were assembled and mapped to reference genomes. *M. phaseolina* was grouped into ten partially recombinant clades, with high admixture in some isolates suggesting on-going recombination. Three of the identified clades clustered with respect to isolate's host of origin (78% of strawberry-derived isolates were in a single clade, whereas 74% of soybean isolates resolved into two clades). This pattern suggests that host specialization may be occurring among isolates in specific clades. Furthermore, pathogenicity tests of select isolates representing each clade suggest strawberry is only highly susceptible to isolates from the "strawberry clade". *Macrophomina* appears to have a one-speed genome. This work provides insight into host specialization and evolutionary mechanisms within this economically important pathogen genus.

C4.3-3

ADAPTIVE EVOLUTION IN VIRULENCE EFFECTORS OF THE RICE BLAST FUNGUS PYRICULARIA ORYZAE

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Text

Plant pathogens secrete proteins called effectors that target host cellular processes to promote disease. Recently, structure-based clustering has identified several families of fungal effectors that share a conserved three-dimensional structure despite remarkably

variable amino-acid sequences and surface properties.
To explore the selective forces that underlie the sequence variability of structurally-analogous effectors, we focused on MAX effectors, a structural family of effectors that are major determinants of virulence in the rice blast fungus Pyricularia oryzae. Using structure-informed gene annotation, we identified 58 to 78 MAX effector genes per genome in a set of 120 isolates representing seven host-associated lineages. The expression of MAX effector genes was primarily restricted to the early biotrophic phase of infection and strongly influenced by the host plant. Pangenome analyses of MAX effectors demonstrated extensive presence/absence polymorphism and identified several candidate gene loss events possibly involved in host range adaptation. MAX effectors displayed high levels of standing variation and high rates of non-synonymous substitutions, pointing to widespread positive selection shaping their molecular diversity.
Our work demonstrates that MAX effectors represent a highly dynamic compartment of the genome of P. oryzae, and suggests that MAX effectors are key players in molecular coevolutionary interactions with plant hosts.

C4.3-4

PATHOGENICITY OF THE CONIFER WILT PATHOGEN, LEPTOGRAPHIUM WAGENERI: GENOMIC INSIGHTS

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Text
Leptographium wageneri is an ascomycete fungal pathogen that causes black stain root disease (BSRD) of conifers. As a primary pathogen that has evolved from closely related saprotrophic species, *L. wageneri* offers an opportunity to identify the possible determinants of pathogenicity. We sequenced the genomes of the three varieties of *L. wageneri* and the closely related non-pathogenic *L. douglasii* and performed comparative genomics between the pathogens and closely related non-pathogenic species. The three varieties of *L. wageneri* were found to have larger genomes, higher gene numbers and a higher content of transposable elements. A putative laccase gene was present only in the three varieties of *L. wageneri* and *L. douglasii*. This laccase gene was horizontally acquired by the common ancestor of *L. wageneri* and *L. douglasii* and encodes for a secreted laccase. Infection of *P. patula* seedlings followed by qRT-PCR analysis indicated that this laccase gene was upregulated in-planta, suggesting its role in pathogenicity. Subsequently, laccase knockout mutants were generated using CRISPR-Cas9 and used in a pathogenicity test. The results showed that the laccase-deleted mutants failed to cause typical symptoms of infection by *L. wageneri*. Collectively, this study illustrates patterns of genome evolution in *L. wageneri* from a non-pathogenic relative. The results also provide some evidence that a horizontally acquired laccase is a key virulence factor in this tree pathogen.

C4.3-5

EXCHANGE OF INTACT NUCLEI PLAYS A MAJOR ROLE IN THE EVOLUTION OF DIKARYOTIC RUST FUNGI

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Text

Many fungi including plant pathogens and ecologically important symbionts are dikaryotic, meaning they carry two separate and different haploid nuclei per cell. Rust fungi are dikaryotic and critical pathogens of many crops, especially wheat and other cereals. Due to the absence of the alternate host required for sexual reproduction and recombination in most parts of the world, rust fungi are restricted to clonal reproduction which should severely limit the generation of genetic diversity. Nevertheless, new races emerge frequently and non-sexual processes such as somatic nuclear exchange have been postulated to play a role, but have been difficult to detect due the lack of genome resolution between the two haploid nuclei. We have recently taken advantage of Hi-C chromatin contact information to develop tools to accurately phase the dikaryotic genomes of rust fungi to their nuclei of origin. This has allowed the detection of numerous instances of somatic nuclear exchange between clonal lineages of cereal rusts. These data suggest that repeated exchange events have shuffled haploid nuclei between clonal lineages leading to global populations consisting of different combinations of a limited number of haploid genome types. Thus, nuclear exchange seems to be the predominant mechanism generating diversity and the emergence of new strain lineages in these otherwise clonal pathogens.

C4.3-6

TRANSPOSONS DRIVE ENVIRONMENTAL ADAPTATION IN A CLONALLY EVOLVING FUNGAL PATHOGEN

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Text

The genomes of many fungal
pathogens are compartmentalized into core regions and accessory regions, which are enriched in transposable elements (TEs). TEs are widely regarded as drivers of adaptive evolution, but direct experimental evidence remains limited. Here we used an evolve and re-

sequence approach to follow environmental adaptation in *Fusarium oxysporum*, a devastating fungal pathogen that attacks more than 150 crops and causes deadly infections in immunocompromised humans. Serial passaging of a clonal isolate through tomato plants or axenic media plates resulted in rapid adaptation and increased fitness under the selection condition. Plate-passaged populations displayed recurrent evolutionary trajectories of sequential loss-of-function mutations that lead to increased proliferation at the cost of reduced virulence. TE insertions accounted for more than half of the variants detected and localized preferentially to sites of histone H3 lysine 27 trimethylation, a hallmark of accessory regions. Our findings reveal that TEs act as the main drivers of adaptation in F. oxysporum and reveal fitness trade-offs between developmental programs stimulating proliferation versus invasion.

F4.3-1

ADAPTIVE GENOME EVOLUTION OF THE CEREAL POWDERY MILDEW FUNGI

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Text
The cereal powdery mildews (Blumeria spp. of the family *Erysiphaceae*) are globally occurring fungal pathogens of grasses and cereals and pose a constant threat for agriculture. *Blumeria* species infect grasses and cereals in a host-specific manner. Ubiquitously distributed transposable elements make up >75% of the genomes of the cereal powdery mildews, which can be a source of genetic variation and genome instability. We study if and how *Blumeria* regulates and repurposes transposable elements to rapidly overcome host resistance. We found transcriptional activity of transposable elements in the barley powdery mildew pathogen *B. hordei* at specific stages of infection, particularly during early host cell penetration and haustoria establishment. Epigenetic profiling in conidia revealed increased 5mC methylated DNA levels in retrotransposons, while small RNA sequencing of isolated mycelia and haustoria indicated accumulation of phasiRNAs in >1,500 retrotransposon loci, suggesting dynamic control of transposon expression through epigenetic mechanisms and RNA interference. We further discovered long spliced antisense RNAs (antisense lncRNAs) at loci of transposon replication genes. These transposon antisense lncRNAs exhibit time point-dependent expression patterns as well as distinct co-expression patterns with transposons, indicative of both positive and negative regulation of transposons by antisense lncRNAs.

P4.3-001

THE MAIZE LATE WILT FUNGUS MAGNAPORTHIOPSIS MAYDIS IN ISRAEL CONSISTS OF AGGRESSIVE STRAINS THAT CAN SPECIALIZE IN DISRUPTING GROWTH OR PLANT HEALTH

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Text

Maize late wilt disease caused by the fungus *Magnaporthiopsis maydis* significantly damages crops in Israel and in other countries. Resistant maize cultivars are the preferred method for disease restraining. However, the pathogen populations of Spain and Egypt have varying aggressiveness, and virulent strains can overcome host resistance. In 2001 and from 2016 to -2019, 17 *M. maydis* strains were isolated from infected maize fields in Israel. The isolates' effects on seed germination, plant development, and disease symptoms severity were evaluated. The isolates from Israel display a diverse degree of aggressiveness that is not linked to their geographic distribution. The virulent strains are found in mixed populations, whereas less virulent *M. maydis* isolates exist. Aggressive strains harmed the development of plants and ears and caused severe wilting and death. In contrast, plants inoculated with less virulent strains exhibited only mild dehydration signs, and crop yield was similar to that of the non-infected control. Interestingly, different host cultivars can evoke specific virulence of *M. maydis* strains. Moreover, some pathogen strains significantly repress plant development, while the impact of other strains was evidenced by wilting symptoms. The current research further increases our understanding of the pathogen and our ability to control it.

P4.3-002

GENOME SEQUENCING AND COMPARISON OF SEVEN STRAINS OF TILLETIA HORRIDA, CAUSAL AGENT OF KERNEL SMUT OF RICE

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Text

Kernel smut of rice, caused by Tilletia horrida, is characterized by the replacement of rice grains with black sooty masses of teliospores. Kernel smut occurs to varying degrees in six continents and 29 countries around the world. In the US, kernel smut has increased its occurrence and severity in the last decade and is currently one of a major disease, threatening the US rice industry. In this study, we sequenced and assembled seven phylogenetically distinct strains of T. horrida. Four (TX1, TX2, TX3, and TX4) of the isolates were assembled to 26 mb in length and the remaining three (TX5, TX6, and TX7) to 20 mb in length. The assembly sizes of the strains in the latter group were similar to previously reported strain QB-1 from China. The genome-based phylogeny analyses confirmed our previous multi-locus analysis results that strains TX1, TX2, TX3, and TX4 were genetically distinct from the strains TX5, TX6, and TX7, along with strain QB-1. Gene prediction and secondary metabolic gene clusters of the seven T. horrida strains ranged from 6, 975 to 8, 108 and 10- to13. These genome resources lay the foundation for future studies on the population genomics of T. horrida and the host-pathogen interactions.

P4.3-003

REOCCURRING WILT, A NEW DISEASE OF COTTON IN AUSTRALIA CAUSED BY NOVEL EUTYPELLA SPECIES

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Text

Reoccurring wilt is a new disease of cotton in Australia which was first detected in the 2017/18 season as a single small patch of wilting and dying cotton plants in a field in Central Queensland. The disease reoccurred in the same location in subsequent cotton plantings, increasing to approximately 1 ha in 2020. Fungal isolations from diseased field grown plants were consistently dominated by one fungus based on culture morphology. Identification was established on sequences of the internal transcribed spacer region of ribosomal DNA and showed that all the isolates had high homology to *Eutypella scoparia*. Further analyses revealed that there were two distinct *Eutypella* species present. Pathogenicity tests showed that a *Eutypella* isolate when inoculated into the stem of healthy cotton caused cankerous growth and necrosis of vascular tissue, typical of trunk disease. The fungus caused a red-brown streaking of the vascular tissue like that observed in diseased field plants. Community profiling of diseased root samples showed that two operational taxonomic units related to *E. scoparia* were the most abundant fungi accounting for 45 to 99% of all sequences. This study shows that the fungal isolates, which form a distinct group within the *Euypella*, are associated with the root and stem of dying cotton and were the dominant fungi of diseased roots. This is the first known case of *Eutypella* affecting cotton worldwide and is considered an expansion of this genus' host range.

P4.3-004

GENOME COMPARISONS BETWEEN EUCALYPTUS LEAF- AND STEM-INFECTING TERATOSPHAERIA SPECIES REVEAL GENE FAMILY EXPANSION IN THE ABSENCE OF REPEAT GAIN

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Text

Several species of the fungal genus *Teratosphaeria* (Dothideomycetes: Mycosphaerellales) are pathogens of *Eucalyptus* trees in plantations globally. While most of these fungi cause leaf and shoot blight, two species are unusual in causing resinous stem cankers. We have sequenced the genomes of all the economically important *Teratosphaeria* species occurring on *Eucalyptus*, providing an opportunity to compare the stem and leaf pathogen lineages at a genomic level. The *Teratosphaeria* genomes were annotated and compared with regards to genome assembly statistics as well as gene content and function. To confidently identify genes unique to *Teratosphaeria* pathogens, the genomes of seven other Teratosphaeriaceae

species were included as outgroups. The stem pathogens were found to have repeat-sparse genomes, yet their gene expansion was at least three times greater compared to the leaf pathogen lineage. Unique functional categories identified in both leaf and stem pathogens included genes that code for CAZymes, proteases, secondary metabolites, and transcription factors. Overall, the results highlight candidate genes that may play an important role in leaf and stem pathogenicity. Furthermore, they suggest that gene expansion has partly driven the evolution of the *Teratosphaeria* stem pathogens from their leaf-associated ancestors.

P4.3-005

FUSARIUM OXYSPORUM EFFECTOR CLUSTERING VERSION 2 (FOEC2): AN UPDATED PIPELINE TO INFER HOST RANGE

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Text
The fungus *Fusarium oxysporum* is infamous for its devastating effects on economically important crops worldwide. *F. oxysporum* isolates are grouped into formae speciales based on their ability to cause disease on different hosts. Assigning *F. oxysporum* strains to formae speciales using non-experimental procedures has proven to be challenging due to their genetic heterogeneity and polyphyletic nature. However, genetically diverse isolates of the same forma specialis encode similar repertoires of effectors, proteins that are secreted by the fungus and contribute to the establishment of compatibility with the host. Based on this observation, we previously designed the *F. oxysporum* Effector Clustering (FoEC) pipeline which is able to classify *F. oxysporum* strains by forma specialis based on hierarchical clustering of the presence of predicted putative effector sequences, solely using genome assemblies as input. Here we present the updated FoEC2 pipeline which is more user friendly, customizable and, due to multithreading, has improved scalability. It is designed as a Snakemake pipeline and incorporates a new interactive visualization app. We showcase FoEC2 by clustering 537 publicly available *F. oxysporum* genomes and further analysis of putative effector families as multiple sequence alignments. We confirm classification of isolates into formae speciales and are able to further identify their subtypes.

P4.3-006

GENOMIC CHARACTERIZATION OF THE MATING-TYPE (MAT1) LOCUS FROM SEVEN SCLEROTINIA SPECIES

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Text

Sclerotinia is a genus in the *Sclerotiniaceae,* a fungal family that includes many important plant pathogens. In filamentous Ascomycetes such as *Sclerotinia,* sexual reproduction is controlled by the mating-type (*MAT*) genes located at the mating-type locus (*MAT1*). This study aimed to characterize the *MAT1* locus of seven previously unstudied *Sclerotinia* species. To achieve this, the locus was annotated from draft genome sequences generated during the study. The *MAT1* locus differed in gene content and arrangement between the different species. The *S. pseudotubarosa MAT1* locus contained the *MAT1-1-1, MAT1-1-5, MAT1-2-1,* and *MAT1-2-10* genes, indicative of homothallism. For *S. spermophila* and *S. sulcata*, the *MAT1-1-5* gene was absent although *MAT1-1-1, MAT1-2-1*, and *MAT1-2-10* were present. *S. sativa* and *S. matthiolae* had all four *MAT* genes, although the presence of inverted repeat sequences suggests an inversion event might occur during meiosis. Such an inversion would truncate *MAT1-1-1*, while inverting the orientation of *MAT1-2-10* and *MAT1-2-1.* The *MAT1* locus of *S. bulborum* and *S. asari* also had four *MAT* genes, although the presence of direct repeats could delete the *MAT1-2* genes through unidirectional mating-type switching. This process would convert a self-fertile isolate to self-sterility. This study produced draft genomes for seven Sclerotinia species, while also shedding light on the evolution of sexual reproduction and the *MAT1* locus in this economically important group.

P4.3-007

FOLDING FEATURES AND DYNAMICS OF 3D GENOME ARCHITECTURE IN PLANT FUNGAL PATHOGENS

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Text

Folding and dynamics of three-dimensional (3D) genome organization are fundamental for eukaryotes executing genome functions but have largely unexplored in non-model fungi. Using the Hi-C data, we generated two chromosome-level assemblies for *Puccinia striiformis* f. sp. *tritici* (*Pst*), a fungus causing stripe rust disease on wheat, for studying 3D genome architectures of plant pathogenic fungi. The chromatin organization of the fungus followed a combination of the fractal globule model and the equilibrium globule model. Surprisedly, chromosome compartmentalization was not detected. Dynamics of 3D genome organization during two developmental stages of Pst indicated that regulation of gene activities might be independent of the changes of genome organization. In addition, chromatin conformation conservation was found to be independent of genome sequence synteny conservation among different fungi. These results highlighted the distinct folding principles of fungal 3D genomes. Our findings should make an important step towards a holistic understanding of the principles and functions of genome architecture across different eukaryotic kingdoms.

P4.3-008

IS HOST JUMPING OF PYRENOPHORA TERES LEADING TOWARDS SPECIATION?

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Text

Barley grass (*Hordeum leporinum*), which often occurs in proximity to commercial barley (*Hordeum vulgare*), is an alternative host to *Pyrenophora teres*, an economically important pathogen causing net blotch in barley. Population and pathogenicity studies of *P. teres* isolates obtained from barley and barley grass have reported that the two populations are genetically distinct and host specific, suggesting that isolates collected from barley or barley grass are two different species. The first successful in vitro sexual recombination event between *P. teres* from barley and barley grass was confirmed by our group using a neighbour-net network and haploblocks based on whole genome sequencing of seven progeny isolates. Pathogenicity assays revealed that *P. teres* isolates from barley grass are not host specific but could infect both barley and barley grass and that the progeny isolates are virulent on commercially grown barley cultivars. Despite the genetic divergence of *P. teres* isolates from barley and barley grass revealed through our phylogenomic, evolutionary and haploblock analyses, there seems to be no complete host or reproductive separation between these populations. Thus, there is a potential for the generation of novel pathotypes through sexual recombination between *P. teres* isolates associated with barley and barley grass, with a risk of increased impacts on commercial barley cultivars that do not carry resistance to these potentially emerging pathotypes.

P4.3-009

DIVERSITY AND PATHOGENICITY OF COLLETOTRICHUM SPECIES CAUSING PASSION FRUIT ANTHRACNOSE IN TAIWAN

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Text

Anthracnose caused by *Colletotrichum* spp. poses a serious threat to the yield and quality of passion fruit, an economically valuable fruit crop of Taiwan. In order to effectively control passion fruit anthracnose, this study aims to identify isolated fungal species, analyze their pathogenicity, and screen for effective disease control materials. In total, 56 fungal isolates were collected from diseased leaves and fruits of passion fruit during 2018 to 2022 from main passion fruit producing areas of Puli Town and Dapingding, with 17 of them further characterized in terms of morphology, pathogenicity, and multi-gene phylogeny. The fungal species identified include *C. brasillense* (11%), *C. fructicola* (18%), *C. karstii* (30%), *C. plurivorum* (23%), and *C. theobromicola* (18%), which belong to *Colletotrichum gloeosporioides* species complex, *C. boninense* species complex, and *C. orchidarium* species complex, respectively. Of note, a severe outbreak of passion fruit anthracnose

occurred at Puli Town in July 2022 and *C. theobromicola* was found to be the main causal agent. Evaluation of candidate fungicides found that both azoxystrobin plus Difenoconazole and cyprodinil plus fludioxonil showed better curative effect for passion fruit anthracnose. Moreover, 4-4 Bordeaux mixture and two cinnamon oil-based non-pesticide agents were able to inhibit the growth of mycelia, thus showing the potential as therapeutic agents. These results will help effective management of passion fruit anthracnose.

P4.3-010

COMPARATIVE SECRETOME ANALYSIS OF ZYMOSEPTORIA TRITICI ISOLATES AND DOTHIDEOMYCETE SPECIES TO IDENTIFY CONSERVED SECRETED EFFECTOR PROTEINS

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Text
Zymoseptoria tritici (Zt) is the cause of Septoria tritici blotch in wheat. Pathogens secrete effectors that modulate innate immunity of the plant and can overcome pattern-triggered immunity mechanisms to facilitate infection. Effector molecules from fungal pathogens are small, secreted proteins (SSPs). They typically are fewer than 300 amino acids in length, are cysteine-rich, contain signal peptides at the N-terminus, and lack transmembrane domains. Proteins of five *Zt* isolates and seven Dothideomycete species were retrieved from JGI Mycocosm and NCBI databases. Functional domain annotations were conducted using BLAST+ 2.12.0., Pfam v.35.0, dbCAN, and the MEROPs database. Secreted proteins were identified by the presence of signal peptides and the absence of transmembrane domains detected by Phobius v1.01, Target P v.2.0. and SignalP v4.1. EffectorP v.3.0, ApoplastP and LOCALIZER were applied to identify effector features and predict the localization of the effectors within the plant cell. The four European isolates of *Zt* showed the highest number of SSPs among all the organisms. The IPO323 strain has similar numbers of SSPs and predicted effectors as *Cladosporium fulvum* and *Parastagonospora nodorum*. A clustering analysis on the predicted effectors revealed two clusters that contain effectors with significant sequence similarity among all the species. We identified shared effectors in 11 species including non-pathogens *Baudoinea compniacensis* and *Cryomyces antarcticus.*

P4.3-011

MATING PHEROMONE AND RECEPTOR GENES ARE GENERALLY CONSERVED IN THE CERATOCYSTIDACEAE, A GROUP INCLUDING IMPORTANT PLANT PATHOGENS WITH DIVERSE MATING STRATEGIES

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Text

The pheromone-receptor system allows fungi to communicate during mating. Heterothallic fungi require opposite partners for sexual reproduction to occur, with compatible mating partners each producing a different pheromone to attract each other and communicate. In contrast, homothallic fungi are able to reproduce without a mating partner. Homothallic fungi also rely on these pheromones and receptors as they have roles other than mate recognition. We identified the a- and α-pheromone genes and their respective receptor genes from the genome assemblies of 34 species in the *Ceratocystidaceae* (*Ascomycota*), which includes many important plant pathogens, and where many are homothallic. Both receptor genes were identified in all species investigated, although the α-pheromone receptor appears non-functional in *Ambrosiella* species as they lack the essential transmembrane domains. The α-pheromone gene was present in all species apart from *Ambrosiella*. In *Berkeleyomyces* species, the α-pheromone gene was duplicated. It was not possible to identify the a-pheromone gene in any of the genome assemblies, likely because of the lack of sequence and locus conservation commonly seen in pheromone genes. The results show that the pheromone-receptor system remains conserved in most members of the *Ceratocystidaceae*, regardless of their sexual strategy.

P4.3-012

MAT1-1-2 GENE KNOCKOUT REDUCES THE FREQUENCY OF UNIDIRECTIONAL MATING-TYPE SWITCHING IN CERATOCYSTIS ALBIFUNDUS

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Text

The African tree pathogen *Ceratocystis albifundus*, exhibits the unusual form of sexual reproduction known as unidirectional mating-type switching. The switching process produces two progeny types that differ in their sexual phenotypes and the gene content at the mating-type (*MAT1*) locus. Self-fertile progeny can complete the sexual cycle in isolation, producing both self-fertile isolates having four mating-type (*MAT*) genes and self-sterile isolates with only two genes. Self-sterile progeny result from mating-type switching, and these isolates require a mating partner for sexual reproduction. While two direct repeats within the *MAT1* locus likely facilitate the deletion of the target region, the role of the *MAT* genes in unidirectional mating-type switching has not been considered. In this study, a CRISPR/Cas9 system was used to delete the *MAT1-1-2* gene from the *MAT1* locus of *C. albifundus*. The deletion resulted in self-sterility while also drastically reducing the ability of the fungus to switch mating-types. Cultures of the transformant have few copies of the switched *MAT1* locus when compared to the wild-type. This likely prevents self-fertilisation from occurring, indicating a role for the MAT1-1-2 protein in *MAT* gene deletion during switching. The gene knockout also resulted in pleiotropic effects in the transformant such as reduced growth rate and lower levels of conidiation, suggesting a role for this gene is the overall fitness of the pathogen.

P4.3-013

BLAZING A TRAIL: UNCOVERING THE MECHANISMS THAT UNDERLIE THERMOTOLERANCE IN THE POST-FIRE FUNGUS RHIZINA UNDULATA

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Text

Rhizina undulata is the causal agent of Rhizina root rot of *Pinus* species in South Africa, and parts of Europe and Asia. The fungus is also known as the “coffee fire fungus” because its ascospores, which rely on heat shock for germination, are activated by camp fires used to prepare coffee along forestry trails. In this study, we aimed to elucidate the genetic mechanisms that underlie thermotolerance in *R. undulata*. This was achieved by sequencing the genome of this pathogen and comparing it to other species within the Pezizales. The *R. undulata* genome harboured 16 heat shock protein 20 (HSP20) genes and 10 glutathione S-transferase (GST) genes. The remaining Pezizales species possessed, on average, four HSP20 genes and five GST genes, suggesting significant expansions of these two protein families in the genome of *R. undulata*. Both HSPs and GSTs have been associated with heat shock defence in other fungi, supporting the hypothesis that the expansion of these protein families may be responsible for the thermotolerance exhibited by *R. undulata*. Given that this fungus requires heat shock for ascospore germination and subsequent host infection, these genes may influence other biological processes of this serious pine pathogen, warranting further investigation and functional characterisation.

P4.3-014

FUSARIUM OXYSPORUM F. SP. FRAGARIAE RACE 2 IN CALIFORNIA DID NOT EVOLVE THROUGH A SINGLE MUTATION IN THE AVRFW1 AVIRULENCE GENE

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Text

In the fall of 2022, *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) race 2 was discovered for the first time in California. This new race was comprised of a single strain that caused severe Fusarium wilt of strawberry and was present in multiple fields at the time of its discovery. The resistance gene it overcame, called *FW1*, was fortuitously present in commercially available strawberry cultivars at the time of *Fof* race 1’s discovery in 2006 and was a critical tool for managing disease caused by *Fof* race 1. This presentation will review what is known about the emergence of this new pathogen, gene-for-gene interactions between *Fof* and *FW1*-resistant strawberry cultivars, and new insights about the evolutionary mechanisms that led to the emergence of *Fof* race 2 in California. Knocking out a single avirulence gene

from *Fof* race 1 confers pathogenicity on *FW1*-resistant varieties, but *FW1*-resistant varieties retain quantitative resistance to the *Fof* race 2 strains generated by single-gene knockouts. By contrast, wild *Fof* race 2 isolates collected from diseased strawberry plants in Japan and California are equally virulent on *FW1*-resistant and *fw1*-susceptible cultivars. These wild *Fof* race 2 isolates share several potentially important pathogenicity factors that are absent in other *Fof* race 1 strains. These data suggest that a fully virulent race 2 phenotype cannot be gained by a single mutation in an avirulence gene; acquisition of other virulence factors is also necessary.

P4.3-015

IDENTIFICATION OF GANODERMA SPECIES ON DIFFERENT HOST IN THE NORTHWEST REGION OF CAMEROON

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Text

Ganoderma is a well-known plant pathogen causing root and stem rots and mortality of a wide range of economically important trees and perennial crops. It is difficult to identify Ganoderma species based on morphology alone due to the resemblance of its macroscopic character, the use of cultural and molecular methods is imperative. This research is aimed at identifying Ganoderma species with its host tree. Ganoderma species were collected using opportunistic sampling. Morphological and cultural identification was done. DNA was extracted and molecular analysis was done using ITS and TEF gene regions. Phylogenetic analyses was done using MEGA. Macromorphological characters of 68 species were described. Species distribution map was produced using ArC GIS 9.3. The colony colour of the different Ganoderma species varied from cream white, green, orange to black. Eight trees namely: Persea americana, Elaeis gineensis, Mangifera indica, Maesopsis eminii, Cola acuminata, Ficus sp., Albizia adianthifolia, and Canarium sweinfurthii were identified as hosts. Twelve species of Ganoderma (G. angustisporum, G. australe, G. orbiforme, G. eickeri, G. multiplicatum, G. weberianum, G. multipileum, G. applanatum, G. brownii, G. cupreum, G. gibbosum and G. lucidum) were identified after nucleotide blast in NCBI. There exists a monophyletic relationship between the species identified. The use of modern taxonomic methods to identify Ganoderma species compliments traditional identification methods.

P4.3-016

RETICULATED EVOLUTION, HYBRIDIZATION EVENTS AND GENE TREE INCONGRUENCE REVEALED IN THE TRICHODERMA HARZIANUM COMPLEX

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Text

Trichoderma harzianum species are infamous biocontrol agents, mycoparasites, and pathogens. Phylogenetic analyses based on ITS, RPB2, and TEF1α have presented T. harzianum as a species complex of cryptic species. We hypothesized that reticulation events and/or poorly delineated reference trees have caused cryptic speciation, due to incomplete lineage boundaries. To answer the problem, we inferred the range of reticulated lineage using ASTRAL-III and Species Network applying Quartets (SNaQ). To evaluate the use of the traditional phylogenetic analysis in inferring evolutionary histories, we compared the three-loci maximum likelihood (RAxML) tree to genome-wide concatenation-based (RAxML) and coalescence-based trees (ASTRAL-III). Phylogenomic relationships of 21 Trichoderma strains were inferred using 4176 orthologous genes. ASTRAL-III and SNAQ revealed reticulated lineages and robust branches. Concatenation-based tree supported the robust branches inferred in the coalescence-based tree. The three-loci ML tree had high incongruence to the phylogenomic trees, including genome-wide inferred robust branches. Detected hybridization events and reticulated evolution among several species offered evidence of gene flow, indicating that the complex consists of only few species, not several. High incongruence of the three-loci ML tree means poor depiction of the genome-wide data for delineation, crucial to predict the evolution of biocontrol, mycoparasitism, and pathogenicity traits.

P4.3-017

CLASSIFICATION AND CHARACTERIZATION OF COLLETOTRICHUM SPECIES ASSOCIATED WITH APPLE BITTER ROT IN KOREA

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Text

Apple is one of the important fruit crops with economic value in Korea. Apple bitter rot caused by *Colletotrichum* spp. is one of the most severe diseases in worldwide. *Colletotrichum* spp. affects the fruits preharvest in the field and postharvest in storage, resulting in considerable economic losses. To analyze the diversity of pathogens causing Apple bitter rot in Korea, a survey was conducted in 2020, and *Colletotrichum* species were identified through analysis of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and internal transcribed spacer (ITS) 2 genes. Among 314 isolates collected from 39 orchards in 22 regions in Korea, new species, such as *C. conoides* (2 of 314) and *C. aenigma* (2 of 314) have been identified. Predominant species of *C. siamense* (183 of 314) and *C. fructicola* (120 of 314) have been isolated. Additionally, 3 species of *C. gloeosporioides* (3 of 314), *C. fioriniae* (2 or 314) and *C. nymphaeae* (2 or 314) have been isolated. All these species caused lesions on apples. A fungicidal sensitivity test was conducted for isolates that have been identified. Understanding these research findings emphasizes the importance of identification and characterization of Colletotrichum spp. within each species complex, which will help in disease management.

P4.3-018

A MULTIPURPOSE TOOLKIT OFFERED PRACTICAL ASSISTANCE TO ADVANCED FUNCTIONAL ANALYSIS OF PHYTOPHTHORA SOJAE GENES

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Text

Oomycetes, represented by *Phytophthora*, have a threat of serious injury to natural and farm ecosystems due to complex pathogenic mechanism. Recently, CRISPR/Cas9-based gene-editing strategy has been established in *Phytophthora sojae*, becoming a powerful tool for oomycete functional gene research. However, an integrated gene research system needs functional complementation and reintroduction of target gene(s). Currently, lacking efficient selection marker for complementation becomes the short board of gene function research. Here, we report that the gene *NAT1* (GenBank: CAA51674.1), which encodes Nourseothricin acetyltransferase and confers resistance to antibiotic Nourseothricin, can be used as a selection marker for *Phytophthora* transformation. Therefore, a new genetic manipulation toolkit is developed based on vectors containing *NAT1* or *NPT II*, offered practical assistance to advanced functional analysis of *P. sojae* avirulence genes. In this study, we demonstrated that the *NAT1* gene can be used as a screening marker and constructed a complete functional genetic research system in *P. sojae*. This report will greatly accelerate the functional genomics of oomycetes.

P4.3-019

COMPARATIVE GENOMICS OF CERATOBASIDIUM THEOBROMAE STRAINS ASSOCIATED WITH VASCULAR STREAK DIEBACK OF CACAO IN ASIA AND RED MAPLE IN NORTH AMERICA.

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Text

Since 1960, vascular streak dieback (VSD) of cacao, associated with Ceratobasidium theobromae (Ceratobasidiaceae, Basidiomycota), has contributed to major yield loss in cacao in SE Asia and Oceania. In the mid 2010's, ornamental trees in nurseries in Eastern North America began to present symptoms of dieback with streaking in xylem and an associated fungus was isolated. Morphology, disease symptoms and a nearly identical ITS DNA sequence led to the diagnosis of C. theobromae. In Summer 2022, symptoms consistent with this disease were reported in red maple (Acer rubrum) in a nursery in Florida. Subsequent analysis at Purdue University, Indiana, USA and Florida Department of Agriculture and Consumer Services confirmed the presence of the same North American strain. Based on ITS, this strain appeared to be more closely related to a strain reported from China in Lonicera japonica, also a widespread invasive plant in Eastern North America, than to cacao strains. We performed whole genome sequencing on the Florida strain (37M 150bp

paired end Illumina reads) and mapped high-quality reads to two previously published genome assemblies from cacao strains from Borneo and Sulawesi. Similarity between Asian and Florida strains was lower (~95%) than among Asian strains (>99%) and numerous putative effector genes present in Asian strains appear to be incomplete or absent in the Florida strain, prompting a more robust evaluation of the latter's functional properties and taxonomic status.

P4.3-022

EXPLORING THE SURPRISING DIFFERENCES IN EFFECTOR COMPLEMENTS OF THE CLOSELY RELATED APPLE AND STRAWBERRY POWDERY MILDEW PATHOGENS

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Text

Podosphaera aphanis is an obligate biotroph and the causal agent of strawberry powdery mildew and is considered the most important aerial disease affecting strawberry cultivation. Similarly, *Podosphaera leucotricha* is an economically damaging pathogen and the causal agent of apple powdery mildew. We have assembled pan-genomes from three *P. leucotricha* and three *P. aphanis* field samples. *In silico* methods have been used to predict genes within these assemblies, particularly candidate 'effector' proteins. The genomes share features with other powdery mildew lineages. Proliferation of similar transposable elements to those in endoparasitic powdery mildew species was found, along with numerous *RALPH* gene orthologues (RNAse like proteins associated with haustoria). Comparable expansion of the *RALPH* gene family has not previously been reported outside of monocot powdery mildews. Enlarged powdery mildew effectorome size has previously been associated with narrow host range and the *Blumeria* lineage. Despite being closely related species, with comparably restricted host ranges, our results reveal a *P. leucotricha* effectorome that is more than four times larger than that of *P. aphanis.* An isolate's effector complement determines its host range. Identifying candidate effector genes in *P. leucotricha* and *P. aphanis* will facilitate investigation into the evolution of these species as well as aiding breeding and diagnostic efforts to combat these economically important pathogens.

P4.3-023

MINI-CHROMOSOMES DRIVE LARGE-SCALE GENOME REARRANGEMENTS AND HORIZONTAL GENE TRANSFER IN THE BLAST FUNGUS MAGNAPORTHE ORYZAE.

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Text

Blast disease epidemics caused by the fungus Magnaporthe (syn. Pyricularia) oryzae are often dominated by clonal pathogen lineages. In absence of sexual recombination, a major driver of genomic diversity and purging of deleterious mutations, these lineages manage to continuously adapt to their host plants. Contrary to their very low genetic diversity in core genomic regions, we observed vast chromosome diversity in clonal blast fungus populations. We found that variable mini-chromosomes (mChr) contribute to megabase-scale genome rearrangements, chromosome duplications, and horizontal transfer of mChr between diverse host-specialized lineages. These transfer events include mChr-encoded virulence effector candidates. We hypothesize that mChr rearrangements and inter-lineage transfer contribute to blast fungus genome diversity and to the adaptive potential of the blast fungus.

P4.3-024

NOVEL PIPELINES FOR ASSEMBLING AND ANNOTATION OF GENOMES FOR PLANT PATHOGENIC FUNGI. APPLICATION ON TWO MAJOR BANANA PATHOGENS.

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Text

An understanding of the evolutionary dynamics that allow plant pathogens to break down host resistance and develop fungicide resistance, is needed to develop durable and efficient control methods. To address the plasticity of fungal genomes, long-read genome sequences and adequate bioinformatic support is required to generate high-quality chromosome-level assemblies. In this study, the variability in the structural genomes of two important banana pathogens, *Pseudocercospora fijiensis* (causing the black Streak) and *Fusarium oxysporum* f.sp. *cubense* TR4 (causing the Fusarium wilt), were investigated. We first developed the workflow « Podium ASM » to assess the quality of long-read genome assemblies, based on contig numbers, genome completeness, and the presence of telomeric sequences. A second workflow, named « EffiCAZ », was then used to improve functional annotation of pathogenicity-related effectors and CAZymes. Chromosome-level genome assemblies were obtained from four Nanopore sequences of each species, and compartmentalised into a core and an accessory part, with contrasting gene densities and effector distribution. A much higher proportion of transposable elements was observed in *P. fijiensis* than in *F. oxysporum* f.sp. *cubense* TR4. These new pipelines pave the way for the comprehensive characterization of the pangenomes of the two banana pathogens.

P4.3-025

MAJOR PROLIFERATION OF TRANSPOSABLE ELEMENTS IN THE LAST 10 MILLION YEARS HAS SHAPED THE GENOME OF THE SOYBEAN RUST PATHOGEN PHAKOPSORA PACHYRHIZI

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Text
The soybean rust disease caused by *Phakopsora pachyrhizi* is a major constraint for soybean production. It can lead to yield losses of up to 80% and it is one of the most economically damaging agricultural disease. This rust fungus is only known to reproduce through clonal propagation of dikaryotic urediniospores on legume species. The dramatically large and highly repeated nature of the genome of *P. pachyrhizi* prevented the production of an accurate assembly, and sequencing attempts in the dawn of fungal genomics have proven unsuccessful. In the frame of a combined effort from ***the international soybean rust genome consortium***, we independently sequenced three *P. pachyrhizi* genomes uncovering a genome size of up to 1.25 Gbp comprising two haplotypes with a transposable element (TE) content of ~93%. We performed a detailed annotation of TEs and showed the rapid genome expansion through the recent proliferation of long terminal repeat (LTR) TEs in the *P. pachyrhizi* genome. We studied the incursion and dominant impact of these TEs on the genome and show how they have a key impact on various processes such as host range adaptation, stress responses and genetic plasticity. The high-quality *P. pachyrhizi* genomes represent a key community resource for developing novel control methods and understanding the molecular mechanisms of *P. pachyrhizi*-soybean interactions.

P4.3-026

HOTSPOT GENOMES SHED LIGHT ON P. ORYZAE EVOLUTION IN SUB-SAHARAN AFRICA

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Text
The rice blast fungus, Pyricularia oryzae, is responsible for the widespread economic losses on rice in several rice producing countries when environmental conditions are favorable. Since its initial outbreak in Uganda in the 1920s, P. oryzae has been a major threat to the SSA rice sector and continues to impede rice cultivation in various countries across the region. We used genomic analysis to demonstrate that P. oryzae in SSA originated from a number of different genotypes that were brought over from Asia. The phylogeny of 42 SSA isolates collected from 13 rice growing countries and 139 global genomes retrieved from sequence archives showed that the blast population had two primary clades, one of which formed three sub clades, all of which at least contained isolates from SSA, although group 1 and 2 are less represented in SSA. Genome analysis strongly supports the notion that East Africa was the initial point of introduction in SSA. Using Bayesian stochastic search variable selection (BSSVS), the relative transition rates between different countries showed that the majority of P. oryzae population found in the world originate from China. Madagascar appears to have the fewest connections of all the samples analyzed. To a significant extent, Burundi acts as a sink for isolates coming from China, the Philippines, and West Africa.

Because multiple genotypes have already been introduced in SSA, preventing any more introductions is crucial.

Germplasm seed movement and global plant health

C3.7-1

REGULATORY FRAMEWORK FOR SEED HEALTH

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Text
Seed production and movement is an increasingly global business where seed lots may move through five or ten countries before they are planted. Phytosanitary certification for international trade of seed specific pests of concern, and each seed lot can be tested or inspected for multiple pests. As an outcome, seed exporters must meet the phytosanitary requirements set by the importing National Plant Protection Organization (NPPO) for each country they enter. To create an alternative phytosanitary mechanism in seed trade, in 2022, USDA-APHIS published a Regulatory Framework for Seed Health (ReFreSH) accreditation standard and supplemental participant manual. The program will accredit a systems approach for managing phytosanitary risk in the seed supply system. A systems approach under ReFreSH would leverage current seed industry best management practices. This allows for flexibility in pest management if entities can provide equivalence in efficacy. The new regulatory framework will allow for an audit-based accreditation for managing seed health as an alternative to consignment-by-consignment inspection or testing for phytosanitary certification. Currently, pilot projects are being established to determine if a systems approach will provide phytosanitary equivalence and an appropriate level of protection against pests of concern. The pilot will also give information about the extent of NPPO resources that are required to implement this program.

C3.7-2

CGIAR GERMPLASM HEALTH UNITS APPLY A SYSTEMS APPROACH TO GERMPLASM SEED HEALTH PROTECTION FOR CONSERVATION AND SAFE INTERNATIONAL DISTRIBUTION

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Text

Germplasm seed exchange from CGIAR genebanks and breeding is important to global agricultural research and development programs. Seed as a pathway for pest spread is an inherent risk for international seed exchanges. Phytosanitary controls have been established in accordance with the International Plant Protection Convention (IPPC) to protect global plant health from transboundary pest invasion. This presentation summarizes pest risks to international germplasm distribution; CGIAR Germplasm Health Units (GHUs) procedures to ensure the production and distribution of pest-free germplasm; bottlenecks to germplasm distribution, including the inadequacy of phytosanitary regulations guided by the International Standards for Phytosanitary Measures (ISPMs) of IPPC; and consequences of delayed germplasm access on crop improvement programs. It also presents the 'CGIAR Greenpass Phytosanitary Protocol (CGPP)' concept as a comprehensive phytosanitary compliance assurance procedure. Based on a systems approach of pest risk identification and pest risk mitigation in the germplasm seed production pipeline and the rigorous implementation of phytosanitary controls in collaboration with national plant protection organizations, the CGPP is expected to fast-track pest-free germplasm distribution to the global community.

C3.7-3

IMPACT NETWORK ANALYSIS (INA) FOR NATIONAL MITIGATION STRATEGIES FOR EMERGING SEED-BORNE PATHOGENS

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Text

National programs tackling emerging seed-borne pathogens need efficient approaches to make the most of often limited resources. Impact network analysis (INA) is a tool for scenario analysis to understand the likely outcomes of the regional management strategies that are being considered (Garrett 2021). INA evaluates the network of pathogen movement through seed trade and/or the movement of vectors or propagules (examples for potato, sweetpotato, and banana: Buddenhagen et al 2017; Andersen et al 2019; Andersen Onofre et al 2021; Nduwimana et al 2022). It can also incorporate the network of communication among stakeholders, which influences decisions about management (example for avocado: Etherton et al 2023). The scenario analyses in INA can be used to evaluate the most important locations for surveillance and mitigation, and the likely outcomes from specific policies that could be implemented to support disease management. INA can also be used to evaluate whether seed systems are providing benefits, such as improved varieties and protection against disease, to all the stakeholders in the system. This presentation will introduce a new user interface for INA, along with new examples of scenario analyses for a range of crops and diseases.

C3.7-4

PHYTOSANITARY TESTING AND SANITATION FOR SAFE TRANS-NATIONAL MOVEMENT OF CLONALLY PROPAGATED CROPS: HISTORY AND FUTURE

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Text

Crops that are propagated through vegetative tissues such as stem cuttings, tubers or roots tend to accumulate virus infections over growing seasons because in contrast to seed crops that can reduce virus infections every generation as most viruses are not, or relatively poorly transmitted through seeds. Other pest and pathogens can also easily be transmitted through vegetatively propagated materials and so material is usually shipped as sterile explants in test tubes, thus eliminating most pests, except those that replicate intracellularly, such as viruses. Virus testing procedures have evolved over the years, starting from evaluating symptoms directly or after transmission to biological indicator plants, evolving to electron microscopy, ELISA, PCR, nucleic acid hybridization and more recently high throughput sequencing (HTS) approaches. We will present the evolution of methods and strategies for virus diagnostics as they occurred at the International Potato Center culminating in the current move to HTS as the novel standard for virus indexing for safe movement of clonal crops.

C3.7-5

PUBLIC-PRIVATE PARTNERSHIP MODEL TO ENHANCE SAFE INTERNATIONAL SEED TRADE IN THE GLOBAL SOUTH: A CASE STUDY IN SOUTH AND SOUTHEAST ASIA

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Text

The importance of public-private partnerships for seed trade cannot be overstated as the bulk of seed production is carried out by the private sector while the public sector is are responsible for vetting and approving to ensure the safe import or export of seed lots. APAARI with the support from STDF/WTO and in active partnership of private seed sector agencies. viz., Asia-Pacific Seed Alliance (APSA), International Seed Federation (ISF), CropLife Asia (CLA) and American Seed Trade Association (ASTA), a project has been launched recently on Strengthening phytosanitary compliances and public private partnership for boosting seed trade for the Asia Pacific region. The pilot countries are Bangladesh, Cambodia, Laos, Nepal, Philippines, Thailand and Vietnam along with engaging New Zealand and Australia as mentoring countries. The initiative is addressing the NPPOs' regulated pest lists, the capacity to adopt and implement ePhyto, and scope for accreditation of private sector seed health testing laboratories, and strengthening of public–private coordination platform for seed trade through capacity building and policy dialogues.

C3.7-6

QUARANTINE OF GERMPLASM FOR PLANT BIOSECURITY AGAINST TRANSBOUNDARY VIRUSES: IMPORTANCE OF DIAGNOSTICS AND PHYTOSANITARY REGULATIONS

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Text

The global movement of germplasm has the potential to introduce new viruses which may pose potential risk to the agriculture of importing country. In India, ICAR-National Bureau of Plant Genetic Resources has been empowered for quarantine processing of imported germplasm including transgenics meant for research purposes. As per the Plant Quarantine (Regulation of Import into India) Order, 2003, 1261 pests including 264 viruses are regulated pests which are of quarantine significance for India. Early, sensitive and accurate diagnosis is necessary for detection of viruses in quarantine. The challenges in virus detection include availability of antisera, virus genome sequences in GenBank, detecting an unknown/exotic virus etc. Adopting a strategy of post-entry quarantine growing/inspection followed by use of combination of physical, serological and molecular detection techniques, 45 viruses of great economic and quarantine importance were intercepted in imported germplasm including transgenics in the last two decades. The interceptions include 19 viruses not yet reported from India and several viruses not known to occur on particular host(s) in India. India should put in place a “National Plant Pests Diagnostic Network” to enhance preparedness. Adopting reliable virus detection techniques and implementing quarantine regulations strictly would go a long way in ensuring plant biosecurity against transboundary viruses through quarantine and exchange of virus-free germplasm.

F3.7-1

SEED PATHWAY FOR PEST DISSEMINATION: THE ISTA REFERENCE PEST LIST, A BIBLIOGRAPHIC RESOURCE IN NON-VEGETABLE PLANT SPECIES.

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Text

Food safety is intimately linked to plant health. It is threatened by pests whose impact varies greatly depending on the crop, agricultural practices, and regions of the world. In a global trade context, the transport of infested plant materials is an important factor in the spread of organisms to new territories, which can cause emerging diseases. It is critical to deploy strategies to limit this from happening. Identifying pests and their vectors is a first step towards risk analysis. International initiatives are bringing together research results on the role of seeds as vectors of pests. The International Seed Testing Association Reference Pest List (ISTA-RPL) focuses on seed-borne pathogenic organisms in about 50 plant species (field crops, legumes, fruit and forest trees, aromatic plants). The aim is to determine, based on scientific results, if these organisms can (or cannot) be transmitted vertically or transferred

in the environment under natural conditions, making seeds a vector of dissemination. The ISTA-RPL currently inventories 333 pests, of which 146 are transmitted or transferred via seeds in 23 host species (v9.0; July 2022). It is a living tool, and an additional set of hosts is under investigation. This literature resource may be a valuable source for risk assessors and policymakers. It also opens avenues of R&D work by seed companies, academic laboratories or industry to develop diagnostics, detection methods, or treatments

P3.7-001

SEED TRANSMISSION OF SPINACH DOWNY MILDEW

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Text
Spinach downy mildew, caused by the oomycete pathogen *Peronospora effusa*, remains a constraint on spinach production. New races of *P. effusa* continue to appear and can overcome cultivar resistance. Spread of the pathogen via airborne sporangia is well established. However, the role of oospores from seed and infected crop debris has been long debated and remained uncertain. We have found oospores to be present in around 19% of evaluated spinach seed lots. To evaluate seedborne downy mildew transmission, we used isolated glass chambers to grow out oospore-infested spinach seeds, and seeds mixed with oospore-infested crop debris in two independent trials. Downy mildew diseased spinach plants were found 37 days after planting in the first trial, and 34 days after planting in the second trial, in glass chambers that contained one of two oospore-infested seed lots or seeds coated with oospore-infested leaves. Spinach plants in glass chambers initiated from seeds without oospores did not show downy mildew symptoms. These findings provide evidence of seed transmission of downy mildew to spinach plants via oospores and suggest management practices such as seed treatments to reduce the primary inoculum of the pathogen are needed.

P3.7-002

DEVELOPMENT AND APPLICATION OF REVERSE TRANSCRIPTION DROPLET DIGITAL PCR ASSAYS FOR DETECTION AND QUANTIFICATION OF MAJOR APPLE VIRUSES FROM IN VITRO MICROPROPAGATED APPLE PLANTLETS

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Text
Most apple cultivars reproduce mainly through asexual propagation, and their replicas have been distributed as vegetative cuttings around the worldwide. The fruit trees, including

apples, are propagated in tissue vegetative and become grafted on root stock. These reproduction methods result in the infection of numerous plant viruses with some of them causes several diseases, fruit crop losses and reducing. Apple stem groove virus (ASGV) and apple stem pitting virus (ASPV) are major viral pathogens of pome fruit crops, such as apple, pear, and causes significant losses to fruit production in many countries. To reduce these economic losses, it is important to develop effective methods to acquire virus-free propagation material and prevent the spread of virus. Accordingly, in order to produce in vitro micropropagated plantlets without viruses, accurate and sensitive detection methods are urgently required. This study purposes a sensitive and accurate method for detection and quantification of ASGV and ASPV from in vitro micropropagated apple plantlets using a reverse transcription droplet digital polymerase chain reaction (RT-ddPCR) assay. Recently, several studies have reported the successful detection and quantification of plant RNA viruses using ddPCR in plants. The RT-ddPCR assay represents a promising alternative for accurate quantitative detection and diagnosis of ASGV and ASPV infection in virus-free certification programs.

P3.7-003

RECOVERY OF BLS-ASSOCIATED PATHOGEN FROM SEED, SEEDLINGS, AND LEAF SAMPLES IN SOUTH DAKOTA

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Text

Bacterial leaf streak (BLS) caused by Xanthomonas translucence pv. (translucens/undulosa), is a significant disease threatening wheat production in South Dakota and its surrounding regions. The pathogen can survive on crop residue and seed, making it a persistent source of infection for future crops. In this study, we analyzed 28 seed samples, 12 leaf samples collected from the field, and 18 seedlings of HRSW, raised from Xanthomonas translucens-infected seed, to check the presence of the Xanthomonas translucence pv. Translucens(Xtt) and Xanthomonas translucencepv. Undulosa (Xtu) . All grain, leaf, and seedlings samples were plated on KB medium for four days. The phenotypic data on the colonies obtained from the 20 seed, 12 leaves, and 10 seedlings suspected presence of Xtt/Xtu. Further, these suspected Xanthomonas translucens - colonies from seed, leaves, and seedlings were genotyped using Xtt and Xtu specific primers. Of 42 samples genotyped 23 samples (13 seeds, 7 leaves, 3 seedlings) were positive with either Xtt or Xtu. The results reveal that seed could be a significant source of inoculum for BLS development in South Dakota. Also, both Xtt and Xtu could be responsible for disease development. In addition, more grain samples and leaf samples collected from various locations and cultivars are under investigation to obtain a broad picture of the pathogen's pathovars and their survival on seed.

P3.7-004

EMERGENCE OF NEW DISEASES IN IMPORTED GERMPLASM OF APPLE

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Text
Transboundary movement of germplasm carries an inadvertent risk of introduction of new diseases and pests into new areas. India is Asia's major apple importer and accessing apple from 29 destinations across the globe. In India, 8 fungal and 13 viral pathogens of quarantine importance have been intercepted during 2015-20 in general. Himachal Pradesh is dominantly an apple State in India with 0.14 million ha area. Apple cultivation is going in a big transformation in the State to replace the more than 50 years of old plantation. In this State, more than 3.0 million plants of apple have been imported for the replacements and also for high density plantation. The State has history of serious threats of diseases like apple scab (*Venturia inaequalis*) and pre-mature leaf fall (*Marssonina coronaria*) causing epidemics resulting in serious losses and their severity is also attributed to the inter-state movement of planting material. In the orchards and nurseries raised out of the imported planting material, many new fungal, viral, and phytoplasma pathogens have been recorded causing leaf spots and blight (*Curvularia* spp.), white thread blight (*Ceratobasidium stevensaii*), root rot (Fusarium solani), fruit rots (*Curvularia lunata*, *C. spicifera*, *Trichoderma gamsii*), flat limb (phytoplasma) and other viral diseases. Incidence of some already prevalent pathogens like pink canker (*Corticium salmonicolor*) and stem brown canker (*Botryosphaeria obtusa* and *B. dotheidea*) has also increased.

P3.7-005

EXPLORATION OF METHODS FOR DETECTING INSECTS IN SEED LOTS

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Text
The relationship between insect pests and seeds is an increasing issue. Insect damage in the field and/or during storage can have a significant impact. Indeed, seeds can be a major vector for the spread of insects across territories. Moreover, the restrictions imposed by countries on the import of seed lots are increasingly stringent.
Currently, ISTA rules do not provide methods to meet the increasing demands of seed testing laboratories for insect detection. For this reason, an ISTA-funded project was developed with the objective of testing and comparing the effectiveness of different methods with potential for detecting insects in all their forms in seed lots.
Based on the results of a survey on the needs of laboratories, several seed/insect combinations were selected with a priority for insects concerned by phytosanitary certificates. Literature searches were conducted to identify existing and potential methods to be tested. The methods to be explored were defined for each combination according to the type of insects and seeds.
The combinations Bruchus sp./Lentils; Acanthoscelides obtectus/Common bean; Zabrotes

subfasciatus/Common bean and Sitophilus granarius/Wheat were tested with different methods: Berlese funnel, sieving, morphological inspection, 2D X-rays, oxygen consumption and multispectral imaging. The results will be presented and compared

P3.7-006

ALFALFA SEED VIROME

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Text
Seed transmission of plant viruses is important due to the role it might play in their dissemination to new areas and subsequent epidemics. Seed transmission largely relies on the replication capacity of a virus in reproductive tissues and its survival during a seed maturation process. It occurs mainly through the infected embryo, although contaminated seed coats can be a source of infection by mechanical means. Alfalfa (*Medicago sativa* L.) is an important legume forage crop worldwide, and except a few individual seedborne viruses infecting the crop, its seed virome is poorly known. The goal of this work was to perform initial seed screenings of alfalfa germplasm accessions maintained as part of the USDA ARS National Plant Germplasm System to identify pathogenic viruses and understand their potential for dissemination. For detection of viruses, we used high throughput sequencing combined with bioinformatic tools. Prior to the experiment, the seeds were scarified with concentrated sulfuric acid and sterilized with 70% ethanol to eliminate surface contamination. Our results suggest that in addition to common viruses, alfalfa seeds are infected by other potentially pathogenic viral species that could be vertically transmitted to offspring. The information gathered will be used to make decisions on whether germplasm distributions need to be restricted based on viral presence. The data will be uploaded to the Germplasm Resources Information Network (GRIN)-Global database.

P3.7-008

SEED POTATO CERTIFICATION ROLE IN DISEASE MANAGEMENT IN AUSTRALIA

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Text
Seed potato certification provides high plant health planting material to the Australian industry to produce commercial potatoes for processing and table stock markets. Phytosanitary data is collected on each seed crop including the incidence and severity of

endemic diseases in addition to the absence of exotic and/or quarantine diseases. This data is important to the production of high health seed potatoes but also is a valuable data set in relation to domestic and international trade of seed potatoes and this data can be used for support pest area freedom.
Seed certification has relied on the visual assessment of seed potato crops for symptoms of disease. For many years, this approach was successful in mitigating Potato Virus Y, however increased occurrence of PVY often without visual disease symptoms, required a different approach of routine leaf testing of seed potato stocks. Initially, a higher rejection of crops occurred but after several years of continued testing the levels of PVY were reduced and seed quality improved.
Potato Spindle Tuber Viroid (PSTVd) is not reported to occur in Australian potato production.. Targeted surveillance for PSTVd in potato crops submitted for certification from 2016 to 2022 was conducted. . Samples of potato leaves were collected from 10% of all seed crops. Leaf samples were analyzed using RT-PCR. PSTVd was not detected during the 7-year surveillance period.

P3.7-009

EVALUATION OF IN SITU AND EX SITU FORAGE GERMPLASM COLLECTIONS REVEALED THE FIRST OCCURRENCE AND SEED-TRANSMISSION OF ALFALFA MOSAIC VIRUS AND SOUTHERN BEAN MOSAIC VIRUS INFECTING BRACHIARIA SPP.

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Text
Brachiaria spp (syn. Urochloa spp) is one of the most important tropical forages grasses of African origin. It is adapted to drought and low fertility soils, and known for palatability and high-quality biomass production, and thus improves livestock productivity. The International Livestock Research Institute (ILRI) forage Genebank maintains 671 accessions of Brachiaria grass, belonging to 28 species, of which 261(39%) accessions are Urochloa brizantha. The susceptibility of Brachiaria grass to diseases and insect pests is a key challenge to the sustainable production of the grass in Africa. In this study, using dot blot assay and RT-PCR methods, we report for the first time the detection of Alfalfa Mosaic Virus (AMV) and Southern Bean Mosaic Virus (SBMV) on 88 % of the tested accessions conserved in situ and ex situ at ILRI forage genebank. In addition, the virus transmission from seed to plant and legume to grass and vice versal was confirmed through bioassay test. The detection of these two viruses on Brachiaria spp. will present a new challenge to germplasm conservation, distribution, and its sustainable production in the regions and demands an immediate attention towards developing an effective virus cleaning method of the conserved germplasm. Furthermore, there is also an urgent need to assess the economic importance of the diseases on the grass and selection of germplasm that has a better resistance to the diseases.

P3.7-010

TAXONOMIC DIVERSITY OF RICE SEED ASSOCIATED BACTERIA IN REPRESENTATIVE SEED IMPORTS FROM VARIOUS COUNTRIES INTO AFRICARICE GHU

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(1) AfricaRice, Bouaké, COTE D IVOIRE

Text

In recent years, seeds have become the primary pathogen inoculum carrier worldwide. Due to the rise in seed-borne diseases, taxonomic charactrization and detection of seed-borne pathogens has become increasingly important. This study isolated and identified culturable bacteria from rice seeds exchanged with AfricaRice over two years, starting in 2021. Metagenomic whole genome shotgun (mWGS) sequencing was used to identify dominant colonies in 1177 seed samples from eight countries: Senegal (139), Tanzania (64), Mali (124), Madagascar (19), Kenya (31), Nigeria (440), Guinea (340), and Philippines (20). Pantoea, Bacillus, and Pseudomonas were the three most prevalent genera in seed samples, with Pantoea being the most common in samples from the Philippines, Mali, Nigeria, Senegal, and Madagascar, and Pseudomonas being equivalent to Bacillus in samples from Tanzania and Guinea. Fifty percent of Kenyan bacterial isolates were Sphingomonas. This study also detected non-pathogenic taxa such Kosakonia, Enterobacter, Microbacterium, Sporosarcina, and Stenotrophomonas. Overall, we found considerable taxonomic diversity among the culturable bacterial isolates from imported rice seeds, as has been reported for many seed exchange microbial analyses. Further analysis of the dorminant species will be conducted to develop techniques for routine identification in countries exchanging seeds with AfricaRice Germplasm Health Unit, to limit the spread of seed borne pathogens.

P3.7-011

SWEETPOTATO VIRUS INCIDENCE AND ELIMINATION IN THE GLOBAL GERMPLASM COLLECTION MAINTAINED AT THE INTERNATIONAL POTATO CENTER (CIP)

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Text

The CIP’s Genebank has the goal of conserving and distributing sweetpotato genetic resources. This implies on the permanent receival of new accessions to sustain the global genotypic diversity of the collection. The introduction of external accessions implies on the permanent development of detection procedures and elimination of plant pathogens prior to the introduction of these genotypes in the *in vitro* collection. The current procedure applied on imported accessions consists of a post entry quarantine, plant indexing, followed by NCM-ELISA and PCR. Serological and molecular tests assess the presence of 10 different virus species and the genus Begomovirus. Samples that are positive to the presence of a pathogen are subjected to thermotherapy and meristem isolation. Data from 175 samples

showed that 76% of the samples were virus infected. Begomoviruses in single or mixed infections were the most prevalent viruses, occurring in 53% of the positive samples evaluated. SPCFV, SPCV, SPFMV were also detected in mixed and single infections representing an overall of 31% of the infected plants. The remaining positive samples were due to the presence of SPMMV, SPVCV and SPVG in single infections or symptoms on the indicator plant with nonidentified causal agent. A single thermotherapy and meristem isolation removed 62% of viruses. However, in some specific virus mixtures up to six meristems were required to be evaluated until a virus free accession line was identified.

P3.7-012

PHYTOSANITARY EVALUATION OF THE IN VITRO CASSAVA COLLECTION (MANIHOT ESCULENTA CRANTZ): COLLECTION HISTORY AND PHYTOSANITARY EVALUATION

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Text

Cassava is an important crop for more than a billion people around the world. Currently, more than 300 million hectares are produced yearly, supporting industrial consumption, and impacting food security. For this reason, the conservation of cassava crop diversity in genebanks is important for crop improvement. The Genetic Resources Program of the Alliance Bioversity and CIAT, conserves and facilitates access to the globally largest collection of cassava (5965 accessions). The collection of cassava was initially established in 1969 under field conditions in Palmira, Colombia. However, the emergence of quarantine diseases, such as Cassava frogskin disease, triggered the establishment of the collection under in vitro conditions. Later, in 1983, the Germplasm Health Unit (GHU) was created to ensure safe distribution of germplasm. The GHU has worked on diagnosing quarantine diseases, using traditional methods (graft-test), serological methods, and molecular techniques. Considering the emergence of new pathogens, the GHU continues working on implementing protocols with more sensitivity, specificity, and faster. As a result of this effort, and with a phytosanitary certification process underway, recognized by the Colombian Plant Protection Organization, the GHU was carried out the evaluation of the 100% of the cassava accessions, for at least one pathogen. This work allowed genebank users to have access to 92% of the germplasm, free of quarantined pathogens for safe distribution.

P3.7-013

IDENTIFICATION, CONSERVATION AND FORMATION OF A BANK OF STRAINS OF QUARANTINE FUNGI ISOLATED FROM BEAN SEEDS AND TROPICAL FORAGES, FROM THE GERMPLASM BANK “FUTURE SEEDS”.

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Text
Reference strains are an important resource that allows guaranteeing the quality of the results used mainly in phytosanitary diagnostic laboratories. Acquiring this biological material is difficult and its transboundary movement is highly restricted, due to the risks of introducing pathogens into disease-free geographic areas. The Germplasm Health Unit (GHU) of the International Bioversity Alliance and CIAT is responsible for evaluating the presence of quarantine-type fungi in the bean and tropical forages collections of the Germplasm Bank "Future Seeds". Having this type of reference material is of vital importance to carry out quality controls in accordance with the ISO 17025 standard. The GHU has built a collection of fungal strains isolated from seeds, as well as plant material collected from phytosanitary monitoring in the regeneration fields. The strains isolated were taxonomically characterized up to the species level, developing a DNA extraction protocol and amplifying the ITS (Internal Transcribed Spacer) region. Several isolates were sequenced using Oxford Nanopore Sequencing Technology. The sequenced genomes will allow the implementation of complementary molecular tests (PCR and/or qPCR) in routine diagnosis, strengthening the evaluation of quarantine-type fungi through phenotypic and genotypic characterization, guaranteeing the quality of the results obtained, which translates in an increase in the availability of the materials evaluated.

P3.7-014

DETECTION AND CHARACTERIZATION OF SEED-BORNE BACTERIAL LEAF BLIGHT IN WHEAT

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Text
Wheat is one of the most important crops which contributes toward global food security and represents a main source of food and income for millions of smallholder farmers worldwide. *Pseudomonas syringae* pv. *syringae* (*Pss*) is the causal agent of bacterial leaf blight wheat disease which can cause up to 50% yield loss or more depending on the time of infection and region. In addition, *Pss* is transmitted by wheat seeds, which can play a role in long-distance spread. Therefore, developing and implementing effective management strategies for bacterial diseases is very important to reduce yield and quality loss. However, rapid and accurate detection of diseases is the first essential step for effective management strategies for control of this disease. Even though molecular tools for *Pss* precise detection and characterization has been developed, the most practical approach for rapid diagnosis is the use of serological assays using specific antibodies. A polyclonal antiserum against a Syrian isolate of *Pss* was produced and its quality was evaluated by Dot-blot Immunoassay using homologous and heterologous antigens. Results revealed that the produced antiserum was able to detect *Pss* up to 1×10^3 CFU/ml dilution using raw antiserum at a dilution of 1/160 with no cross reactivity with other bacterial species (e.g. *Xanthomonas*).

P3.7-015

EFFECTIVENESS OF NATURAL COMPOUNDS AND LOW RISK ACTIVE INGREDIENTS FOR THE CONTROL OF FUNGAL DISEASES ON SEED-BEARING CABBAGE

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Text

Seed-bearing crops represent a sector with considerable economic and strategic importance worldwide. The resurgence of fungal diseases, need for high quality seeds and increasing restriction on the use of synthetic pesticides, have spurred the search for alternative solutions to protect seedbearing vegetable crops from seedborne pathogens. This work aimed to evaluate the main fungal pathogens on seedbearing cabbage and to control them under farming conditions applying four innovative active substances in different protection strategies: chitosan, chito-oligosaccharides and oligo-galacturonides (COS-OGA), mixture of terpenes and *Bacillus amyloliquefaciens*. The field-trials were carried out involving two companies located in the Marche region, Central-Eastern Italy. According to the laboratory investigations on different symptoms observed in the experimental field, it was isolated several pathogens from different leaves symptoms. The main fungi detected were: *Alternaria alternata*, *Alternaria brassicicola*, and *Stemphylium* spp. In both cabbage fields, all innovative management strategies provided a good protection against leaf necrosis due to fungal infections, better than conventional application with the standard management strategies.

P3.7-016

GERMPLASM SEED MOVEMENT AND GLOBAL PLANT HEALTH

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Text

Global seed transfers through trade, and collection and distribution of genetic resources by genebanks are important pathways for the transboundary spread of seed-borne pests, especially viruses that the insect vectors can further transmit upon introduction. Various phytosanitary procedures, including the IPPC International Standard Phytosanitary Measures, have been established to minimize the risk of seed transmission and provide access to quality seeds crucial for food production and biodiversity conservation. This session will summarize the current state of efforts in minimizing the seed-transmission risk and measures to overcome bottlenecks to comply with phytosanitary standards. Presentations will cover pest risk to seed pathways and implications to global plant health,

strategies for minimizing seed-transmission risk, advances in diagnostic techniques for characterization and sensitive detection of seed-borne pests, and efforts to enhance phytosanitary capacity, especially in low- and middle-income countries to enable safe seed exchanges. The session will also highlight policy and regulatory limitations/bottlenecks necessary to improve safe exchange of germplasm and boost seed trade.

P3.7-017

PHYTOSANITARY PROCEDURES FOR THE CONSERVATION AND USE OF PLANT GENETIC RESOURCES CONSERVED IN THE IITA'S INTERNATIONAL GENEBANK

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Text

Phytosanitary procedures are essential for the sustainable conservation of plant genetic resources and their safe international distribution. This presentation summarizes the methods used at the International Institute of Tropical Agriculture (IITA) to support the collection, conservation, and distribution of genetic resources of some of sub-Saharan Africa's most important food and nutritional security crops. It includes seed crops such as cowpea, soybean, maize, and several indigenous wild Vigna species and vegetatively propagated crops such as cassava, banana/plantain, and yam. These collections comprising over 33,000 accessions acquired over 50 years from over 100 countries, have been conserved as seed, in vitro plants, on the farm, and in third-party genebanks as part of the safety duplication. Procedures used include bioassays, ELISA and PCR-based methods, high-throughput sequencing (HTS) to assess the seed health status, and physical or chemical treatments to eliminate/inactivate pests or regenerate healthy propagation materials in-vitro and inspect for pests and diseases before germplasm can be exchanged. We will also discuss the challenges posed by the integrated viruses and cryptic viruses identified by the HTS to germplasm distribution and the development of a decision framework to evaluate the pest risk and decisions on distribution, regulatory challenges to international germplasm distribution, and the future prospects.

High throughput sequencing approaches for the detection of pathogens

C6.2-1

TO TRUST OR NOT TO TRUST ? THAT IS THE QUESTION WHEN DETECTING A PATHOGEN WITH HIGH THROUGHPUT SEQUENCING : CONSIDERATIONS FOR IMPROVING THE RELIABILITY OF DETECTION

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Text

High-throughput sequencing (HTS) technologies and the bioinformatics analysis of the generated data have brought tremendous improvements in the ability to detect plant pathogens. As any promising technology becoming widespread, there is a key question that needs to be addressed by the scientific community: how reliable are the generated results? Indeed, bias can occur at any step of the analysis.
The presentation will review nearly a decade of collaborative efforts to improve the reliability of HTS protocols for detecting plant pathogens: viroids, viruses, bacteria, fungi, oomycete…. Starting by demonstrating the huge impact of bioinformatics analysis on virus detection, the presentation will go through publications that focused on performance criteria evaluation for viruses (based on shotgun sequencing) and fungi (based on amplicon sequencing) detection by HTS.
In addition, the recent publication of international guidelines for reliable plant pathogen detection, published by more than 50 plant pathologists during Valitest project, has been a cornerstone in our collective path toward improved reliability. These guidelines will be summarized with a strong focus on the identified bias and on the controls to be used with HTS test. In addition, the usefulness of an alien control, a new external control to monitor the contamination burden between samples and to determine an adaptative threshold of detection, will be shown as well as the doubts that can arise in its absence.

C6.2-2

HIGH THROUGHPUT SEQUENCING: RESEARCH TO REALITY – THE AUSTRALIAN POST ENTRY QUARANTINE JOURNEY.

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Text

The ability to detect all viruses and viroids present in plants undergoing post-entry quarantine is the ‘holy grail’ for both plant health scientists and regulators alike. For the Australian Government Post Entry Quarantine (PEQ) facility, the aspiration has become a reality after a near-decade long journey from proof-of-concept and validation through to operationalisation. In December 2022, small RNA sequencing and VirReport bioinformatics were deployed as

the primary screening tool for virus and viroid detection in imported Prunus, Rubus, Fragaria and clonal grass species at PEQ. The outcomes from this deployment include the potential to reduce quarantine lag times, thousands fewer PCR tests per year, reduced use of biological indexing, and increased capacity for higher volumes of plant imports as a result of increased availability of glasshouse bench space. We anticipate that adopting high throughput sequencing (HTS) will enable plant industries to remain competitive and access more rapidly emerging high-value market opportunities.

C6.2-3

VALIDATING SMALL RNA SEQUENCING AND ASSEMBLY AS A GENERIC METHOD FOR VIRUS INDEXING IN POTATO AND SWEETPOTATO GERMPLASM.

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Text

Metagenomic sequencing has been used extensively for identification of viral infections by known and novel viruses in plants since over a decade. Different nucleic acids can be targeted in metagenomic approaches each with their benefits and draw-backs. Small RNA sequencing and assembly (sRSA) has shown to be a sensitive and relatively rapid method for detection of any viral infections in plants, fungi and insects, but includes some technical challenges related to the use of short sequences and quality and detection thresholds to implement in routine testing. Virus testing for international movement of potato and sweetpotato germplasm has traditionally been performed by a combination of technologies, including expensive and time-consuming host range testing that has been a bottleneck to rapid availability for international distribution. The International Potato Center has developed procedures, software and analysis approaches for sRSA and performed several years of validation experiments representing several hundred sweetpotato and potato accessions. Results indicate that using the developed procedures, sRSA generated essentially equivalent results to current routine indexing procedures and can be adopted to replace them at a similar cost, but significant reduction in time to result.

C6.2-4

ROSE VIRUSES: UNDERSTANDING THE CURRENT STATUS AND PROTECTING THE FUTURE OF THE UNITED KINGDOM ROSE SECTOR

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Text

Rosa spp. is the national flower of England and one of the most valued ornamental flowering shrubs grown around the globe. Despite the spread of rose viruses, they have not been studied in detail in the United Kingdom (UK) since the 1980s. Molecular methods have evolved since then, and as a result they have rarely been deployed to study these viruses. In the UK many viruses have been reported previously infecting roses such as arabis mosaic virus (genus *Nepovirus*). However, numerous viruses have been identified infecting roses in recent years, especially with the application of high-throughput sequencing (HTS). Diagnosis is fundamental to facilitate the management of plant diseases, and early detection is essential for successful biosecurity campaigns, for example against rose rosette virus (RRV; genus *Emaravirus*). In this project, different molecular and serological methods have been used to understand the baseline of viruses present in roses in the UK. Detailed experiments were performed to compare various targeted and non-targeted methods, including two different pipelines for HTS data analysis. The performance of this study has allowed the estimation of the prevalence of some previously reported viruses in the UK but also the identification of *Rosa* spp. as a new host for viruses that are widespread in the country. This work resulted in three first virus records in the UK, and the discovery of a new virus species.

C6.2-5

HIGH THROUGHPUT SEQUENCING APPROACHES FOR FASTIDIOUS BACTERIAL DETECTION AND IDENTIFICATION

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Text

Detection and identification of some fastidious bacteria, such as phytoplasmas, can be achieved by metagenomic sequencing. Metagenomic sequencing is highly valuable as it can produce high quality draft genomes for diagnostic and surveillance purposes as well as informing taxonomy, biology and epidemiology. However, this approach is not always effective for diagnostics due to the overwhelming presence of host genomic DNA, especially when the fastidious bacteria are present in the host in low titre. Additionally, depending on the application, full genome assembly of a bacteria is not always required. In this presentation an overview will be given about the use of metagenomic sequencing and alternative approaches, from pre-sequencing enrichment of the target to targeted high throughput sequencing (HTS) methods, to improve detection and identification of fastidious and other bacteria using HTS. These methods show potential in reducing limitations of HTS detection and identification of fastidious bacterial pathogens.

C6.2-6

METAGENOMIC SEQUENCING FOR TOMATO AND PEPPER BACTERIAL SPOT DIAGNOSTICS

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Text

The first step in combatting plant diseases is accurate, swift disease diagnosis. Metagenomic sequencing (MGS) has the capability to provide diagnostic results to describe the entire phytobiome in the context of disease. Although a common research tool, MGS has not yet been regularly adopted for diagnostic purposes in clinics. We are working towards establishing MGS-based disease diagnostics by using bacterial spot of fresh market tomato and pepper as a model pathosystem. Bacterial spot caused by Xanthomonas spp. is one of the most significant and yield-reducing diseases of tomato and pepper worldwide, and management options are limited as bacterial populations have acquired copper bactericide resistance. To advance MGS for plant diagnostics, we conducted a survey of bacterial spot in Ohio fresh market tomato and pepper production systems and elucidated the current distribution of bacterial species, new potential variants, virulence factors, and copper resistance statewide. We assessed growers' perceptions of MGS based diagnostics before and after interacting with MGS diagnostic information from their farms. Our data show that MGS can successfully identify the disease-causing agent in both tomato and pepper bacterial leaf spot samples in two days and we can recover near complete pathogen genomes from MGS data. MGS has the capability to transform plant disease diagnostics, and we are laying the groundwork for MGS-based diagnostics for any pathosystem.

F6.2-1

PLASMIDS PLAGUE PELARGONIUMS: A TALE OF BACTERIAL BLIGHT AND CONVERGENT EVOLUTION

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Text

The way in which we diagnose and respond to bacterial disease outbreaks has transformed over the past several decades. Serological and molecular methods were once the gold standard for diagnostics; however, these methods are labor-intensive and can make it difficult to identify and characterize pathogens. To combat these challenges, we used in-house, low-cost whole genome sequencing (WGS) to rapidly define and track an outbreak in real-time of Xanthomonas hortorum pv. pelargonii (Xhp) on US geraniums (Pelargonium x hortorum) that occurred in the spring and summer of 2022. We used short- and long-read sequencing to assemble 31 Xhp isolate genomes from the 2022 outbreak, and five Xhp genomes from previous decades. We compared the content and size of the core and plasmid

genomes in order to investigate the evolution of virulence. The outbreak strains (Xhp2022) clustered in a new lineage and showed plasmid expansion when compared to older Xhp isolates. The new, larger plasmid acquired genes via horizontal gene transfer and cointegration of other plasmids, leading to expansion. These additional genes may have led to enhanced virulence and fitness, such as heavy metal resistance, and we speculate these factors led to the outbreak of 2022. WGS gave us insight within 2-3 weeks of receiving samples and allowed us to quickly characterize the outbreak, leading us to conclude that rapid sequencing can be used as a model for the surveillance and tracking of other pathogens.

F6.2-2

FINDING A NEEDLE IN A HAYSTACK USING NGS AND ASSOCIATED BIOINFORMATICS TOOLKIT

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Text

Emerging and re-emerging plant diseases pose an enormous threat to agricultural production and global food security. Early detection and identification of outbreaks using advanced high-throughput sequencing (HTS) technology and bioinformatics tools are playing increasingly important roles. At CFIA, the PolyChrome (PC) system and the Clasnip platform (www.clasnip.com) are developed for the early detection and identification of bacterial ring rot, zebra chip and soft rot of potato, as well as potato wart disease. The PolyChrome system, is comprisied of two command-line pipelines (PCC and PCD), an integrated state-of-the-art bioinformatics software and a high-quality genomic reference database. The analysis system allows for timely and accurate detection and identification of high-risk pathogens at the species/subspecies levels. The Clasnip platform is a web-based platform to quickly classify pathogens and their closely-relatives based on SNPs and/or whole-genome sequences. It was developed to allow users with minimum bioinformatics background to compare SNPs with curated, high-quality reference databases. Clasnip can accurately identify CLso haplotypes based on specific genetic signatures in seconds, and is also available for identifying bacterial ring rot pathogen from its close relatives of Clavibacter spp, differentiating different species of soft rot and blackleg bacteria, and different phylogroups of Potato Virus Y (PVY).

F6.2-3

AUTOMATED PIPELINE FOR GENOMIC EPIDEMIOLOGICAL DIAGNOSES OF PHYTOPATHOGENIC BACTERIA

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Text
The emergence of new and re-emergence of extant plant pathogens can lead to severe disease outbreaks in situations such as nurseries. Rapid responses to limit pathogen spread require quick and robust detection methods coupled to timely and appropriate management strategies. Whole-genome based diagnoses allow for fine-scale resolution and transmission-chain tracking, with potential to dramatically reduce the response time to an outbreak. However, the availability of infrastructure, tools, and expertise have delayed adoption in many diagnostic settings. To address this gap, we are developing an automated pipeline that processes raw whole genome sequencing reads, performs genome assembly and annotation, calls variants, generates a core genome phylogeny, and mines genomes for features of interest such as those implicated in virulence, antibiotic resistance, and management. We are using the Nextflow platform to develop this automated and reproducible bioinformatic pipeline. As a proof-of-concept, we applied the pipeline to analyze strains of the geranium pathogen *Xanthomonas hortorum* pv. *pelargonii* reportedly implicated in a common source outbreak that spread US wide within the nursery industry in 2022. This new bioinformatic pipeline is expected to support surveillance efforts and improve early detection as well as reduce response times to accelerate deployment of adequate management strategies against plant pathogens that afflict agriculturally important crops.

P6.2-001

IDENTIFICATION OF VIRUSES INFECTING HORTICULTURAL CROPS IN KOREA BY NANOPORE SEQUENCING

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Text
The development of several molecular diagnostic tools for pathogens has a remarkable increase over the past few decades. Diagnostic methods commonly used to detect plant viruses have limitations in that prior knowledge of the pathogen is required and the ability to simultaneously detect multiple viruses is limited. Therefore, in this study, we used MinION, a portable sequencing device based on Oxford Nanopore Technologies (ONT), was used to rapidly detect plant viruses in various horticultural crops; lily (Lilium spp.), spearmint (Mentha spicata), moth orchid (Phalaenopsis), Prunus mume and Malva verticillate. Plant virus populations for each crop were identified with the What's In My Pot workflow and BLASTn. Three plant viruses (lily mottle virus, lily symptomless virus and plantago asiatica mosaic virus) in lily, cucumber mosaic virus in spearmint, three viruses (odontoglossum ringspot virus, cymbidium mosaic virus and nerine latent virus) in moth orchid, five viruses (mume virus A, cycas necrotic stunt virus and asian prunus virus 1, 2, 3) in Prunus mume and three viruses (turnip mosaic virus, clover yellow vein virus and lettuce mosaic virus) in Malva verticillate were identified by nanopore sequencing. The ONT platform can be used as an effective strategy for efficient monitoring of plant pathogens, including fast run times, portability, low cost, and possibility to be used in any laboratory and field conditions.

P6.2-002

ENDOPHYTIC MYCOBIOME CHARACTERIZATION IN COWPEA (VIGNA UNGUICULATA) USING ILLUMINA SEQUENCING

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Text

Cowpea is an important crop for small-scale farmers in poor areas but is also being developed for commercial agriculture as a possible substitute for commercial legumes. Endophytic fungi are omnipresent and play crucial but diverse roles in plants. This study characterized the endophyte component of the cowpea mycobiome from leaves, main and crown stems and roots using Illumina MiSeq of the ITS2 region of the ribosomal operon.Ascomycetes exhibited the highest diversity, with Molecular Operational Taxonomic Units (MOTUs) assigned as Macrophomina, Cladosporium, Phoma, Fusarium and Cryptococcus, among the most dominant genera. Certain MOTUS showed preferential colonization patterns for above or below ground tissues. Several MOTU generic groups known to include phytopathogenic species were found, with relative abundances ranging from high to very low. Phylogenetic analyses of reads for some MOTUs showed that a level of identification could be obtained to species level. It also confirmed the absences of other species, including phytopathogens. This is the first study that adopted a holistic metagenomic typing approach to study the fungal endophytes of cowpea from a single location, a crop that is so integral for low-income households of the world.

P6.2-003

ANCIENT POWDERY MILDEW DNA FROM REFERENCE COLLECTIONS: THE EFFECT OF A CENTURY ON POWDERY MILDEW DNA PRESERVATION

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Text

The use of molecular techniques to investigate herbarium specimens has increased over the last decade. However, fungal obligate biotrophic plant pathogens such as powdery mildews present a massive knowledge gap in next-generation sequencing from reference collections. This study analysed powdery mildew reference collection specimens over a 117-year period to better understand how powdery mildew DNA decays. We examined DNA base substitution, fragmentation and investigated the microbial community present on the powdery mildew specimens. We found that powdery mildew DNA degraded at a similar rate to plant

herbarium DNA regarding nucleotide misincorporations at DNA break points and excess cytosine to thymine substitutions. The microbiome profiling revealed the dominant fungal families were *Erysiphaceae*, *Aspergillaceae* and *Saccotheciaceae* and the dominant bacterial families were *Microbacteriaceae*, *Pseudomonadaceae* and *Sphingomonadaceae*. Abundance plots showed *Saccotheciaceae* and *Pseudomonadaceae* were more abundant in recent herbarium samples and *Aspergillaceae* being more abundant in ancient herbarium samples. These findings will provide baseline knowledge for future diagnosticians and researchers how to extract and amplify obligate biotrophic plant pathogen DNA from reference collections.

P6.2-004

TOBACCO RINGSPOT VIRUS: A NEW EMERGING VIRUS INFECTING COTTON (GOSSYPIUM HIRSUTUM L.) IN THE UNITED STATES

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Text
Cotton (Gossypium spp.) is an economically important crop in the United States (U.S.) More than >11.5 million acres of cotton is cultivated in the southwestern states of the U.S. Among them Oklahoma cotton's acreage exceeds 490,000 acres during 2021 growing season. However, various virus diseases are recently emerging in the U.S. cotton which could pose a potential threat to the productivity and yield of cotton. Recently, cotton leaf roll dwarf virus (CLRDV) was first reported from Alabama in 2017 and subsequently the infection was recorded in >12 states. The purpose of this study was to monitor virus-like disease in the cotton fields of Oklahoma. During 2021 growing season, symptomatic cotton leaf samples were collected and tested by RT-PCR against CLRDV. A few symptomatic samples were negative to CLRDV by RT-PCR and two of them were subjected to high-throughput sequencing (HTS). A total of 17,542,322 and 22,572,118 trimmed pair-ends reads for both samples were assembled using CLC Genomics Workbench and subjected to BLASTn analysis. Blast results showed 91-100% nucleotide identities with different genes of Tobacco ringspot virus (TRSV) RNA1 of isolates WA-AM1 (MW495243.1), and IA-1-2017 (MT563078.1). Further confirmation of TRSV was achieved by specific RT-PCR. Our results showed the first natural infection of cotton by TRSV which could pose a new threat to cotton crops in Oklahoma, and the U.S in the coming growing seasons.

P6.2-005

METAGENOMIC ANALYSIS OF VIRUSES INFECTING GRAPEVINE (VITIS VINIFERA) IN MEXICO

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Text

Although the grapevine industry is relatively small in Mexico, some of the oldest vineyards in the American continent are situated in the country. Here, the first search for grapevine viruses via high throughput sequencing (HTS) was conducted in Mexico. In 2021, 48 plants displaying virus-like symptoms were sampled in an important grapevine-producing area of Mexico, and later analyzed for the presence of viruses using HTS. Virus screening was verified by real-time RT-PCR following the grapevine disease testing protocol 2010, a certification scheme developed at University of California-Davis. As a result, fourteen different viruses were identified, including grapevine Pinot gris virus (GPGV), grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine Syrah virus-1 (GSyV-1), grapevine rupestris vein feathering virus (GRVFV), grapevine fanleaf virus (GFLV), grapevine fleck virus (GFkV), grapevine virus B (GVB), grapevine asteroid mosaic-associated virus (GAMaV), grapevine red globe virus (GRGV), grapevine Cabernet Sauvignon reovirus (GCSV) and grapevine leafroll-associated viruses 1, 2, 3, 4 (GLRaV-1, 2, 3, 4). In addition, divergent variants of GLRaV-4 and GFkV, and a novel Enamovirus-like virus were discovered. This is the first report of GPGV, GLRaV-4, GRVFV, GSyV-1, GRGV, GAMaV and GCSV infecting grapevines in Mexico. Virus infection in Mexican vineyards represent a concern due to the potential for economic losses and management strategies should be implemented.

P6.2-006

COMPLETE GENOME OF AN ITALIAN TOMATO BROWN RUGOSE FRUIT VIRUS ISOLATE FOLLOWING A NEW TARGET-SPECIFIC NANOPORE SEQUENCING APPROACH.

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Text

Tomato brown rugose fruit virus (ToBRFV) is an emerging and rapidly spreading RNA virus that infects tomato and pepper causing severe crop losses and threatening their production worldwide. ToBRFV is transmitted mainly via contaminated seeds and mechanical contact. A ToBRFV infection was discovered in a commercial tomato and pepper greenhouse in southern Italy and confirmed by RT-PCR and qPCR. RNA extracted from infected samples was processed for high-throughput sequencing with a nanopore MinION device. In order to reconstruct the full ToBRFV genome, two libraries were synthesized by using six specific primer, custom-designed along the whole sequence, during the reverse transcription phase. This original target-specific protocol resulted in an enrichment of reads mapping to the viral genome (30% of total reads) and consequently, in the obtainment of the complete ToBRFV genome (Acc No. OK624678). This new isolate shared a high nucleotide sequence identity (99.82%) with the ToBRFV-Israeli isolate, while maximum-likelihood phylogenetic analysis showed the closest relationship to a Swiss and a British isolates. Moreover, the complete genome of pepino mosaic virus (PepMV;Acc. No. OL362110) was also obtained from the same libraries suggesting that this technology could have enough sensitivity even towards non-target sequences. Our study discloses that a low number of off-target reads can provide clear evidence on unforeseen mixed virus infections.

P6.2-007

SEQUENCING DRAFT GENOMES OF CYST NEMATODE SPECIES ENDEMIC TO AUSTRALIA

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Text

Cyst nematodes form a major taxon of plant-parasitic nematodes causing a significant economic impact globally. They are characterised by the female's ability to retain hundreds of eggs in its body after the completion of its life cycle. They are notorious agricultural pests, classified into eight genera of which *Heterodera* and *Globodera* are two of the most economically important. Traditionally, detection of cyst nematodes is reliant upon morphological identification. However, this is time consuming and requires expertise, so, there has been an increase in the use of molecular diagnostic strategies. Whole genome sequencing (WGS) provides information to identify unique molecular barcodes to distinguish species as well as underlying mechanisms of host invasion. However, WGS of cyst nematodes is difficult since it is very challenging to extract DNA from an individual juvenile. Therefore, millions of juveniles are pooled together to generate enough data to obtain a high-quality sequence. To date, only six draft genomes have been sequenced which have given an insight into the diverse biological processes associated with cyst nematodes. Our study aims to sequence, assemble and annotate draft genomes of cyst nematodes that are endemic to Australia. The goal of this genome sequencing effort is to expand on the existing genomic resources and provide usable data of sufficient quality to the nematology community and develop diagnostic assays for species identification and management.

P6.2-009

THE DIVERSITY OF ALFALFA PATHOBIOME

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Text

Through the recent advances of modern high-throughput sequencing technologies, "one microbe - one disease" concept is being gradually replaced with the principle of "pathobiome". A pathobiome is a diverse community of pathogenic microbes associated with reduced host fitness. This consortium may affect the viability of the plant collectively, through complex interactions between different pathogens and the host, leading to increased disease incidence and severity. To date, a concept of pathobiome as one of the major players in limiting the productivity of alfalfa (*Medicago sativa* L), the most frequently grown forage

legume, is non-existent. We approached this task by characterizing the biodiversity of the alfalfa pathobiome using high throughput sequencing technologies. Our metagenomic study revealed a remarkable abundance of different pathogenic communities associated with alfalfa in field production settings. Plant viruses constituted a significant proportion of the alfalfa pathobiome, representing a ubiquitous background for all other host–pathogen interactions. In many cases, alfalfa samples analyzed in this study also carried bacterial and fungal coinfections, many of which are known plant pathogens of agricultural importance. This initial research on characterizing the alfalfa pathobiome is a starting point in addressing the complexity of plant microbial interactions, their impact on crop health, and insights into the development and evolution of plant pathogenesis.

P6.2-010

HIGH-THROUGHPUT SEQUENCING FOR PATHOGEN INDEXING TO SAFEGUARD THE INTERNATIONAL MOVEMENT OF CLONAL CROPS

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Text
The exchange of genetic resources is vital to the improvement of crops, the diversification of food supply, the adaptation to climate challenges and threats from biotic agents. The international transfer of clonal crops carries the inherent risk of spreading diseases along with vegetative propagules hence stringent testing is required to assure that germplasm is free of pests and diseases prior to movement. The risk associated with movement of tissue culture (TC) materials of clonal crops is that pathogens at low concentrations may be present that escape testing and are thus passaged with TC. Growing-on tests during transit or post entry quarantine are therefore prescribed to assess the plants during active growth by inspection and testing. This results in a lengthy and cumbersome process that is further accompanied by uncertainties associated with latent or unknown viruses. We therefore included high-throughput sequencing to screen cassava germplasm from South America for presence of pathogen sequences and compared the results with the commonly used growing-on indexing. We found several new viruses and highly diverse sequences of known viruses that did not result in pronounced symptoms in cassava and thus would probably be overseen. HTS of total RNA pools prepared from TC proved sensitive and robust to detect RNA as well as DNA viruses in a straightforward and robust workflow. The use of HTS in transit quarantine testing of clonal crops will be discussed.

P6.2-011

METAVIROMICS REVEALS THE PRESENCE OF NOVEL VIRAL DIVERSITY ASSOCIATED WITH CULTIVATED OLIVES IN SOUTH AFRICA

READ David. (1), SLIPPERS Bernard. (1), STEENKAMP Emma. (1)

(1) Forestry and Agricultural Biotechnology Institute, Pretoria, SOUTH AFRICA

Text
Woody Mediterranean crops appear disproportionately affected by viral pathogens. Few virological studies have been carried out on olive (*Olea europaea*) when compared with their *Vitis* and *Citrus* counterparts, with concomitantly fewer known viruses. South Africa has a small but growing olive industry (~3000 ha) producing both table olives and oil. During 2021 and 2022, leaf samples were collected from orchards in the Stellenbosch area. Virus-like symptoms included leaf yellowing, leaf mosaic and decline were observed. RNAtag-seq was used to generate metaviromes and the genomes for five viruses were identified among the *de novo* assembled contigs. These are considered novel based on shared amino acid identities with related viruses and currently accepted species demarcation thresholds. Four of these are putative members of the *Closteroviridae* family and one from the *Solemoviridae* family. Their presence was confirmed with reverse-transcription PCR. The closteroviruses are tentatively named olive virus A (OlVA) (*Amplelovirus*, 21,087 nt), olive virus O (*Olivavirus*, 16,514 nt), olive virus P (*Olivavirus*, 16,589 nt) and olive virus V (*Velarivirus*, 17,050 nt). Based on current demarcation thresholds, the solemovirus (4,226 nt) may represent a new viral genus. While no specific symptoms could be ascribed to these viruses, many closteroviruses are yield limiting pathogens. The OlVA genome represents the longest, non-segmented RNA genome of any plant virus reported thus far.

P6.2-012

NOVEL VIRAL DIVERSITY ASSOCIATED WITH HELIANTHUS ANNUUS L. IN SOUTH AFRICA

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Text
Helianthus annuus L. (sunflower) is one of the world's most important oilseed crops. Diverse viruses from the *Potyvirus*, *Begomovirus* and *Umbravirus* genera are known to infect sunflower, however little to no data is available regarding the sunflower-associated viral diversity in South Africa. During the 2021 and 2022 growing seasons, sunflowers showing symptoms of either severe leaf mottle, mosaic, or ringspots, were collected from major sunflower production areas. Total RNA from each sample was used to generate RNAtag-seq libraries. *De novo* assembly of trimmed reads was performed using metaSPAdes and blast analyses of the resulting contigs, showed that samples were infected with either bidens mottle virus (BiMoV) (*Potyvirus*), pepper ringspot virus (PepRSV) (*Tobravirus*), or a novel member of the *Umbravirus* genus. RT-PCR assays were performed to confirm the presence of the respective viruses. This is the first time that BiMoV is being reported in South Africa and is represented by diverse variants. This is also the first report of PepRSV outside of Brazil and on sunflower. The novel umbravirus has been tentatively named sunflower chlorotic mottle virus. Phylogenetic analyses suggest the virus is most closely related to Ixeridium yellow mottle virus 2. Although all three viruses have been associated with characteristic foliar symptoms, further research is required to determine their capacity to cause yield limiting disease.

P6.2-013

VIROME ANALYSIS OF MULTIPLE SAMPLE TYPES BRINGS EXTENDED INSIGHTS INTO THE PLANT VIRUS PRESENCE IN THE ECOSYSTEM

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Text

Shotgun high-throughput sequencing approaches enable virome analyses of a wide array of sample types, spanning from host tissues to environmental samples. To obtain an extended insight into the virome of selected agroecosystems linked with tomato farms, we analysed viromes of tomatoes, wild and volunteer plants growing in the vicinity of tomatoes, and irrigation water sources used for irrigation of crops on the analysed sites. Over a span of two years, we collected more than 400 plant samples and 24 water samples. We isolated total RNA from the samples and prepared each sample type appropriately for shotgun sequencing using Illumina platform. After in-depth bioinformatics analysis of the obtained data, we detected many known, and even higher number of novel viruses in different sample types. Most of the previously unknown viruses were detected in wild plants, nevertheless, several were also discovered in tomatoes and water samples. Some stable plant viruses (e.g., tobamoviruses) were detected in across diverse sample types. Supplementing the information obtained from analysis of plants with analysis of water samples, provided markedly extended information about the prevalence of some newly discovered viruses in the analysed ecosystems. This multiple sample type virome study brings rare insights into the epidemiological links between plants and environmental waters, and helps to better understand possible future emergences of viral diseases in tomato and other crops.

P6.2-014

ANTIQUE OLIVES HAVE ANTIQUE VIRUSES THAT ARE STILL NEW TO US?

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Text

Olive (*Olea europaea* L.) or its wild relatives have been a part of the Mediterranean diet and trading routes since antiquity. The shallow Pupak off the island of Palagruža was on the ancient trans-Adriatic waterway and is the site of the shipwreck where a large number of olive stones and well-preserved fruits were found in two amphorae. The biological material was carbon- dated and confirmed to be 2200 years old. Total nucleic acids were extracted

from olive embryos found in some of the stones after opening, and DNase treated. Preamplification was performed for these low-biomass samples prior to shotgun high-throughput sequencing (HTS). In-house developed bioinformatic pipeline was used for the detection of viruses. Near-complete genomic sequence of a putative alphacarmovirus was obtained from the data. Based on the comparison of sequence identities, the RNA-dependent RNA polymerase sequence of the new virus is most similar to that of Cichorium alphacarmovirus 1 (62 % identity) and honeysuckle ringspot virus (61 % identity). Phylogenetic analysis confirmed that the new virus belongs to the genus *Alphacarmovirus*, family *Tombusviridae*. RT-PCR primers were constructed and amplicons were obtained from different samples to validate the HTS data. Further tests are planned to ascertain the ancient origin of viral RNA. If confirmed, this finding represents unprecedented insight into the sanitary status of olives in antiquity and possible olive viruses over the span of two millennia.

P6.2-015

APPLICATION OF MINION SEQUENCING FOR THE DETECTION OF VIRUSES IN SEED YAM SYSTEMS

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Text

The use of virus-free planting materials is the most effective method to control the spread of yam viruses, including Yam mosaic virus, able to cause up to ~50% yield losses. Sensitive nucleic acid-based tests have been developed to detect a large range of yam viruses. Yet, there is the potential for new viruses or emerging variants to escape detection because existing methods target only known viruses and sequence diversity. This would result in the dissemination of infected materials with potential threats to crop production and food security.

High throughput sequencing (HTS) technologies can detect known and unknown viruses in any plant sample, overcoming the limitations of standard tests. This research aimed to develop an HTS workflow to detect and characterise viruses in yams (Dioscorea spp.). MinION, a portable high-throughput sequencer from Oxford Nanopore Technologies, offers a potential adoption of this technology in low-resource laboratories where other HTS platforms are impractical or too expensive. This will improve molecular diagnostic testing capability and support the production and sustainable supply of high-quality seed yams in Sub-Saharan Africa.

RNA preparation methods and MinION sequencing protocols were compared and optimised to develop a sequencing workflow adaptable for virus indexing in yam seed systems in Ghana. The study established MinION sequencing workflows that can be used to detect and accurately sequence up to full-length genomes of yam viruses.

P6.2-016

DOUBLE-STRANDED RNA: A UNIVERSAL TEMPLATE FOR VIROME (VIRUS AND VIROID) CHARACTERIZATION USING SECOND AND THIRD-GENERATION SEQUENCING TECHNOLOGIES

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Text

Viral diseases represent a major threat to food production, worldwide. Treating virus-infected plants is impractical, unlike bacterial or fungal diseases. Therefore, reducing the impacts of viruses on crop production relies on our capacity to monitor and anticipate, outbreaks. Early detection is a critical step in defining upstream mitigation strategies to facilitate viral disease management. Because viruses lack shared conserved regions for identification at the species level, virion-associated nucleic acid (VANA) and metagenomic sequencing are commonly used instead to harness the virome. VANA sequencing systematically favors DNA and enveloped RNA viruses, while metagenomic sequencing is influenced by large-genome organisms and their prevalence. We have improved double-stranded RNA (dsRNA) extraction methods by optimizing the existing cellulose-based method and developing new methods employing dsRNA-binding proteins. These methods were used to sequence dsRNA using the Illumina MiSeq and Nanopore MinION platforms. The results demonstrate that dsRNA sequencing is a powerful universal tool for the detection and genomic characterization of viruses regardless of the genome type, size or the genomic heterogeneity. In the One health context, which requires the monitoring of known and unknown viruses throughout the virosphere (plants, animals, insects and soil), dsRNA sequencing provides a unique opportunity to increase our capacity for monitoring and anticipating, viral outbreaks

P6.2-017

EVIDANSES: A QUALITY MANAGEMENT-FRIENDLY BIOINFORMATICS PIPELINE FOR VIRUS DETECTION IN PLANTS

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Text

High throughput sequencing is now used in routine for many applications, and the diagnostic of plant pathogens is not an exception. It enables the detection of poorly characterized pathogens even in co-infected samples composed of multiple organisms. However, the analyses after sequencing remain a black box for labs without bioinformatics competences. To ensure that each step is in line with quality management requirements, we chose to develop a complete analysis workflow from the wet lab, including library preparations and sequencing, to the bioinformatics.
The eVIDanses bioinformatics pipeline was written to analyze automatically raw reads from single end or paired ends sequencing, throught different stages: quality trimming, de-novo

assembly, contigs identification, mapping on reference genome, and metagenomics analysis, via Krona. The script allows the creation of a pdf report that includes the main results of the analysis and the traceability (versioning of the script, software, virus database, etc.).
This bioinformatics pipeline was tested successfully on Ion torrents Proton and Illumina sequencings. Next steps will be to include specific analyses for MinION sequencing, and to include the complete workflow in a quality management system.

P6.2-018

PATHOBIOME ANALYSES IN VEGETABLE FOOD PRODUCTS

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Text

Vegetable food products can be contaminated with human and plant pathogens from production to consumption. Such contaminations can have serious effects on plant health or human food safety. The aim of this collaborative work was to develop a method based on high throughput sequencing, using MinION sequencer, to identify all pathogens (virus, bacteria and fungi) in contaminated vegetable food products. The efficiency of the method was tested on mock DNA communities and on vegetable food products artificially contaminated with relevant human and plant pathogens. Metabarcoding analyses allowed the detection of some plant pathogen genera and of human pathogens (*Listeria monocytogenes* and *Salmonella enterica*) at very low concentration, close to infectious doses while metagenomic analyses were demanding on high quality DNA extracts. Concerning RNA and virus detection, the method was very efficient on phytovirus, but not on human virus even inoculated at high doses. The phytosanitary risk of 15 plant pathogens of tomato absent from France was also evaluated: one bacteria and four viruses showed high potential of introduction. In conclusion, this method is very interesting compared to targeted methods as it can give information on pathobiome without *a priori* and identify potential emergence, although it needs optimizations on DNA extraction for metagenomic analyses and gene selection for metabarcoding analyses.

P6.2-020

MONITORING PLANT AND SOIL HEALTH BY ASSESSING BIODIVERSITY USING HIGH-THROUGHPUT SEQUENCING

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Text
At ILVO, one of our key missions is to ensure healthy crops for a sustainable agricultural production. In this context it is important to monitor the biodiversity of the environment in the vicinity of the plants, for both harmful and beneficial (micro-)organisms. Here, we highlight some high-throughput-sequencing (HTS) applications related to biodiversity monitoring. In a first application, we study the genetic diversity within or between species by means of genotyping-by-sequencing (GBS), allowing the investigation of genetic relationships between individuals or pools of individuals using thousands of genetic markers. In a second application, we study the taxonomic composition of communities using metabarcoding. This can be done to monitor soil health, for example by investigating the microbial community composition (fungi and/or bacteria) and bio-indicator organisms such as nematodes, or to detect plant pests and their natural enemies in pitfall traps or on sticky plates. In a third application, we use an untargeted approach (RNA-seq) to study biodiversity by taxonomically classifying all sequences present in a plant, insect or environmental sample. This can be used to find the biological cause of certain disease symptoms and is particularly interesting for large-scale virus and viroid scanning. In conclusion, HTS-based methods have greatly expanded our toolbox to investigate the biodiversity of the plant's environment, its microbiome, and its pathogens and pests.

P6.2-021

ASSESSING THE RISK OF VIRUSES FROM NICHE TUBER CROPS OF ANDEAN ORIGIN

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Text
Andean root and tuber crops (ARTCs) are a group of diverse crops belonging to a variety of families, that originate from the Andean. FroIn Europe, these plants often enter the country for planting via internet trade, which is may be a phytosanitary risk. The CIP Genebank has identified developing tools for phytosanitary diagnostics of ARTCs as priority area.
This study follows on from a High Throughput Sequencing (HTS) paper on (Ullucus tuberosus) plants grown in the UK. Work so far has been focused on determining the virome of ARTCs imported through internet trade and determine the potential risk of this commodity to UK plant health. This project used HTS to study the viruses present in oca (Oxalis tuberosa) plants purchased from two mainland European countries via eBay. Using an Illumina MiSeq two bulks of these plants were sequenced, uncovering six novel virus candidates from different genera. Following in silico molecular characterisation based on genome sequence, RT-PCR and qRT-PCR assays were designed to these viruses. Additionally, biological characterisation of new findings is lacking. A review of 78 papers reporting on fruit tree viruses examined the biological data included with virus discovery. Only 8% included transmission studies, a vital part of biological characterisation. This talk will discuss the virome findings so far, as well as consider challenges of modern diagnostic

biological characterisation when applied to HTS novel findings as shown by this project.

P6.2-022

RNA-FULL THE PIPELINE FOR FULL LENGTH GENOME SEQUENCING OF PLANT RNA VIRUSES

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Text

Accurate pathogen characterization in infected plants is critical for agriculture production that could prevent economic losses and spread of viral diseases. Over the recent years the Illumina sequencing platform has become an important standard in HTS of plant viruses and has led to the identification and characterization of many previously unknown viruses. In the meantime, the platform is based on the use of relatively short read sequencing (up to-300 base pair read lengths) and subsequent assembly of these reads into so-called consensus sequences that could prevent in-depth profiling of full-length virus genomes. Viruses are often present in complex populations of highly similar sequences and accurate assembly of virus genomes of several kilo-bases in length using only ~100 base pair reads is challenging. To address this constrain we have developed a protocol for full-length genome sequencing of RNA viruses (RNA-full) that is based on recently emerged MinION technology. Furthermore, RNA-full was used for identification and accurate profiling of dsRNA viral intermediates that are produced during virus replication.

P6.2-023

REVISITING HIGH THROUGHPUT SEQUENCING DATA USED FOR PLANT VIRUS DETECTION IN ORDER TO FIND EVIDENCE OF NON-VIRAL PLANT PATHOGENS AND PESTS

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Text

High-throughput sequencing (HTS), more specifically RNA-seq of plant tissue, has become an indispensable tool for plant virologists to detect and identify plant viruses. During the data analysis step, plant virologists typically compare the obtained sequences to reference virus databases only, which lead to our hypothesis that they might be missing possible traces of other pathogens in the data. In this study, we set up a community effort to re-analyze existing RNA-seq datasets used for virus detection to check for the potential presence of non-viral pathogens or pests. In total 101 datasets from 15 participants derived from 51 different plant species were re-analyzed, of which 37 were selected for subsequent in-depth analyses. In 29 of the 37 selected samples (78%), we found convincing traces of non-viral plant pathogens or pests (>100 reads per million). The most observed organism categories were fungi (15/37 datasets), insects (13/37) and mites (9/37). Nematodes were not observed and only a few samples showed the presence of plant pathogenic phytoplasmas (1/37), bacteria (3/37) and oomycetes (4/37). In conclusion, we were able to show that it is possible to detect non-viral pathogens or pests in these metatranscriptomics datasets, in this case primarily fungi, insects and mites. With this study, we hope to raise awareness among plant virologists that their data might be useful for fellow plant pathologists in other disciplines (bacteriology, mycology, entomology) as well.

P6.2-024

PLANT VIRUSES DIAGNOSTIC FROM HIGH-THROUGHPUT SEQUENCING (HTS) DATA USING VIROSCOPE: A FIELD-SCALE PILOT STUDY TO IMPLEMENT HTS FOR FAST-TRACK QUARANTINES.

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Text

Plant trade and food consumer demand has greatly increased the global movement of plants. Current post-entry quarantines programmes facilitate testing the imported plants for virus pathogens but are highly extensive, cost demanding and viruses diagnostics are subject to conventional PCR bias. Although the HTS-based virus diagnostics is considered as the gold standard in molecular diagnostics, challenges related to both the data analysis and at the regulatory level implementation are still discussed.
In this work, we performed a robust study (n=200) using the Viroscope.io web-service to provide viral diagnosis from HTS data for phytosanitary purposes of fast-track quarantines. The leaves samples of *Prunus sp.*, *Malus sp.*, *Fragaria sp.* and *Citrus sp.* were provided by the SAG (Servicio Agrícola Ganadero), and sequenced using Illumina®. The samples were processed *in-house* (total RNA extraction; ribodepletion RNA; library construction). Simultaneously, the samples were processed at the SAG laboratory for virus detection by using its own RT-PCR protocols.
Here we report the side-by-side comparison with conventional techniques currently used for phytosanitary post-entry quarantines. Further, functional annotation provided by Viroscope provides enhanced biological insight for diagnostic certainty in cases of low abundance.

P6.2-025

CHARACTERIZATION OF THE SOIL, RHIZOSPHERE AND ROOT MICROBIOME ASSOCIATED TO KIWIFRUIT VINE DECLINE SYNDROME IN ITALY

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Text
Kiwifruit vine decline syndrome (KVDS) has been threatening kiwifruit cultivation in Italy for the last decade. Symptoms lead to severe root decay and plants usually die within a few weeks. Aboveground symptoms include leaf curl, necrosis, and twig wilting, which appear long after root impairment. KVDS is commonly identified as a multifactorial disease, caused both by abiotic and biotic factors. The copresence of different stresses impacts plant growth and yield, leading to rapid decline. Pathogens associated to disease development are soilborne oomycetes. This work focused on defining the microbial communities characterizing soil, rhizosphere, and root population present both in affected and healthy fields in North-West Italy, by analyzing the whole pathobiome, as bacteria, fungi, and oomycetes, through metabarcoding. Bacteria and fungi showed a wide diversity, even when grouped by sampling location. Focusing on oomycetes, Phytopythium spp. was the main genus across all infected matrices. Phytopythium spp. was present in significantly higher abundances in diseased orchards, compared to healthy ones, showing a statistically significant correlation with the occurrence of the syndrome. From the same sites, isolation was performed through the years revealing the presence of Phytopythium spp. mainly associated to symptomatic roots. Bacteria, fungi, and oomycetes were considered together for the first time, reinforcing the role of Phytopythium spp. in KVDS.

P6.2-026

VIRTAB: ASSESSING THE RISK OF WHITEFLY-TRANSMITTED VIRUSES FOR BELGIAN CROPS

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Text
The whitefly *Bemisia tabaci* is an important crop pest and causes over a billion euro of damage per year worldwide. It is well-known to transmit plant viruses and more than a hundred plant virus species are known to be vectored by *B. tabaci*. In the EU, 'non-European' populations of *B. tabaci* - those found in plant commodities imported from non-European countries - are considered as quarantine pests (EU directive 2019/2072). According to EPPO, *B. tabaci* is present in Belgium but its current distribution is unknown. In addition, it is unknown whether the Belgian and non-European populations carry whitefly-transmitted viruses that could pose a phytosanitary risk to Belgian greenhouse crops. In 2022, the Flanders Research Institute for Agriculture, Fisheries and Food and the Vegetable Research Centre joined forces in a FPS Public Health funded project, called VIRTAB, to fill this knowledge gap. During the second half of 2022, more than 20 non-European *B. tabaci* populations were intercepted at ports of entry, while two Belgian *B. tabaci* populations could be collected from local greenhouses. Both classical molecular techniques and high-throughput sequencing were used to identify and characterize the whitefly itself, as well as the viral load they carry. The identification of these viruses will allow to assess the potential impact of *B. tabaci* on Belgian greenhouse crops and the possible measures that have to be taken.

P6.2-027

TRACKING BUNT AND SNOW MOLD PATHOGENS IN SWITZERLAND USING SOIL MONITORING

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Text
Long-term environmental monitoring projects are essential to understanding human-driven effects on ecosystems and detecting threats such as pollution, erosion, and biodiversity loss. High throughput sequencing (HTS) has revolutionized biological soil monitoring, enabling expansive data collection on soil biodiversity, including pathogenic fungi. In this study, we tracked the occurrence of seed-borne cereal pathogens that can also spread through contaminated soils using the Swiss Soil Monitoring Network. The network's sampling for microbial biodiversity was conducted in 30 sites composed of an equal number of forest, grass- and arable land sites during five consecutive years. We focused on the taxa of snow mold (*Microdochium nivale* and *M. majus*) and bunt pathogens (*Tilletia* spp.). These fungi are regularly evaluated in cereal seed health testing, but their presence in soil can contribute to disease occurrence. The *Microdochium* spp. were found in all sites, though most abundant in grass- and arable lands. Reads matching *Tilletia controversa*/*caries* were limited to 2 arable land sites, while other *Tilletia* spp. were found at 13 grass- and arable land sites. The pathogens' distributions may have implications for disease avoidance, and future work should compare fluctuations of pathogen abundance in the soil with disease occurrence.

These pathogens serve as a case study for adapting biological soil monitoring networks to survey plant diseases.

P6.2-028

SURVEILLANCE OF CROP-ASSOCIATED MICROBES USING HIGH-THROUGHPUT SEQUENCING OF ENVIRONMENTAL AND SEED SAMPLES

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Text

Phytopathogens & Invasive Alien Species pose an increased risk to plant health & food security, threaten biodiversity and exacerbate phytosanitary issues. Their effects are amplified by climate change led by more permissive conditions for their establishment in previously inhospitable areas. More restrictive international trade regulations are among the repercussions of the establishment of adventive species. High-Throughput Sequencing is a powerful approach to pre-screen phytopathogens and locate hotspots. We use metabarcoding (fungi, oomycetes) & Whole-Genome Shotgun sequencing (WGS; bacteria) to develop surveillance tools, increase plant health and enhance agriculture sustainability (beneficial endophytes). First, preliminary results from a metabarcoding workflow for analyzing spore and suction trap samples collected biweekly will be introduced (abundance, diversity, incidence & distribution forecasting models). Then, results from the metabarcoding of endophytic community in barley seeds will be shared. Next, results obtained by WGS bacterial profiling of canola seed washes will be shown. Taxa detected by culture-independent methods were compared with culture-dependent communities. This study could provide a baseline of Canadian microbes in crops/seed washes which may be instrumental in international plant trade disputes and crop improvement (endophytes). Combined results will help understand pest incidence in space & time and improve risk readiness & response measures.

P6.2-029

TILED AMPLICON PCR AS A VIROLOGY DIAGNOSTIC TOOL FOR POST-ENTRY QUARANTINE TESTING IN NEW ZEALAND

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Text

Post-entry quarantine is one of the most valuable tools for protecting New Zealand from

unwanted plant diseases. High-throughput sequencing technologies allows the testing of imported plant germplasm for pathogens present in a sample with high sensitivity comparable to Polymerase Chain Reaction (PCR) based diagnostics. It also offers strain-specific detection not offered by PCR-based assays. Tiled Amplicon PCR (TA-PCR) has been tested as an enrichment technique to amplify partial or complete genomes of viruses present in low titres. We developed a series of primer panels targeting three viruses of Fragaria: strawberry chlorotic fleck-associated virus (SCFaV), strawberry mottle virus (SMoV) and strawberry vein banding virus (SVBV). These represent two positive single stranded RNA viruses (SCFaV and SMoV) with different replication strategies, and a double stranded DNA virus (SVBV). The primer panels for each virus can be combined as a multiplex assay to detect all three viruses from samples with low viral titres around the limit of detection for quantitative PCR. Illumina and Oxford Nanopore platforms were successfully used for sequencing of the tile amplicons, covering up to 100% of the reference genomes for each virus. TA-PCR allows for targeted strain-specific regulation of unwanted organisms. The limit of detection of TA-PCR is comparable to that of quantitative PCR assays, the current gold standard for diagnostic of plant pathogenic viruses.

P6.2-030

COMPARING SHORT- AND LONG-READ HIGH-THROUGHPUT SEQUENCING METHODS WITH TRADITIONAL PLATING FOR THE CHARACTERIZATION OF TREE SEED MYCOBIOMES

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Text

Tree-associated mycobiomes have traditionally been analyzed by isolating fungi on agar media from plant host material. A shortcoming of this approach is that not all fungal species, in particular obligate biotrophs, can be grown in culture. Nowadays, fungal microbiome analyses are increasingly conducted with high-throughput amplicon sequencing using Illumina platforms, which allows the identification of fungal species without culturing them. However, with the short-read sequencing using Illumina platforms, usually only a small fraction of reads can be assigned to fungal species. A better assignment might be obtained with long-read sequencing using Oxford Nanopore Technologies. However, few studies exist in which the same biological samples were compared using these three different approaches. Here, we compared the diversity and community composition of tree seed mycobiomes captured by traditional culturing, with diversity and composition captured by both Illumina and Nanopore amplicon sequencing. Seeds from 13 tree species native to Europe, North America, or Asia were analyzed. Preliminary results show that a main proportion of the dominant fungal taxa obtained by Illumina sequencing comprised of culturable fungal species. Further analyses will reveal potential differences across methods, and determine the most appropriate method for identifying potentially pathogenic fungal species in tree seeds, which will improve the safety of tree seed movement.

P6.2-031

FAST HIGH-RESOLUTION PLANT PATHOGEN IDENTIFICATION THROUGH MULTIPLEXED PCR AND HTS

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Text

The Ministry for Primary Industries' Plant Health and Environment Laboratory is responsible for the identification of all suspected exotic, new, and emerging pests and pathogens affecting plants and the environment in New Zealand.

Current identification of bacteria and fungi isolated from diagnostic samples is performed by a 2-round Sanger sequencing workflow: First, a general phylogenetic marker is sequenced to allow identification to the genus level. Typically, these general markers do not provide enough resolution to determine species but allow to select taxon-specific markers for the second round of sequencing to generate data for species level identification. This sequential identification process is time consuming and challenging when results are urgently required.

To facilitate faster sample processing, we are developing multiplex PCR assays and HTS workflows to enable rapid identification of fungal and bacterial plant pathogens from cultured isolates in a single HTS step. We developed two sets of degenerate primers which amplify diagnostically informative genes across a broad range of bacterial and fungal genera. We combined these into a single multiplexed reaction for bacteria or fungi, amplifying up to six taxonomically relevant markers. To enable flexibility, sequencing workflows were developed for both Illumina and ONT platforms. This new workflow will reduce turnaround time, costs, and provide greater taxonomic resolution for isolate identification.

P6.2-032

ASSESSMENT OF ONT SEQUENCING (MINION) FOR PLANT VIRUS DETECTION AND COMPARISON WITH ILLUMINA-BASED SEQUENCING

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Text

General plant health can be improved by the use of virus-tested starting material for vegetative propagation. HTS-based untargeted detection replacing specific molecular methods, or even biological indexing, is extremely promising for this purpose. Illumina sequencing has already proven to be a reliable and sensitive method for virus detection, yet

also the ONT MinION has gained interest as a promising tool over the last years. ONT sequencing generates long reads, can be performed in every lab and decreases the costs. For MinION sequencing a protocol based on Liefting et al. (2021) and similar to our standardized Illumina sequencing protocol, was used. In short, total RNA extraction was followed by rRNA depletion and random primed double stranded cDNA synthesis. Barcodes and adapters were added, samples were pooled and sequenced for 72 hours on a MinION Flow Cell. Reads were classified by comparing against the Genbank non-redundant Nucleotide database with Kraken2 and mapped to virus reference genomes with Minimap2. Five plant samples, previously screened for viruses with Illumina sequencing, were selected for this study. Overall, most of the viruses could be detected and were identified correctly with this MinION sequencing protocol. Viruses detected below 50 rpm with Illumina sequencing could only be detected with poor genome coverage or failed to be detected. It can be concluded that correct virus detection and identification is possible but sensitivity is still lower.

P6.2-033

MULTILOCUS GENES AND WHOLE GENOME SEQUENCING TO IDENTIFY AGROBACTERIUM ARSENIJEVICII, CAUSAL AGENT OF CROWN GALL DISEASE IN RASPBERRY PLANTS

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Text

Raspberry is a widely cultivated fruit species with increasing demand in national and international markets. However, its production can be severely impacted by crown gall disease, which is commonly attributed to the soil-borne bacterium Agrobacterium radiobacter, causing stunted growth and reduced yields. It spreads through infected nursery propagative material and is maintained by the use of contaminated soil. However, the symptoms are not always indicative of A. radiobacter, making it challenging to identify the bacterium responsible for the disease. In this study, multilocus genes atpD, gyrB, glnA, rpoB, and recA were used to identify the actual causal agent of crown gall disease in raspberry plants from plantations in the states of Michoacán and Jalisco, Mexico. The results indicated A. arsenijevicii as the causal agent. Furthermore, the whole genome sequencing of sample CPO J19 revealed a genome size of approximately 5.7 Mb, with a G+C content of 58.45 %. The assembly produced 46 contigs, with N50 of 305 kb. Annotation analysis identified a total of 5578 protein-coding genes. A comparative genomic analysis showed a high degree of similarity with the A. arsenijevicii type strain KFB 330, confirming the identity of the causal agent responsible for crown gall disease in raspberry plants. The identification of the causal agent is critical to improve disease management strategies. The use of certified nursery propagative material can help prevent the spread of the disease.

Immune receptors: activation, signaling & evolution

C5.4-1

CONNECTING THE DOTS OF PRR-MEDIATED IMMUNE SIGNALING

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Text
Plant immunity relies on both cell-surface and intracellular immune receptors. Recent studies showed that signaling triggered upon activation of these receptors is more connected than previously thought, and that both types of immune receptors can induce congruent cellular immune signaling outputs. Yet, the exact molecular mechanisms and components involved in the generation and regulation of such outputs are not fully understood. I will present our recent efforts to gain a better understanding of the molecular basis of early immune signaling, particularly that mediated by cell-surface receptor kinases acting as pattern-recognition receptors (PRRs).

C5.4-3

ENZYMATIC ACTIVITIES OF TIR DOMAINS: MOLECULAR BASIS AND IMMUNE SIGNALLING IN PLANTS AND BEYOND

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Text
TIR (Toll, interleukin- receptor, resistance protein) domains feature in a subset of plant NLRs (nucleotide-binding – leucine-rich repeat receptors - TNLs) that respond to plant pathogen effectors and trigger effector-triggered immunity (ETI). Following the demonstration that the TIR domains from the human protein SARM1 have enzymatic activity - cleavage of NAD+ (nicotinamide dinucleotide), such activity was also demonstrated for a number of plant TIR domains (1). We have studied the molecular and structural bases of these enzyme reactions and the role of the corresponding enzymatic products in signalling. In particular, we have defined the chemical structures of two cyclic ADP ribose isomers produced by NAD+ hydrolysis, 2′cADPR and 3′cADPR, which are cyclized by O-glycosidic bond formation between the ribose moieties (2). While 2′cADPR appears to be associated with ETI signalling, 3′cADPR appears to be associated with suppression of plant immunity. Our work

has provided new information about how TIR domains work, how they produce signalling molecules and how these products signal, but much future work is needed to fill the gaps.
(1) Horsefield et al (2019) Science 365, 793
(2) Manik et al (2022) Science, eadc8969

C5.4-4

THE MLA IMMUNE RECEPTOR AGAINST BARLEY POWDERY MILDEW MIMICS THE BINDING INTERFACE OF EFFECTOR TARGET TO CONFER RESISTANCE AGAINST THE BLAST FUNGUS

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Text
The plant immune system heavily relies on intracellular NLR immune receptors, which recognise pathogen secreted effectors to trigger a robust immune response. In barley, resistance to powdery mildew conferred by *Mildew locus a* (*Mla*) is mediated by an NLR that exists as an allelic series wherein each allele recognises its cognate sequence-unrelated mildew effector. Recent findings have shown that different *Mla* alleles confer resistance to divergent fungal pathogens in addition to barley powdery mildew. We established that the *Mla3* allele confers resistance to *Magnaporthe oryzae* by recognising *PWL2*, an effector that conditions host-specificity towards weeping lovegrass. We aimed to dissect the molecular basis of Pwl2 recognition by Mla3. We established that Pwl2 associates with Mla3 in planta, and that the last 83 amino acids of Mla3 are sufficient for this interaction to occur. Association in yeast-two-hybrid and co-purification from *E. coli* of Pwl2 with this C-terminal region of Mla3 strongly suggest that Mla3 directly recognises Pwl2. Pwl2 targets an HMA-containing plant protein as virulence target. The predicted structure of Pwl2 bound to the C-terminal region of Mla3 shows that the binding interface with Mla3 resembles the actual interface of Pwl2 bound to the HMA target. This prediction was validated by mutagenesis experiments, suggesting that a singleton NLR recognising multiple pathogens evolved to mimic the binding interface of an effector target to confer resistance.

C5.4-5

HIGHLY VARIABLE PLANT IMMUNE RECEPTORS ARE WIDESPREAD IN PLANTS AND SHARE DISTINCT GENOMIC AND EPIGENOMIC FEATURES

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Text

Plants rely on population level diversity of the innate immune receptors to recognize rapidly evolving pathogen-derived molecules. The Nucleotide Binding Leucine Rich Repeat-containing plant immune receptors (NLRs) can recognize pathogens through either direct binding of pathogen-derived effectors, indirect guarding of plant proteins or integration of additional protein domains that act as effector baits. Availability of high-quality plant pan-genomes allowed us to identify subsets of highly variable NLRs (hvNLRs) and to predict their ligand binding sites. hvNLRs show a strong overlap with hybrid incompatibility loci, suggesting that generation of new immune specificities comes at the cost of autoimmunity. We have now extended these analyses to pangenomes of maize, soybean as well as to other gene families, uncovering other highly variable receptors, such as Receptor like kinases and receptor like proteins, and protein families. In our current work, we investigated genomic features that differentiate highly variable NLRs from their conserved paralogs. Using whole genome bisulfite and RNAseq extracted for the same leaf in Arabidopsis, we found that highly variable NLRs are more expressed, less methylated, and closer to TEs than non-highly variable NLRs. This provides a strong foundation for understanding the diversity generation mechanisms in these rapidly evolving genes.

C5.4-6

RECEPTOR-LIKE CYTOPLASMIC KINASES FROM VARIOUS SUBFAMILIES POSITIVELY AND DIFFERENTIALLY REGULATE AVR4/CF-4-TRIGGERED REACTIVE OXYGEN SPECIES PRODUCTION IN NICOTIANA BENTHAMIANA

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Text

Cell-surface receptors form the first layer of the plant innate immune system. Receptor-like cytoplasmic kinases (RLCKs) bridge activated receptor complexes with their downstream signaling components. Arabidopsis thaliana RLCK class VII subfamilies 4, 5, 6, 7 and 8 are generally involved in immunity, among which is the accumulation of ROS. It is largely unknown what the role is of the different RLCK-VII subfamily members downstream of the tomato RLP Cf-4. Cf-4 is the receptor of Avr4, an apoplastic effector secreted by Cladosporium fulvum. Cf-4 is functional in Nicotiana benthamiana and by studies involving rlck knockouts in N. benthamiana, we show that RLCK-VII subfamilies 6, 7 and 8 are required for the Avr4/Cf-4-triggered ROS production. Typically, the Avr4 protein triggers a biphasic ROS burst in the leaves of N. benthamiana:Cf-4. Interestingly, the first ROS burst is strongly reduced, whereas the second ROS burst is completely abolished in rlck-vii-6

knockouts. In addition, ROS production is overall strongly compromised in rlck-vii-7 knockouts. Furthermore, both the first and second phases of the Avr4/Cf-4-triggered ROS burst are highly impaired in rlck-vii-8 knockouts, with the first burst also being delayed. These observations indicate that there are different ROS regulatory mechanisms in N. benthamiana. Further studies show that RLCK-VII-6, 7 and 8 are also essential for ROS production triggered by flg22, chitin, nlp20/RLP23, and pg13/RLP42 in N. benthamiana.

F5.5-1

THE BASAL EXPRESSION OF IMMUNE RECEPTORS DEPENDS ON SALICYLIC ACID LEVELS REGULATED BY THE DMR6 FAMILY OF HYDROXYLASES

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Text
Immune receptors alarm the host plant about the presence of invading pathogens. Despite advances in understanding how receptor proteins are activated by non-self molecules, little is known about the regulation of their expression at the transcript level. Broad-spectrum disease resistance in Arabidopsis and crops can be conferred by mutations in the susceptibility genes *DOWNY MILDEW RESISTANT 6* (*DMR6*) and its close paralog *DMR6-LIKE OXIDOREDUCTASE 1* (*DLO1*). The DMR6 and DLO1 proteins are salicylic acid (SA) hydroxylases that act as negative regulators of immunity. RNAseq profiling of the transcriptomes of Arabidopsis *DMR6*/*DLO1*mutant and overexpression lines revealed a role of basal SA levels in the regulation of the expression of immune receptor genes. These *DMR6*/*DLO1*-affected and SA-dependent genes belong to specific groups of surface and intracellular immune receptors. We will present further experimental evidence for the importance of the expression control by SA through *DMR6*/*DLO1* for early immune signaling. We propose that immunity in the *dmr6* and *dlo1* mutants involves enhanced basal expression of immune receptor genes that boosts the plant recognition potential.

Immune receptors: activation, signaling & evolution

C5.4-2

A TREE-SPECIFIC FAMILY OF DEFENSE PEPTIDES SHOWS ANTI-RUST FUNGI AND ELICITOR ACTIVITIES

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Text

Plant secreted signaling peptides known as phytocytokines regulate immunity via cell-surface receptors. Recently, a handful of phytocytokines have been shown to display also antimicrobial activities against pathogens, suggesting that such peptides may function as multifunctional molecules reminiscent to host-defense peptides in animals. RISPs (RUST INDUCED SECRETED PEPTIDEs) constitute a family of small secreted cationic peptides from poplar trees. *RISP* genes systematically cluster with receptor-like protein (RLP)-encoding genes in poplar genomes, that we termed RISP-ASSOCIATED RECEPTOR-LIKE PROTEINs (RALRs). We show that RISPs display anti-fungal activity towards Pucciniales (rust fungi) *in vitro* and *in planta*. In addition, RISPs trigger stomata closure in poplar. Notably, while the Solanaceae plant species *Nicotiana benthamiana* is insensitive to RISPs, expression of RALRs is sufficient to confer RISP-responsiveness, suggesting RALRs are RISP receptors in poplar. We will also discuss the convergent evolution of eukaryotic defense peptides as multifunctional molecules.

P5.5-001

ARABIDOPSIS THALIANA CELL SURFACE RECEPTOR SIGNALLING FOR RECOGNITION OF ELICITORS OF FUSARIUM SPP.

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Text

Fusarium spp. cause severe economic damage in many species of cultivated plants exemplified by Fusarium Head Blight or Panama Disease. Microbe-associated molecular patterns (MAMPs) can be perceived by plants supporting disease resistance via the activation of pattern-triggered immunity (PTI). However, knowledge of MAMPs or corresponding plant immunity components is largely lacking for Fusarium spp.. We identified a new peptide elicitor fraction present in Fusarium and related fungal species, which elicits PTI responses in monocots and dicots. We then mapped the causal mutation in an elicitor-insensitive Arabidopsis mutant (fere1) to a leucine-rich receptor-like kinase (MIK2). PTI loss-of-function in fere1 was fully complemented with the full-length FERE1/MIK2 protein. The strength of the phenotype in fere1 and independent mik2 mutants supports that MIK2 is a new key component in sensing Fusarium. MIK2 also contributes towards basal resistance to Fusarium wilt. We now widened MIK2 functions to the perception of comparable elicitors from a broader spectrum of fungal species. Genetic interaction of MIK2 with PTI signalling components, new data on elicitor-interaction and biochemistry further establish MIK2 as a potential pattern-recognition receptor. Because MIK2 was also described as a receptor for endogenous SCOOP peptides that act as phytocytokines, MIK2 may represent an integrator of endogenous and exogenous danger peptides for an optimal immune response under fungal attack.

P5.5-002

FIND THE NEEDLE IN THE HAYSTACK – IDENTIFICATION OF THE IMMUNE RECEPTORS REQUIRED FOR THE RECOGNITION OF RALSTONIA SOLANACEARUM EFFECTORS IN NICOTIANA BENTHAMIANA

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Text

Harnessing plant genetic resistance to disease contributes to sustainable improvement of crop production. Plant defense responses to pathogens are initiated by two large families of immune receptors that function in an integrated network. Immune receptors directly or indirectly monitor the presence of specific pathogen-derived molecules. Genomic approaches have revealed the extent of the nucleotide-binding leucine-rich repeat receptors (NLRs) repertoires in diverse plant species but the identification of receptor genes involved in recognition of the devastating bacterial wilt disease agent, *Ralstonia pseudosolanacearum* (*Rps*), remains a challenging task. Here, we have characterized several type III secreted effectors present in Korean *Rps* isolates that trigger robust defense responses in the model Solanaceae *Nicotiana benthamiana*. Using the combinatorial silencing of ~ 300 NLRs genes in *N. benthamiana*, we were able to rapidly identify and clone NLRs activating effector-triggered immunity. Further genetic and biochemical characterization of these receptors are ongoing. Altogether, our findings expand the pool of resource to improve bacterial wilt disease resistance in Solanaceae crops.

P5.5-003

ESTABLISHING A HAPLOTYPE-RESOLVED NLROME FOR THE CAVENDISH BANANA

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Text

Musa acuminata cv. Cavendish is the most important export banana cultivar worldwide. It is, however, susceptible to a variety of diseases, which can lead to losses in yield and fruit quality. Although resistant banana germplasm has been identified for some important diseases, little is known about the molecular basis underlying that resistance. The most predominant type of resistance (R) genes contain nucleotide binding site and leucine rich repeat (NBS-LRR) domains. The identification and characterisation of R genes in Cavendish bananas would enhance the knowledge needed for its genetic improvement and possibly uncover the basis of its resistance to Fusarium oxysporum f.sp. cubense (Foc) race 1. We have generated a high coverage genome sequence of Cavendish and this is a valuable resource for analysing the genomic organisation of R genes in this important cultivar group. In this study, we identified 361 NLR genes in the Cavendish genome and characterised their

protein motifs, gene structures and phylogenetic relationships. Our study establishes a haplotype-resolved NLRome for Cavendish banana which is an important resource for the identification of functional R genes against various pathogens. This is a stepping-stone for improving this cultivar and may aid the development of gene-edited Cavendish resistant to Foc Tropical race 4 in the future.

P5.5-004

HIGH-THROUGHPUT ANALYSIS OF RPI GENES IN POTATO CULTIVARS, BREEDING LINES AND WILD SOLANUM SPECIES

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Text
Phytophthora infestans causes late blight, a highly destructive potato disease which has challenged global agriculture for centuries. Genes involved in resistance to *P. infestans* (*Rpi* genes) have been discovered in wild potato species (*Solanum* spp.), and some of these *Rpi* genes have been introduced into potato cultivars. However, we do not know which *Rpi* genes are present in the many different potato genotypes. The goal of our work is to investigate the incidence of 11 *Rpi* genes and to analyse their diversity in potato cultivars grown in Poland and Norway using an Amplicon Sequencing (AmpSeq) approach. The 183 potato cultivars were selected based on resistance to late blight and acreage of cultivation. In addition, 98 breeding lines and 54 genotypes of wild potato species were included.
Plant material was initially screened for the presence of *Rpi* genes using PCR targeting short fragments of *Rpi* genes (1-3 primer pairs per gene). Subsequently, the entire coding sequences of the genes in 316 of 335 potato genotypes were amplified. So far, 243 amplicons of *R1*, *R2*, *R3a*, *R3b*, *Rpi-phu1*, *Rpi-blb1* and *Rpi-ber1* were sequenced using PacBio high fidelity long read technology. The first batch of *Rpi* gene sequencing resulted in more than 4 million reads with an average length of 4,086 bp. The AmpSeq strategy proved to be reliable and efficient, and will allow us to obtain data on the nucleotide diversity of genes crucial for the potato defence against *P. infestans*.

P5.5-005

THE I RESISTANCE GENE AGAINST BCMV AND BCMNV IN COMMON BEAN: IDENTIFICATION OF THE MOLECULAR BASIS THROUGH TWO INDEPENDENT MUTANTS

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Text

Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) cause severe economic losses in common bean (Phaseolus vulgaris), which is one of the most important grain legumes for human consumption worldwide. The dominant resistance gene *I* confers hypersensitive-type resistance to BCMV and BCMNV and has been introduced by breeders in many bean cultivars. The *I* gene is located at a complex multiparasitic cluster with dramatic structural variation in the number of TNL genes, ranging from one to 34, depending on the genotypes. Because of its agronomic importance, many teams have tried unsuccessfully to clone the *I* gene, due to suppression of recombination and/or repeated sequences at this resistance cluster. Our team identified the TNL gene encoding *I* through two independent BAT93 mutants: a tilling mutant and a natural mutant, linked to the insertion of a transposable element (TE). The *I* gene encodes a TNL presenting a C-JID domain in C-terminal. The TE belongs to the LTR/Gypsy/Retand family and is inserted in the *I* gene near repetitive and palindromic sequences. This TE is not present in the same TNL gene in other BAT93 genomic resources and LTR of the TE are 100% identical. This suggests a recent TE insertion event in certain BAT93 seed batches in our laboratory. Since another spontaneous mutant of the *I* gene has been reported in the literature, we suspect that the *I* gene could be a hot spot of mutation.

P5.5-006

RESISTANCE TO FUSARIUM WILT IN HEIRLOOM CULTIVAR “EARLIGLOW” IS CONFERRED BY UNLINKED RESISTANCE GENES ON CHROMOSOME 2B IN STRAWBERRY

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Text

Fusarium wilt, a soil-borne disease caused by the fungal pathogen *Fusarium oxysporum* f. sp. *fragariae* (Fof), has become an increasingly important cause of strawberry plant death and yield losses in many other parts of the world. The pathogen colonizes vascular tissue, causing wilt, collapse, and death in susceptible accessions. Several sources of resistance to Fof have been identified through genome-wide association studies of genetically diverse cultivars and ecotypes. However, no resistance gene has been characterized in strawberry. To date, five resistance loci are known to confer innate immunity to Fof and are hypothesized to encode well-known resistance (*R*) proteins. The cultivar “Earliglow” was previously shown

to be resistant to California race 1 and Japanese race 2 isolates of the pathogen. An Earliglow F2 population was screened for resistance to a race 1 isolate, and a 15 resistant to 1 susceptible phenotypic segregation ratio was observed. QTL-mapping revealed that “Earliglow” harbors a dominant *R*-locus (*FW6*) in a near-telomeric cluster of *R*-loci on chromosome 2B and a second dominant *R*-locus (*FW7*) approximately 20 Mb downstream of *FW6*. The proteins encoded by *FW6* and *FW7* are currently unknown; thus, we plan to utilize HiFi long-read sequencing and transcriptomics to identify candidate genes and uncover the *FW6* and *FW7* genes. These epistatically interacting *R*-genes expand the arsenal of *R*-genes available for developing strawberry cultivars resistant to race 1.

P5.5-007

PRESENCE OF HOMOLOGUES OF THE PVY RESISTANCE GENE RYSTO IN WILD RELATIVES OF POTATO

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Text

Potato virus Y (PVY) is among the top ten economically important plant viruses. It causes potato tuber necrotic ringspot disease, leading to decreased yield and tuber quality. Wild relatives of potato are valuable sources of genes involved in resistance to many pathogens that attack potatoes, including PVY. The gene Ry_{sto} derived from *Solanum stoloniferum* confers extreme resistance to PVY. The aim of this work is to screen Ry_{sto} homologues and to analyze their diversity in wild relatives of potato (298 genotypes representing 29 accessions of 26 wild potato species, IHAR-PIB’s collection) using PacBio Circular Consensus Sequencing (CCS) technology. Using PCR primers targeting part of the Ry_{sto} gene, fragments of the Ry_{sto} homologues were detected in 102 out of the 298 wild potato genotypes. Subsequently, the full coding sequences of the Ry_{sto} gene from 88 amplicons representing 72 genotypes (12 accessions, 10 wild potato species) were obtained by CCS. More than 1.7 M HiFi reads with an average length of 4741 bp were generated, representing 56 unique amplicon sequence variants (ASVs) and 41 different protein sequences with 76.4 – 99.9 % (54 ASVs) and 100% (2 ASVs) identity to the reference Ry_{sto} protein (QEL52752.1). Among them, 17 ASVs were detected in PVY-resistant wild potato genotypes, 20 in susceptible ones and 5 in both. Knowledge on PVY resistance and resistance gene contents in analyzed accessions will enable their exploitation in potato breeding programs.

P5.5-008

THE SOYBEAN (GLYCINE MAX) LYSM RECEPTOR KINASES GMNFR5A AND GMCERK1 MEDIATE CHITOOLIGOSACCHARIDES-TRIGGERED IMMUNITY

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Text
Chitin is a major component of fungal cell walls and serves as a molecular pattern for the recognition of potential pathogens in the innate immune systems of plants. Previous research suggested that chitin has different immune signaling pathways in Arabidopsis and rice, including extracellular receptor recognition and intracellular signal transduction. The mechanism of induced resistance of chitin oligosaccharide (COSNAC) and its deacetylated product chitosan oligosaccharide (COS), collectively referred to as chitooligosaccharides, is not clear in soybean. Herein, we report that chitooligosaccharides trigger immune responses and plant disease resistance in soybean. GmNRF5a and GmCERK1 are required for chitooligosaccharides recognition in soybean. Unexpectedly, COSNAC is directly recognized by GmNFR5a and GmCERK1, whereas COS only binds GmNFR5a. In addition, we confirmed that GmCERK1 and GmRLCK5 transduce intracellular signals of chitooligosaccharides through proteins interaction and phosphorylation. Taken together, our results suggest GmNFR5a and GmCERK1 play a key role in the perception of chitooligosaccharides elicitors, and the existence of a complete phospho-signaling transduction pathway from GmNFR5a and GmCERK1 mediated chitooligosaccharides recognition to GmRLCK5 activation in soybean.

P5.5-009

A NOVEL SOYBEAN APOPLASTIC PROTEIN TRIGGERS RESISTANCE TO PHAKOPSORA PACHYRHIZI

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Text
Soybean (*Glycine max*) is a worldwide economic oilseed crop and could be infected by various pathogens at different growth stages. Soybean rust caused by obligate, biotrophic fungus *Phakopsora pachyrhizi* leads to severe yield losses and raises the strategy to protect soybean production via plant immunity. Plants have a two-tier immune system mediated by plasma membrane-localized pattern recognition receptors and intracellular receptors, which recognize apoplastic effectors as well as damage-associated molecular patterns (DAMPs) and intracellular effectors, respectively. However, DAMPs and their cognate receptors in soybean are barely known. This study aims to identify *P. pachyrhizi*-triggered soybean DAMPs (PTSDs). With the combined strategy of mass spectrometry, RNA-seq, bioinformatic prediction and *Agrobacterium*-mediated transformation in *Nicotiana benthamiana*, 2 out of 7 candidates were able to trigger hypersensitive response and PTSD1 was further analyzed. Infection of *P. pachyrhizi* triggers PTSD1 expression and plant immunity activation enhances PTSD1 protein abundance in the apoplast. In addition, PTSD1 activates soybean immune responses and triggers resistance to *P. pachyrhizi*. Moreover, PTSD1 could also be recognized by *Solanum* species and relies on yet unknown leucine-rich repeat receptor-like proteins to trigger immune responses. Collectively, this study identifies that PTSD1 acts as a DAMP and triggers immunity against *P. pachyrhizi*.

P5.5-010

BIRTH, DEATH, AND PERSISTENCE IN NLR DIVERSITY IN THE ARABIDOPSIS IMMUNE SYSTEM

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Text

Plant pathogens have a major impact in both natural and agricultural ecosystems, inducing widespread disease, reduced fitness, and mortality. Genes encoding nucleotide-binding leucine-rich repeat (NLR) proteins are the major class of disease resistance (R) genes in plants, and encode receptors that directly or indirectly detect the molecular signals of pathogens and activate defense response. NLR genes are among the most variable in plant genomes, exhibiting tremendous diversity in sequence and structure. This structural diversity makes NLRs difficult to study but with long read sequencing we can directly sequence complex gene clusters. Here, we assembled the genomes of 18 diverse lines of Arabidopsis thaliana using the PacBio HIFI sequencing technology. We performed comprehensive genome annotation integrating full-length transcript data generated with Iso-Seq, pan-TEome (transposable elements) annotation, CG-methylation, segmental duplications, and recombination to investigate the processes that lead to the birth, death and maintenance of NLR diversity across the species. We found that TEs play a major role in generating structural diversity and that pseudogenization is a major force in moderating the genomic load of active NLRs. We also unravel hidden NLR diversity generated through isoform variation. Our findings give a better understanding of the different strategies used by plants to compete in the defensive arms race against pathogens.

P5.5-011

ATLAS OF TANDEM KINASE PROTEINS ACROSS THE PLANT KINGDOM

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Text

The Tandem Kinase Protein (TKP) family, discovered by our lab, emerged as new regulators of plant immunity.

Plant pathogens can modulate plant metabolism and immune responses to their benefit by secreting effector proteins into plant cells. In turn, plants have numerous intracellular nucleotide-binding leucine-rich repeat (NLRs) receptors that are powerful components of effector-triggered immunity. Most functionally characterized plant disease resistance genes encode NLRs specific to certain pathogen races, which rapidly evolving pathogens may overcome.
The discovery of the wheat stripe rust resistance gene *Yr15* and barley stem rust resistance gene *Rpg1* encoding a rather nontypical resistance protein with a combination of two kinase domains prompted a new chapter in plant immunology. To date, numerous TKPs from cereal crops have been discovered. However, both their evolutionary history and mechanism of action remain poorly understood.
The creation of the TKPs' Atlas shall play a pivotal role in the future discoveries of TKP evolution and molecular function. Defining TKPs as protein sequences with two or more protein kinase domains, we scanned the genomes of 105 plant species and discovered TKPs across all Plant kingdoms. We found that over half of the discovered TPKs have kinase domains lacking critical catalytically important residues (thus, representing pseudokinases) likely serving as decoys for the effector recognition in TKP-related immune responses.

P5.5-012

DISSECTION OF A RAPIDLY EVOLVING WHEAT NLR RESISTANCE GENE CLUSTER BY ONT LONG-READ GENOME SEQUENCING FACILITATED THE CLONING OF PM69

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Text
Wild emmer wheat (WEW), the progenitor of common wheat, is a valuable genetic source for resistance breeding. However, gene cloning in repeat-rich polyploid genomes remains challenging. Previously, we used the "Durum as a bridge" approach for map-based cloning of WEW genes, such as the yellow rust resistance genes *Yr36*, and *Yr15*. The cloning of *Yr15* have shed light on the novel plant tandem kinase-protein family, which is a new player in plant immunity. Here we describe a new strategy for overcoming the major bottlenecks encountered during the cloning of the WEW powdery mildew (Pm) resistance gene *Pm69*. Conventional positional cloning approach encountered structural variations that suppressed recombination, while chromosome sorting was compromised by insufficient purity. A *Pm69* physical map, constructed by assembling Oxford Nanopore Technology long-read genome sequences, revealed a rapidly evolving nucleotide-binding leucine-rich repeat (NLR) gene cluster. Transcriptome sequencing of susceptible mutants revealed a candidate NLR that contains Rx_N with RanGAP interaction sites, NB-ARC, and LRR domains. The candidate

gene, located within a rapidly evolving R-gene cluster was validated by the virus-induced gene silencing approach. *Pm69* is a very rare allele found only in one location across the distribution range of WEW natural populations. We introgressed *Pm69* into common wheat lines currently available for wheat resistance breeding.

Impact of scientific advances in plant health

C4.7-1

THE TOOTHPICK PROJECT: THE IMPACTS OF A COMMERCIALIZED BIOHERBICIDE INNOVATION

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Text
The International Congress of Plant Pathology One Health venn diagram displays environmental health, human health, and animal health, all overlapping in a unified One Health strategy to promote interdisciplinary collaborations and communications. We see the United Nations codify human, environmental, and animal health through the Sustainable Development Goals, with seventeen impact goals defined with measurable indicators. Are these impact indicators linked to the One Health research strategy we are seeing and vice versa? With the opportunity to talk about the real-life impact and exactly how the Venn diagram could look, a commercialized bioherbicide development in Africa stands as a successful example of multi-layered and intersecting impact stemming from plant pathology research. Using a fungal pathogen to target a parasitic weed that reduces staple crop yield by 20-100%, the bioherbicide clearly addresses human health by preventing crop loss. In deeper evaluation and intentionality during the design and strategic development process, the innovation also addresses poverty alleviation with particular attention to women; biodiversity and nutritional diversity; access to technical training; ensuring women's participation; reducing chemical pollution; promotion of local economic growth; reduce the production of greenhouse gas emissions associated with pesticide production and use; fostering collaboration, partnerships, and innovation.

C4.7-2

THE PLANT DISEASE PYRAMID: PLANT DISEASE AND EPIDEMIC MANAGEMENT REQUIRES A HOLISTIC APPROACH

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Text

The plant disease triangle is a central tenet of plant pathology. The field has undergone transformative changes since this simple but powerful concept was originally proposed in the 1960's. Six decades of accumulated knowledge and powerful current tools now provide deep insight into plant-pathogen interactions at very fine physical and molecular scales, as well as how abiotic factors affect these interactions across time and space. This rich knowledge base is, however, also leading to changing perspectives on the disease triangle. Recently, for example, researchers have argued that the model is incomplete without a fourth dimension that consider the influence of biotic factors such as the microbiome on disease development. We argue that another dimension is needed in this model, namely the critical role that socio-economic systems play in the emergence, evolution and severity of disease and epidemic developments. The influence of all these elements of the system needs to be considered if successful interventions are to be developed in the context of interrelated local, regional and global systems. We argue that such a holistic approach is urgently needed in the context of the increasing pressure on food security and environmental health.

C4.7-3

GLOBAL CHALLENGES FACING PLANT PATHOLOGY: IMPACT OF SCIENTIFIC ADVANCES

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Text

Scientific advances continue to be made by plant pathologists on topics in plant health, environmental protection, and food security. It is important for the scientific community to evaluate the impact of these advances given the global challenges of the 21st century. Impact will depend on recognition of the multidimensional nature of these challenges and the ability to transcend discipline-based research, integrating studies from the molecular to the ecological in a systems level approach. The adoption of high throughput sequencing for diagnosis and detection will make little impact unless providing the agricultural and ecological context and combined with improved surveillance. Deployment of novel resistances to specific pathogens needs to be aligned with a greater appreciation of genetic diversity and the complementary contribution made by tolerance of plant disease. Epidemiological understanding of the temporal and spatial spread of plant diseases can be enhanced by population dynamic and genetic approaches for established, invasive, and emerging plant pathogens. Recent emphasis on holobiont research can invigorate soil microbial studies especially for disease complexes and declines. The challenge of climate change cannot be

met with single crop disease studies but requires these to be placed in the context of shifting populations of new crops, wild plants, and soil microbes. Advances in informetric analysis illustrate the global impacts of plant disease introductions.

C4.7-4

SYSTEMATIC MAPS FOR IDENTIFICATION OF SCIENTIFIC EVIDENCE FOR DISEASE CONTROL METHODS IN OATS, OILSEED RAPE AND POTATO

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Text
Systematic maps are a tool for compiling available and relevant research results in a structured way. We have produced systematic maps for disease control methods in oats, oilseed rape and potato. The aim was to identify scientific literature to support crop protection strategies for the crops. For oats 58 articles, for oilseed rape 118 and for potato close to 1,000 articles were identified. At the same time there are common trends within the crops studied, as there is a clear increase in the number of publications over the last decade, which can be linked to the general increased volume in scientific publishing. The extent of the research is largely based on which crop(s)/diseases that attracts funding. This is reflected in the fact that the most serious crop diseases are also the most studied, for oilseed rape black leg (*Leptosphaeria* spp.) and Sclerotinia stem rot (*Sclerotinia sclerotiorum*) for oats Fusarium head blight (*Fusarium* spp.) and crown rust (*Puccinia coronata*) and for potato late blight (*Phytophthora infestans*). Overall, the two most common control methods against plant diseases are the use of pesticides and resistant varieties. Biological control is included in only a few studies for oats, while several products were tested in oilseed rape and potato. Various cultivation and tillage methods were also identified in all three maps. We show that systematic maps can be used to identify both knowledge and knowledge gaps relevant in plant protection of different crops

C4.7-5

DATA-DRIVEN DECISION MAKING TO REDUCE GLOBAL CROP LOSSES

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Text
According to the IPCC, activities related to agriculture amount to 8.5% of all greenhouse gas (GhG) emissions with a further 14.5% attributed to land use change (mainly deforestation

and clearing land for food production). GhG emissions from agriculture are predicted to increase 30-40% by 2050, as food systems are under constant pressure of producing more food to meet growing demand of rapidly increasing population number. At the same time, farmers face challenges due to climate change and associated weather shocks and increasing pest pressures. An estimated 20-40% of crops are lost due to pests before they're even harvested – so the potential for closing this gap is enormous. The Global Burden of Crop Loss (GBCL) initiative identified the need for rigorous crop health metrics and methodology to assess annual losses of the world's most important crops. The goals of the initiative are to provide actionable baseline estimates of crop losses to inform multi-scale decision making towards assessing agricultural resilience to climate change and the introduction of mitigation strategies. Our ambition is to build a comprehensive database of reported pest and abiotic impacts on crops, including their evolution due to climate change, to underpin our assessment.

C4.7-6

NOVEL PLANT GROWTH PROMOTING RHIZOBACTERIAS (PGPRS) AS POTENTIAL INDICATOR OF SUSTAINABLE AGRICULTURE DEVELOPMENT

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Text

Biostimulants can be used as a tool to complement the use of chemical inputs, by involving living-based products, containing beneficial rhizosphere bacteria, such as plant growth-promoting rhizobacteria. Increase in soil fertility, plant growth promotion, and suppression of phytopathogens are the targets of the bioformulation industry that leads to the development of eco-friendly environment. To scope this cope, we investigated the potentiality of PGPRs (Plant Growth Promoting Rhziobacterias) from different hot agroclimatic states of India, characterized and correlate them with the potential effects on seed germination under unfavourable stress. Results indicate that *Bacillus safensis FO-36b* MMAPL-W, *Dyella jiangningensis* MMAPL-Fa and *Agrobacterium tumefaciens* MMAPL-Q strains exhibited high levels of qualitative and quantitative assays for biological Nitrogen fixation, Phosphorus (solubilisation index =7.8; 8.7; 6.9) and Potassium solubilizing bacteria (solubilisation index =2.85; 3.30;2.89), Siderophore production activities along with Biofilm characters. These strains render multifaceted benefits and presence of peculiar traits to the plants by several mechanisms. The inoculation of wheat, chickpea and moong seeds with culture filtrate directly during germination as well after two weeks of growth, were the most efficient methods of protecting seeds from growth inhibition. These PGPR strains can make it potential liquid biofertilizer candidates.

P4.7-001

REMOTE SENSING IN THE CONTRIBUTION TO THE STUDY OF FOLIAR DISEASES OF CEREALS "APPROACH TO THE EPIDEMIOLOGICAL STUDY AND IDENTIFICATION OF BARLEY DWARF YELLOWS" IN ALGERIA

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Text

Cereals continue to be at the center of the concerns of governments, regional and international institutions, constitute the staple food, of populations throughout the world and of constantly growing needs.
Thus, the investigations carried out over the last ten years in certain potential cereal-growing regions of eastern Algeria (Constantine, Mila, Guelma, Annaba, etc.) indicate the risks of epidemic development of certain cryptogamic diseases (yellow rust, Helminthosporioses, Septorias , etc.), a large number of cereal samples from the surveyed regions and its severity is linked to its epidemiology correlated with a high activity of aphid vectors such as: Rhopalosiphum padi, Rhopalosiphum maidis, etc. Remote sensing is efficient technique for acquiring and analyzing the spectral properties of plants and the earth's surface at different distances. This modern technology shows promise in agricultural production, including crop protection. The variability of plant reflectance spectra resulting from the occurrence of diseases and pests, allows their identification using remote sensing data. Various techniques such as infrared, multiband, multispectral imaging and hyper-spectral imaging, have been studied for the detection of plant diseases. In this work we use data from the satellites ALSAT, LANDSAT and SENTINEL sensor for the diagnosis of wheat disease and thus make agriculture more sustainable and safer, avoid the costly use of pesticides in crop protection.

P4.7-002

BIOLOGICAL AND MOLECULAR CHARACTERISATION OF CITRUS VIROID VII

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Text

Citrus is notable for being susceptible to a broad range of viroids, including citrus exocortis viroid, hop stunt viroid and the recently discovered citrus viroid VII (CVd-VII). CVd-VII was first detected in Australia in ‘Lisbon’ lemon plants held at a citrus germplasm field collection in south-western New South Wales by RT-PCR using universal primers designed against members of the genus apscaviroid. The viroid is enigmatic, as it has not been found beyond these original trees, either in Australia or overseas, raising questions regarding its origin. Host range studies suggest that CVd-VII can experimentally infect many types of citrus but not plant species outside the *Rutaceae*. Most infections appear to be asymptomatic, but in ‘Etrog’ citron, downward leaf curling and plant stunting occurs. Field and pot trials have been established to determine the long-term effect of CVd-VII on yield, and to investigate potential synergistic interactions with other viroid species. miRNA expression studies are investigating how CVd-VII may disrupt normal plant metabolism to induce symptoms in 'Etrog' plants. Finally, the regions of the viroid genome that are prone to mutation are being identified to guide design of PCR primers for diagnosis.

P4.7-003

FOXES IN CHARGE OF HENHOUSES: COMBATTING SUGARCANE RATOON STUNTING DISEASE (RSD) AND ITS DEFENDERS IN AUSTRALIA

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Text

Management of sugarcane ratoon stunting disease (RSD) in Australia continues to falter, despite significant diagnostic advances. This failure has far-reaching deleterious impacts on sugarcane production and the environment. The inadequacies of current management are in part due to the symptomless presentation of RSD that baffles farmers, but also on long-standing but untested perceptions within the industry that the disease is under control. The development of the leaf-sheath biopsy (LSB) qPCR diagnostic has cast RSD through a new lens as it is quicker, samples more stalks in a field, and is more sensitive than previous methods. This has led to changes in the diagnostic platform offered in Australia, in turn revealing that far from being under control, RSD is rampant in most regions. This presentation discusses the consequences of the problematic nature of RSD on the profitability and sustainability of sugarcane production in Australia, and its impacts on sensitive river catchments and the Great Barrier Reef.

P4.7-004

EVALUATING THE OILSEED RAPE GROWTH-STATUS USING NDVI AND NDYI OBTAINED FROM UAV-BASED RGB IMAGERY

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Text

The study aimed to evaluate the potential of the normalized difference vegetation index and the normalized difference yellowness index derived from RGB-imaging to monitor the growth status of oilseed rape. Subsequently, collected values were used to evaluate their correlations with the yield. Field trials with different seed densities and nitrogen rates were conducted for two years. The images were taken by an unmanned aerial vehicle carrying a multi-spectral camera. The NDVI and NDYI values for each plot were calculated from the reflectance at RGB and NIR bands' wavelengths pictured in a re-constructed and segmented orthomosaic. During both seasons, the NDVI increased significantly from the seedling stage to the beginning of the winter season, then decreased slightly after winter, again increased to reach the first pick before crop flowering, then significantly decreased during flowering stages. The NDVI approached the highest pick at the full pod development stage and then reduced strongly until maturity. In contrast, the NDYI accessed saturation around flowering time, decreased during pod development stages, shortly increased at the end of pod development, and then decreased until plant maturity. The strongest correlations were found between the final yield and NDVI of full leaf development before winter and the final yield

with NDVI of full pod development stages. A significant correlation was observed between NDYI at the full flowering stage with the final yield.

P4.7-005

MOLECULAR EVOLUTION OF CHEMOSENSORY GENES IN BEETLES (COLEOPTERA): IMPLICATIONS FOR THEIR ADAPTATION AND SPECIATION

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Text

Insects' olfactory systems guide important behaviours throughout their life, such as host preference and mate choice. The chemosensory superfamily is the largest gene family in genomes and has been seen as an ideal model for understanding evolutionary divergence and speciation. It has been hypothesised that ecological adaptation, such as changing host species and range, may correlate with the 'birth-and-death' evolution of olfactory genes. Beetles are the most diverse order of insects, providing an ideal model for comparative evolutionary studies. This study focuses on flea beetles (*Alticinae*), some of which are agricultural pests, such as the cabbage stem flea beetle *Psylliodes chrysocephala* L. From a feeding preference perspective, *Phyllotreta vittula* R. is of special interest since it represents one of the closest polyphagous relatives of the monophagous *chrysocephala*. With a whole-genome assembly of *vittula*, we hope to better understand their host interaction for developing better pest control methods.

Aims (1) Sequence and assemble the genome of the polyphagous flea beetle *Ph. vittula*. (2) Test the hypothesis that host adaptation drives chemosensory gene evolution using a comparative genomic approach.

We will compare the olfactory gene families of the polyphagous with the newly acquired genome of *Ps. chrysocephala* and other closely related beetle species to identify key olfactory genes involving host selection and pave the way for our further knock-out verification.

P4.7-006

INVESTIGATING GRAPE SOUR ROT DEVELOPMENT IN A COMMERCIAL VINEYARD IN MARYLAND, USA

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Text

Grape sour rot (GSR) is a disease complex involving fungi and bacteria that can cause significant yield losses of susceptible cultivars. It is widely spread in eastern U.S. and other grape-growing regions globally. Previous studies suggest that damaged fruit skin and feeding

insects like *Drosophila* spp. are required for the disease to occur. Synthetic insecticides and fungicides are recommended for GSR management, but the specific insects and microbes involved in GSR development are not well understood. We aimed to (i) determine the bacterial and fungal species associated with GSR development using culture-based and high throughput sequencing techniques and (ii) conduct in vitro assays to investigate the pathogenicity of the core communities. So far, we have isolated about 700 bacteria and fungi combined from healthy and infected grape berries of three cultivars collected at multiple time points from a commercial vineyard. We found five bacterial species in the genera *Pantoea* and *Curtobacteria* associated with GSR as opposed to *Gluconobacter* and *Acetobacter* species frequently reported in literature. Among the core fungi involved in GSR were many undescribed yeast species including *Pichia*, *Hanseniaspora*, *Rhodotorula*, *Starmerella*, and *Sporobolomyces* species. Filamentous fungi like *Cladosporium*, *Pestalotiopsis*, and *Neopestalotiopsis* were also isolated from GSR-infected berries. These results will form a basis for downstream analyses to elucidate the mechanism of GSR development.

P4.7-007

TAKING A TOUR INSIDE THE KIWIFRUIT MICROBIOME: A STUDY ON THE ETIOLOGY OF KIWIFRUIT VINE DISEASE SYNDROME (KVDS)

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Text

Since 2012, Italian kiwifruit orchards and overall production have been particularly threatened by a new complex disease known as Kiwifruit Vine Decline Syndrome (KVDS). The main symptoms associated with this syndrome are the reduction and browning of roots, xylem necrosis, progressive loss of capillitium, hypertrophy, and separation of the cortical layer. These symptoms can quickly spread systemically, inducing the collapse and death of plants within the same season. The etiology of KVDS is not yet understood and, to date, no causative agents have been significantly associated to it. Thus, a field trial has been carried out in an experimental kiwifruit orchard in Lazio region. Soil and root samples from plants showing KVDS symptoms have been collected at different time-points, from vegetative renewal to flowering, and compared to healthy-looking samples. To deeply investigate the causative agents related to this syndrome, total DNA extracted from both healthy and symptomatic samples was sequenced using an Illumina platform for downstream metagenomics analysis. The results gave the first insights into the different microbiome composition and how it is influenced by the different experimental conditions. In future, a transcriptomic and a metabolomic analysis will shed light on the plant defense mechanism against KVDS, narrowing down the circle of possible causative agents and helping to design more specific control strategies.

P4.7-008

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF THE IMMUNE ACTIVATION-BOOSTING DOMAIN OF LILIUM DEFENSE-PRIMING PROTEIN LSGRP1

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Text
Defense priming of induced resistance is a form of plant immunological memory[K1] to certain biotic or abiotic stimulations, which leads to faster and stronger immune activation upon subsequent pathogen challenges without causing growth retardation. Herein, the resources capable of triggering defense priming are considered potential disease control agents to reduce chemical pesticide usage. Lilium LsGRP1 is a defense-priming protein involved in both growth promotion and immune activation, crucial for fighting off Botrytis elliptica. LsGRP1-transgenic Arabidopsis driven by constitutive promoter shows better growth and confers resistance to various pathogens accompanied with enhanced defense responses triggered by pathogen-associated molecular patterns (PAMPs) and effectors, revealing this system could help to identify the functional region of LsGRP1 for immune activation. Through comparing the levels of Pseudomonas infection, PAMP flg22-triggered callose deposition and FRK1 expression, and effector AvrRpm1-triggered hypersensitive response among Arabidopsis transformants of wild-type and different region-deleted mutants of LsGRP1, the essential region for LsGRP1 boosting immune activation was located. Besides, the synthetic peptide of this region was proven to enhance B. elliptica secretion-triggered callose deposition in Lilium leaves that this peptide alone did not cause, revealing the potential of this immune activation-boosting domain of LsGRP1 in plant health management.

P4.7-009

OCCURRENCE STATUS OF NATIONAL MANAGEMENT VIRUS IN CHUNGCHEONGBUK-DO PROVINCE, KOREA FROM 2020 TO 2022

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Text
In this study, high-risk viruses and quarantine viruses, which are national-managed viruses, were investigated. The investigation was conducted for 3 years from 2020 to 2022, and 7 types of viruses were investigated in the Chungcheongbuk-do Province of Korea. The types of viruses are BRRV(Blueberry red ringspot virus), INSV(Impatiens necrotic spot virus), CNSV(Chrysanthemum stem necrosis virus), TSWV(Tomato spotted wilt virus), TYLCV(Tomato yellow leaf curl virus), CABYV(Cucurbit aphid borne yellow virus), CCYV(Cucurbit chlorotic yellows virus). As a result, TSWV was detected in red pepper, TYLCV in tomato, CCYV, and CABYV in melon, cucumber, and watermelon. The most frequently occurring virus was CABYV in melon, with an incidence rate of 81.8 to 100% over 3 years. CABYV was found in watermelons, cucumbers and melons. CCYV was also found in watermelons, cucumbers, and melons, and the incidence rate over 3 years was highest in melons, ranging from 9.7 to 25%. TYLCV had an incidence of 0-20% for 3 years in tomato, and TSWV had an incidence of 14.3-22.2% for 3 years in pepper. Three types of viruses, BBRV, CSNV, and INSV, did not occur.

P4.7-011

CHLOROPHYLL FLUORESCENCE TECHNIQUE FOR SCREENING THE COLD HARDINESS OF OLIVE (OLEA EUROPAEA L.) CULTIVARS

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Text

The olive is an evergreen tree with low and cultivar-dependent frost tolerance. In winter, air temperatures below 0ºC can cause damage during dormancy. Also, the olive cold hardiness is affected by the plant's age, sanitary and nutritional stages or tissue. Indeed, olive leaves are considered more sensitive than shoots. Here, we evaluated the cold-hardiness of 12 olives (*Olea europaea* L.) cultivars ('Arbequina' 'Arbosana' 'Arroniz' 'Cornicabra' 'Empeltre' 'Frantoio' 'Hojiblanca' 'Koroneiki' 'Manzanilla Cacereña' 'Manzanilla de Sevilla' 'Picual' and 'Sikitita') using the electrolyte leakage method as standard, and a chlorophyll fluorometer. We collected fully expanded, uniformly sized leaves from 1-year-old shoots from 10-year-old trees in the dormant period (winter pause) and exposed them to low temperatures at 0, -3, -6, -9, -12, and -18ºC for one hour each. We assessed the leaf frost damage by measuring the electrical conductivity of the cell electrolytes released into an aqueous medium and the frost impact on the leaf fluorometry. Both evaluation methods correlated for classifying the cultivar's cold hardiness. The optimum temperature to classify varieties was -6ºC. This year, a freezing event during January-February (2 weeks with -2 to 0ºC) in the Olive Germplasm Bank of the University of Cordoba permitted evaluate most varieties (300 approx) by fluorometry. This information is essential for selecting olive cultivars for regions with elevated frost risk.

P4.7-012

THE IMPACTS OF PLANT PROTECTION PRODUCTS ON PRIMARY PRODUCERS: AN OVERVIEW FROM THE LAST DECADES' LITERATURE

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Text

Synthesis of the international scientific literature available from the 30 last years regarding impacts of Plant Protection products (PPP) on biodiversity and ecosystem functions was conducted on behalf of the French Ministries in charge of the Environment, Agriculture and Research through a collective scientific assessment. We present the main highlights regarding the impacts of PPP on primary producers, from cyanobacteria, microalgae to terrestrial plants, in various environments such as agroecosystems, freshwater, estuarine, marine ecosystems. Our literature review evidenced a general lack of knowledge at the community level, whatever the type of organism or environment, particularly for estuarine,

marine waters and soil photosynthetic microorganisms.
Unintended effects of PPP on field edge plant communities are still insufficiently known to allow a robust assessment of the nature and extent of risks posed by herbicides.
Toxicity of herbicides on marine phototrophs is still poorly documented, especially in overseas territories.
Most toxic compounds to aquatic primary producers were photosynthesis inhibitors, terrestrial plants were mostly affected by acetolactate synthase inhibitors. Copper was also highlighted as potentially toxic to photosynthetic microorganisms at environmentally realistic concentrations. Only a few studies actually address the impacts of PPP on a few ecosystem functions of primary producers, the main one being the function of organic matter production.

P4.7-013

CHARACTERIZATION OF AN ISOLATE OF THE POTYVIRUS PASSIFLORA VIRUS Y NATURALLY INFECTING SOYBEAN IN BRAZIL

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Text
Soybean (*Glycine max*) is one of the most important crops worldwide. Since 2020, Brazil has been the largest producer of soybeans in the world, producing 114.2 million tons in the 2019/2020 growing season. Several diseases affect soybean production, and viruses cause major problems, with at least 46 viruses identified infecting soybean worldwide. This study reports an isolate of Passiflora virus Y PaVY naturally infecting soybean plants growing near a commercial passion fruit crop. The nearly complete genome sequence is 9679 nt long and shares 84.4% nt sequence identity with a previously reported PaVY isolate from Passiflora sp. The putative ORF of PaVY starts at an AUG codon at nucleotide positions 170–173 and ends with an UGA codon at nt 9419–9422, encoding a polyprotein of 3083 amino acids (aa) with a molecular weight of 352.16 kDa. PaVY-Br induced chlorotic spots and systemic mosaic on soybean and chlorotic local lesions on yellow passion fruit (*Passiflora edulis*) and sesame (*Sesamum indicum*). The virus was successfully transmitted by *Myzus persicae*, indicating that this aphid vector can contribute to the spread of PaYV from passion fruit to soybean plants. It is worth mentioning that PaYV had never been reported in Brazil, even infecting passion fruit. The presence of PaYV was reported to the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) as required by federal law. Its distribution in soybean production areas in Brazil needs to be studied further.

P4.7-014

IDENTIFICATION OF TWO NOVEL BREVIPALPUS-TRANSMITTED VIRUSES (BTV) AND RECOGNITION OF A KITAVIRUS AS THE CAUSAL AGENT OF THE CITRUS ZONATE CHLOROSIS DISEASE

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Text

Viruses transmitted by *Brevipalpus* mites belong to the genera *Cilevirus*, fam. *Kitaviridae* [ss(+)RNA] and *Dichorhavirus*, fam. *Rhabdoviridae* [ss(-)RNA]. They produce non-systemic infections in crops and ornamental plants, and some of them cause citrus leprosis, a serious disease of citrus in the Americas. We analyzed the virome of large periwinkle (*Vinca major*) plants collected in Chile in 2019, exhibiting chlorotic and ring spots; and sweet orange (*Citrus sinensis*) trees collected in Brazil from 1933 (herbarium samples) to 2022, with symptoms of zonate chlorosis. Plant RNA extracts were sequenced using HTS technology. In periwinkle, the genome of two bi-segmented viruses with genomic organizations of cileviruses and dichorhaviruses were identified. Sequence analyses indicated that both viruses represent new species in their respective genera. In citrus plants, the genome of a tri-segmented virus with >98% nucleotide sequence identity with the kitavirus hibiscus green spot virus 2 (HGSV2, genus *Higrevirus*) was identified. Sixty mites collected in periwinkle plants were anatomically and molecularly identified as *B. chilensis*, while in citrus, *B. yothersi* and *B. papayensis* were detected. HGSV2 was transmitted to healthy Arabidopsis plants using specimens of both species. Here we report, for the first time: the presence of BTVs in Chile, *B. chilensis* as a potential viral vector, transmission of a higrevirus by *Brevipalpus*, and HGSV2 as the causal agent of citrus zonate chlorosis.

P4.7-015

NUTRIENTS' EFFECT PREDICTION ON WHEAT PHYSIOLOGY BY USING MACHINE LEARNING

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Text

Nutrient management involving wheat has been a matter of interest for a long time owing to the unique production environment of wheat. Machine learning is increasingly being used in agriculture to classify plants, identify pests, predict the weather, and track yield. Here, a machine learning-based prediction model was developed to understand the role of individual nutrients (N, P, K, Zn, and S) on different plant parameters (plant height, tiller number, dry matter production, leaf area index, grain yield, and straw yield) of wheat. A feed-forward

neural network with back-propagation training was developed using the neural network toolbox. For the training of the model, data obtained from two consecutive crop seasons over two years (four crops of wheat) were used. In the present study, an attempt was made to understand the role of individual nutrients in achieving crop growth and yield using an artificial neural network-based prediction model. The model predicts that growth parameters such as plant height, tiller number, and leaf area index often achieve their maximum performance at below the maximum applied dose, while the maximum yield in most cases is achieved at 100% N, P, K, Zn, and S dose. In addition, the present study attempted to understand the impact of individual nutrients on both plant growth and yield in order to optimize nutrient recommendation and nutrient management, minimizing environmental pollution and the wastage of nutrients.

P4.7-016

PHENOTYPING FOR QUANTITATIVE RESISTANCE TO LEPTOSPHAERIA MACULANS IN BRASSICA NAPUS (RAPESEED): A FRAMEWORK USING MACHINE LEARNING AND ARTIFICIAL INTELLIGENCE (MLAI)

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Text

Phenotyping of plant diseases has not kept pace with the rapid progress in genetic and genomic characterisation and is a bottleneck for breeding. Blackleg crown canker (caused by L. maculans) of B. napus causes large economic losses but breeding for quantitative resistance is challenging due to the large number of genes involved and the lack of an economically viable non-subjective phenotyping method deployable at the scale required for in-field screening in a breeding context. To overcome this issue, we developed machine learning and artificial intelligence (MLAI) techniques for automated assessment of crown canker severity in cross-sections of canola stems at plant maturity using RGB images. The MLAI algorithm was trained on a data set of 4000 images to extract the region of interest (ROI), resulting in an overall accuracy of 88%. Moreover, the disease was quantified based on the pixel values extracted from the images. To ensure the accuracy of our approach, we validated the results by comparing them with standard visual assessments. Overall, the MLAI framework provides an automated disease assessment approach which could form the basis to identify the genes underlying QR and help growers to have resistant varieties of canola to reduce Blackleg related yield loss.

P4.7-017

CAL POLY STRAWBERRY CENTER IS A MODEL FOR INDUSTRY-UNIVERSITY PARTNERSHIPS

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Text
In 2014, the California Strawberry Commission (CSC) and Cal Poly State University entered a partnership to increase the sustainability of the California strawberry industry through applied research and workforce training that is aligned with industry needs. The campus is centrally located to California's 16,823 ha of strawberries and 47 km from Santa Maria, where 42% of the state's strawberries are grown. The Center's laboratories and fields are located within walking distance from campus, facilitating student participation. The Center has grown to 6 Ph.D. scientists and 8 support staff who work in three research areas: plant pathology, entomology, and automation. An industry advisory council composed of industry leaders, meets biannually to provide feedback on the Center's programs and outcomes. Over 125 students have received hands-on work experience in the field and lab and 20 students received master's degrees in plant pathology or entomology. The Center leverages financial support from the CSC to compete for grant funding and has obtained $3.7 million USD, resulting in 16 peer-reviewed publications and 35 published product efficacy trials. More importantly, direct improvements to pest and disease management and labor efficiency have been made to the industry. The Strawberry Center is poised to have greater impact in the future through an expansion of lab and field facilities and national and international research collaborations.

P4.7-018

BASIC SUBSTANCES AS AN ENVIRONMENTALLY FRIENDLY ALTERNATIVE TO SYNTHETIC PESTICIDES FOR PLANT PROTECTION: THE EXPERIENCE OF EUPHRESCO BASICS PROJECT

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Text

Improving the sustainability of agriculture and, at the same time, reducing the adverse effects of synthetic pesticides on human health requires effective alternatives that improve the productivity while maintaining the food quality and safety. Basic substances are relatively novel compounds that can be used in plant protection without neurotoxic or immune-toxic effects and are still poorly known by phytosanitary consultants, researchers, growers, consumers, and decision makers. The BasicS project takes together the experience of 30 Research Units from 19 Countries representing all continents, to test and validate the use of 24 basic substances currently approved in the EU and further potential basic substances. Most of these substances have a fungicidal activity (calcium hydroxide, chitosan, chitosan hydrochloride, *Equisetum arvense* L., hydrogen peroxide, lecithins, cow milk, mustard seed powder, *Salix* spp., sunflower oil, sodium chloride, sodium hydrogen carbonate, *Urtica* spp., vinegar, and whey). Considering the increasing requests from consumers of fruits and vegetables with no or a reduced amount of pesticide residues, basic substances can complement and, at times, replace the application of synthetic pesticides with benefits for users and for consumers. Large-scale trials are important to design the best dosage and strategies for the application of basic substances against pathogens and pests in different growing environments and contexts.

P4.7-019

APPLICATIONS OF CHITOSAN ALONE, ALTERNATED OR COMBINED WITH COPPER FOR GRAPEVINE DOWNY MILDEW MANAGEMENT IN LARGE SCALE TRIALS

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Text

The implementation of the sustainable practices in viticulture has become an important issue for the wine industry. In organic grapevines, copper-based treatments are widely used to control grapevine downy mildew (GDM) and this can affect the quality of wine due to the presence of residues on the grapes. This study was conducted in three commercial vineyards and years (2019-2021) to evaluate the effectiveness of chitosan against GDM. Strategies were based on application of chitosan alone, chitosan alternated with copper, and chitosan combined with copper at half rate each were tested, using copper sprayed alone and untreated plants as controls. Our results showed that all the strategies applied were able to reduce GDM McKinney Index on bunches compared to untreated control. Chitosan alone provided a good protection against GDM, and when alternated with copper have the same effectiveness of copper. Chitosan combined with copper at half rate provided a protection by GDM better than copper alone. All innovative strategies based on chitosan were able to reduce copper amount on harvested bunches. These investigations demonstrated that

chitosan can be a good alternative to complement and even replace copper in GDM management strategies. Further investigations are needed to test the effectiveness of the different chitosan formulations available on the market and the effects on the quality of the wine.

This work was conducted within the framework of the PSR Marche Vitinnova Project

P4.7-020

METAGENOMIC ANALYSIS OF RUSSIAN AMPELOGRAPHIC COLLECTIONS

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Text

Grapevine germplasm collections are unique repositories of grape cultivars; therefore, it is necessary to minimize their infection with pathogens, including viruses, and develop various programs to maintain them in a virus-free state. In our study, we examined the virome of the largest Russian grapevine germplasm collections using high-throughput sequencing of total RNAs. As a result of bioinformatics analysis and validation of its results by reverse transcription PCR and quantitative RT-PCR, we identified 28 viruses and 4 viroids in 120 libraries. All samples were infected with 1 to 12 viruses and viroids, including those that cause economically significant diseases: leafroll, fleck, and rugose wood complex. Two new grapevine viruses were discovered in these germplasm collections. One of them is member of the genus Umbravirus with the provisional name Grapevine umbra-like virus. The second virus is pararetrovirus from the genus Caulimovirus, which was tentatively named Grapevine pararetrovirus.

The research was made possible with support from the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement ? 075-15-2022-318 date 20 April 2022 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center “Agrotechnologies for the Future”.

P4.7-022

EFFECT OF SILICON ON PLANT GROWTH AND REDUCING DISEASE IN BLACK PEPPER (PIPER NIGRUM L.)

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Text

Silicon in bio-available form (silicic acid) application effectively suppressed diseases caused by fungi in a variety of plant species. Various fungicides had been used to control Phytophthora foot rot diseases and Fusarium wilt of black pepper (Piper nigrum L.) but ineffective for a better sustainable agriculture. Thus, this study to confirm the silicon (Si) uptake ability and other nutrients accumulation in leaves and to determine the passive defense pathways (cuticles thickness and wax of the leaf surface). Silicon had been applied once a week on pepper plants variety Kuching at different concentration; T1 [0.5% Si (v/v)], T2 [1.5% Si (v/v)] and T3 [2.0% Si (v/v)] for six months. Although P. nigrum L. is known as Si intermediate accumulators, results proven that it could uptake Si and enhance plant growth. Plants showed the greatest plant height, diameter and chlorophyll content compared to control (without Si). Moreover, disease severity was reduced with increasing tissues concentration of Si whereas nutrients such as K, P, Ca, Mg, Mn and B accumulated more in leaves. The passive defense pathways had mechanically deterred hyphae invasion of the pathogen by strengthening the leaf cell walls. Undoubtedly, Si can be included in disease management plans as an important component of the integrated disease management.

Impact of war and conflicts in plant pathology research and food safety of countries

C1.7-1

RESEARCH AND EDUCATION IN PLANT VIROLOGY IN UKRAINE: THE PRESENT IS FOGGY, THE FUTURE IS BRIGHT?

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Text

Ukraine has been at war for a year now. Writing these lines in February 2023, we really hope that when we report back in summer, this absurdity will be over.

The war has greatly affected research and education in general, including that in plant virology, especially in the affected areas of the country. Research and collaboration ties have been disrupted, making it difficult for Ukrainian scientists to share their expertise.

A brain drain of Ukrainian scientists remains an important issue, as many were forced to leave the country in search of safer options and better opportunities. This led to a loss of experience and knowledge in the field of plant virology in Ukraine. Dozens of universities have been relocated to other regions of the country, and many students preferred to pursue academic mobility programs abroad.

The war also led to the destruction of some of the country's security systems related to plant pest control, where it became difficult or impossible for Ukrainian authorities to monitor and control the spread of plant diseases.

Despite these challenges, some Ukrainian scientists continue to work in plant virology research and education, especially in regions of the country not directly affected by the conflict or abroad. International cooperation and support have played an important role in maintaining our capacities in plant virus research and nurturing future generations of plant virologists. Here we discuss these challenges and how these are mitigated.

C1.7-2

IMPACT OF WAR AND CLIMATE CHANGE ON VIRAL DISEASES OF WINTER WHEAT A THREAT TO FOOD SECURITY

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Text

Ukraine is one of the 5 largest exporters of grain, supplying more than 45 million tons of grain to the world market every year. Plant viruses are an important economic factor. Our research showed that the isolates of WSMV, BYDV, WDV are circulating in Ukraine and have high epidemic potential, because they lead to a decrease in wheat yield (by 30-50%) and significant deterioration in the products` quality. Our research has shown that species composition of wheat viruses is influenced by climate changes. In recent years, winter wheat in Ukraine suffers from significant temperature drops in phases of emergence into tube and beginning of earing, which leads to significant economic losses. Due to climate change, it is often difficult to distinguish viral infections symptoms from other abiotic factors.
In addition to decrease in crop yield and quality due to damage by plant pathogens and climate changes, the following problems arised in today's realities of the war waged by russia against Ukraine:i) reduction of sown areas due to active warfare, occupied territories, mined fields (by 45 % compared to 2021);ii) mined fields cannot be cultivated, so they are source of virus-carrying insects and plants. The forecast for the gross production of winter wheat in 2023 is decrease of 15-20%, without occupied territories by 30%.Thus, the main goal of plant pathologists, geneticists and breeders in Ukraine should be creatiion of wheat varieties resistant to viruses, fungi and abiotic factors

C1.7-3

KEEP CALM AND GROW PLANTS, OR HOW HORTICULTURE SURVIVES IN WAR IN UKRAINE

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Text

Agriculture is one of the sectors that has suffered the greatest losses during the full-scale war of russian federation against Ukraine. The direct and indirect damages to the industry resulting from the nine months of hostilities are estimated to exceed $40 billion. Due to military actions, productive plantations and farms' facilities for growing fruits and berries were damaged or destroyed, when the machinery was either destroyed or stolen. In 2022, the production of certified planting material saw sharp decrease over 40%, paralleled by respective reduction in the number of registered nurseries. At least two horticultural germplasm collections with over 900 varieties and forms remain inaccessible and in an uncertain condition. The loss of production potential and stock plants will create a shortage of healthy planting material in the future. Lack of plant protection products leads to the impossibility of proper phytopathogens' control in orchards and non-adherence to recommended treatment regimens. Funding for institutions responsible for phytosanitary supervision and monitoring of phytopathogens has substantially decreased. In current situation, the support of the Ukrainian government and international organizations is important for specialized enterprises and research institutions since it will contribute to mitigating risks to the food security and preserving the production potential, which is important for the post-war reconstruction of Ukrainian horticulture.

C1.7-4

CROP PATHOGEN SEVERITY AND PESTS IN BANANA, CASSAVA, POTATO, AND SWEETPOTATO PRODUCTION IN THE LAKE KIVU REGION OF RWANDA AND BURUNDI

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Text

The Ukraine crisis has caused major disruptions in the availability of wheat flour in Africa. Many African bakers have turned to banana, cassava, potato, and sweetpotato flours. This shift will likely increase pressure on the seed systems of these crops in the coming years. Since these crops are vegetatively propagated, the accumulation of pests and pathogens is a serious concern. The risk of epidemics in seed systems can be affected by the geographic distribution of crops and climate. Here we evaluated cropland connectivity of these crops in the Lake Kivu region of Rwanda and Burundi. The analysis of cropland connectivity allowed us to develop a risk map of locations that are candidate priorities for disease/pest surveillance in the Lake Kivu region. We surveyed 292, 50, 194 and 211 fields for banana, cassava, potato, and sweetpotato, respectively. We evaluated the severity and community structure of major pathogens and pests of these crops as a function of altitude, climate

variables, and the geographic structure of croplands. There was high variability in pests and pathogens severity, but effects of altitude, climate and cropland structure were observed. We used several machine learning algorithms, including support vector machines and random forests, to predict severity. Climate variables and the geographic structure of croplands can be used to predict severity. Models to guide surveillance and mitigation can improve responses to new epidemics and global change.

C1.7-5

PHYTOSANITARY MANAGEMENT OF ICARDA'S GERMPLASM SEED COLLECTIONS FOR BETTER FUTURE USE

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Text

Recent years have witnessed an increasing global concern about the loss of plant genetic resources, as a result of conflicts, epidemics, earthquakes, etc., which led to disrupting access to some germplasm and undermining social protection systems, and thus an increase in global awareness to preserve germplasm for their current and future use. This led genebanks all over the world to create disaster risk reduction policies to organize activities in the safekeeping, conservation, and dissemination of germplasm resources. Safety duplication of base collection at different geographic sites, such as Svalbard Global Seed Vault, is one of the essential measures. Any procedure must comply with phytosanitary regulations to enable direct and rapid response for safe germplasm exchange and retrieval. In order to make sure that germplasm is viable to combat challenges, ICARDA's Germplasm Health Unit (GHU) exercises maximum effort to maintain the health status of germplasm collections, ensure compliance with phytosanitary regulations in international germplasm distributions, and develop methods to detect and manage seed-borne pathogens to guarantee minimum loss of genetic resources. In addition, maintaining plant health during germplasm regeneration is essential to reducing the risk of seed-borne pathogen spread via future germplasm distributions. The role of ICARDA's GHU in preserving germplasm through conservation, seed regeneration, exchange, and retrieval will be presented.

C1.7-6

EMERGING AND (RE)EMERGING VIRAL THREATS FOR COMMERCIAL PLANTS IN UKRAINE: WAR AND OTHER PROBLEMS

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Text
Here we review different risk factors which are or may be important for emergence and establishment of plant virus infections in a new environment/host(s), as well as their subsequent spread and reoccurrence with a focus on Ukraine. In this context, virus properties, transmission routes including vector- or seed-born spread, host range, availability of wild-growing reservoir plants, and effect of some anthropogenic factors (i.e., heavy metals, radioactivity and war activities) are covered.
To showcase this, we analyzed particular plant pathogens which are of specific importance to Ukraine, and suggested major issues possibly contributing to the emergence or further successful persistence of viral diseases of important crops. Additionally, we also show that abiotic stressors (heavy metals and radionuclides) may be essential drivers of virus abundance in the ecosystems, as well as of higher incidence rate of plant infection. Main risk factors favouring the appearance and spread of viruses in Ukraine are summarized.

Latest advancements in knowledge and management of Ralstonia species

C8.3-1

DESCRIBING THE KNOWN GLOBAL DISTRIBUTION, HOST RANGE, AND GENOMIC DIVERSITY OF THE RALSTONIA SPECIES COMPLEX THROUGH COHORT-BASED UNDERGRADUATE RESEARCH

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Text
The Ralstonia species complex is a genetically diverse group of plant wilt pathogens with a wide distribution around the world. Collectively, the global plant pathology community has carried out hundreds of population surveys for Ralstonia. However, this rich biological data is fragmented into tables of hundreds of papers. To unify this information, we created a cohort undergraduate research experience (CURE) where we train junior scientists to consolidate data on the reported global distribution and host range of Ralstonia clades. We have cataloged information from almost 8,000 strains isolated from almost 400 plant species in 105 geographic regions. The Global Ralstonia Diversity Database is currently available as a BioRxiv preprint with the strain metadata organized into an Excel spreadsheet. The aggregated data shows that the pandemic brown rot lineage (IIB-1 and IIB-2) is the most widely dispersed lineage. Phylotype I and IIB-4 lineages are also widely distributed and these lineages have the broadest natural host range. Recently, we have created a parallel CURE where junior scientists generate whole-genome resources of Ralstonia isolates. We are regularly submitting new Ralstonia genome sequences to public databases. Moreover, to improve the accessibility of carrying out RSSC phylogenomic studies, we have also created an open graphical user interface (a KBase narrative) where colleagues can identify phylogenetic relationships of new strains with 250 public RSSC genomes.

C8.3-2

THE SPECIAL CASE OF RACE 3 BIOVAR 2: WHY IS RALSTONIA SOLANACEARUM IIB-1 SO EFFECTIVE?

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Text

R. solanacearum phylotype IIB-1 strains, also known as R3bv2, cause destructive potato brown rot and tomato bacterial wilt in cool zones. But are all cool virulent strains R3bv2? To improve regulation of threatening Ralstonia strains, we built a robust phylogenetic tree using genomes of >275 Ralstonia strains. Measuring virulence and colonization of diverse strains on tomato and potato at 22°C and 28° revealed cool virulence is a quantitative trait not limited to R3bv2. Still, epidemiological data indicate that a clonal lineage of S. American origin causes the rapidly spreading brown rot pandemic. This brown rot pandemic lineage (BRPL) can be identified by whole genome sequencing and has a unique LINbase identifier. Host resistance is the best control for bacterial wilt, but mechanisms of the widely used Hawaii7996 tomato breeding line are unknown. We found H7996 resistance is overcome by BRPL strain UW551. Unlike other Ralstonia strains, UW551 grew well in ex vivo xylem sap from Ralstonia-infected H7996 plants. Further, other Ralstoniastrains could grow in sap from H7996 plants previously infected by UW551, which detoxifies inhibitors in H7996 xylem sap. Metabolomics suggested the inhibitors in sap are phenolic compounds. Culturing UW551 in this sap reduced total phenolic levels, indicating that the resistance-breaking BRPL Ralstonia strain degrades these chemical defenses. Thus, H7996 tomato wilt resistance depends in part on inducible phenolic compounds in xylem sap.

C8.3-3

PROTEASES AND STRUCTURAL COMPONENTS THAT RESTRICT R. SOLANACEARUM COLONISATION IN RESISTANT TOMATO

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Text

We have used live monitoring together with grafting of susceptible and resistant varieties to investigate the spatio-temporal *R. solanacearum* colonization dynamics in tomato1. Our work reveals four different restrictions to the bacterium in resistant tomato and that structural constraints are key for resistance to bacterial wilt both in root and shoot tissues. We have investigated the physico-chemical nature of the induced plant barriers as ligno-suberin coatings and tyramine-derived hydroxycinnamic acid amines. In agreement with these findings, overexpression of the ligno-suberin pathway in a susceptible tomato enhanced resistance by restricting R. solanacearum movement inside the plant and delaying disease

progression2,3.
In parallel, we have studied tomato apoplastic proteases activated in response to R. solanacearum infection. We described important papain-like cysteine proteases, hidrolases and especially the P69 clade of serine proteases as strongly activated upon pathogen challenge. We will present the biochemical characterisation of the P69 family and their role in resistance to bacterial wilt.
Our findings open new avenues to engineer resistance against vascular wilt pathogens.

1 Planas-Marquès M. et al., 2020. J Exp Bot, doi.org/10.1093/jxb/erz562
2 Kashyap A. et al., 2021. J Exp Bot, doi:10.1093/jxb/eraa444
3 Kashyap A. et al., 2022. New Phytol, doi: 10.1111/nph.17982
4 Planas-Marquès M. et al., 2018. Mol cell Proteomics, doi: 10.1074/mcp.RA117.000052

C8.3-4

THE EXPANSION OF THE TROPICAL RALSTONIA PSEUDOSOLANACEARUM (PHY I) TO THE TEMPERATE CLIMATES AND CONSIDERATIONS ON THE NEW RISKS

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Text
In a given geographic area, a shift in climatic conditions may result in major effects on plant pathogens. Ralstonia pseudosolanacearum (phy I) populations mainly originate from Asia. It is thought that this group of bacteria is not pathogenic at low temperatures although they occur frequently as latent infections in the plant. Given the high recombination frequency and the high virulence plasticity of these strains, adaptation to various new host plants and diversification to cope with different environmental conditions in many geographic areas worldwide could easily be achieved. Over the past decade new plant species, including many ornamentals, have been reported as hosts, particularly in North and South America and Asia. In 2015 an I-seq 33 population was detected in roses in the Netherlands showing typical bacterial wilt symptoms; this population was highly virulent to many plant species, including roses and potato. Recently R. pseudosolanacearum (phy I) has been found in aquatic environments in temperate climates in Europe. These findings, in combination with the pattern of dissemination of this pathogen through irrigation water, crop debris, common agricultural practices and increased trade in plant products, pose a serious threat to agricultural production in the temperate climates and could result in major outbreaks of this disease on known and new host plants. The results obtained from our work on Ralstonia pseudosolanacearum (phy I) will be discussed.

C8.3-5

BACTERIAL WILT OF POTATO: A THREAT TO FOOD SECURITY IN SUB-SAHARAN AFRICA

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Text
Potato is an important food security crop and source of household income for many rural smallholder farmers in sub-Saharan Africa (SSA). Despite the importance of potato, potato productivity remains at 4-9 t ha-1, largely due to bacterial wilt caused by Ralstonia solanacearum species complex (RSSC). Latently infected propagation material such as potato seed tubers are known to be a source of long distance spread of the pathogen and pose a threat to crop production and contribute to pathogen dispersal. The potato seed system in SSA is largely informal, and unrestricted in regional and cross-border movement of seed potato. The unregulated seed movement poses a great threat in disseminating RSSC strains to uninfected areas and have been introducing new strains from abroad as well. Here, we will give insight into the potato seed system in sub-Saharan Africa; distribution, pathogenic diversity, epidemiology of RSSC strains, and the state of the art for management of bacterial wilt under small holder farmers' prospective. Components of the bacterial wilt management strategies examined include diagnostics and surveillance, prevention and control of infection using phytosanitation and use of bacterial wilt free seed, and management of disease through the breeding and promotion of varieties, and cultural practices. We highlight key research areas that need prioritization and conclude by examining the future outlook for bacterial wilt disease management in potato from SSA prospective.

C8.3-6

ELUCIDATION OF INFECTION MECHANISM OF RALSTONIA SOLANACEARUM ON GINGER USING ASEPTICALLY REGENERATED PLANTS

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(1) Kochi University, Nankoku, JAPAN; (2) Ehime University, Matsuyama, JAPAN

Text
Ginger (Zingiber officinale) is an important spice crop vegetatively propagated through rhizomes. *Ralstonia solanacearum* hampers ginger production. It is laborious and time-consuming to prepare enough numbers of ginger plants for the pathogenicity test. We developed a new pathogenicity test of *R. solanacearum* using aseptically regenerated ginger plants. Ginger plants were regenerated in vitro using 6-benzyl adenine and 1-naphthalene acid (NAA) in Murashige-Skoog (MS) agar media from shoot tips. The regenerated plants were cultured in liquid MS media with NAA and used to evaluate the pathogenicity by root dipping inoculation. When a wild-type strain MAFF 211479 was inoculated, wilt symptoms such as yellowing of leaves were observed within 15 days of post-inoculation (dpi), and ginger plants died at 28 dpi. MAFF 301069, a non-virulent strain of ginger, did not show symptoms on ginger plants. These results demonstrated that new method using the regenerated ginger plants in vitro is suitable for the virulence assessment of *R. solanacearum*. We constructed MAFF 211479 mutants defective in type III secretion system. All the mutants lost pathogenicity in the regenerated ginger plants as well as in the eggplants. The mutant cells proliferated less efficiently than the wild type in the inoculated ginger plants. Based on these results, we conclude that the aseptically regenerated ginger

plants could be used to elucidate the infection mechanism of R. solanacearum in ginger.

P8.3-001

CONTRIBUTION OF THE QUORUM SENSING OF RALSTONIA PSEUDOSOLANACEARUM STRAIN OE1-1 TO ITS INFECTION IN TOMATO ROOTS AND VIRULENCE

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Text
The soil-borne Gram-negative β-proteobacterium *Ralstonia pseudosolanacearum* strain OE1-1 secretes methyl 3-hydroxymyristate (3-OH MAME) as a quorum sensing (QS) signal. In the active state of QS, the LysR family transcriptional regulator PhcA regulates virulence-related genes. Our omics analysis of the strain OE1-1 showed that QS consists of the 3-OH MAME-dependent cascade for the PhcA activation and the 3-OH MAME-independent cascade for the PhcA production. To elucidate how QS contributes to the infection process of strain OE1-1 in tomato roots, we developed an in vitro pathosystem using 4 days after sowing-tomato seedlings. The microscopic observation showed attachment of the strain OE1-1 to surfaces of the meristematic and elongation zones in tomato roots and a detached epidermis. The strain OE1-1 colonized intercellular spaces between the epidermis and cortex, then infected cell wall-degrading cortical cells adjacent to the epidermis, followed by forming mushroom-shaped biofilms. The strain OE1-1 next progressed through intercellular spaces of the cortex and endodermis, infecting pericycle cells and xylem vessels. The *phcA*-deletion mutant lost its infectivity in cortical cells and the following infection in xylem vessels to lose its virulence. Taking results together, infection of the strain OE1-1, which attaches to surfaces of the meristematic and elongation zones, in cortical cells dependently on QS leads to its subsequent infection in xylem vessels and virulence.

P8.3-002

PLANT SIGNALS THAT INDUCE THE HRP REGULON IN RALSTONIA SOLANACEARUM AND THE COGNATE R. SOLANACEARUM RECEPTORS

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Text
Genes encoding a type III secretion system in *Ralstonia solanacearum* are regulated by HrpB as a *hrp* regulon, which is induced only in the plant. This study aims to identify plant

signals inducing the *hrp* regulon and confirm the recognition mechanism of signals.
Signal molecules inducing the *hrpB* expression were screened with an assay system using resting cells of the *hrpB-lacZ* reporter strain. The soluble and insoluble fractions were prepared from tobacco seedlings. Only the soluble fraction induced the *hrpB* expression. The heated soluble fraction retained the *hrpB*-inducing activity, indicating that active compounds were not proteins. When the soluble fraction was fractionated into acidic, neutral, and basic components, the acidic and neutral fractions induced the *hrpB* expression. Among organic acids in the acidic fractions and sugars in the neutral fractions, malic acid and sucrose mainly induced the *hrpB* expression.
The sucrose-induced *hrpB* expression was significantly reduced in a *prhA* mutant, suggesting that sucrose might be perceived with the outer membrane protein PrhA. This result agrees that the *hrpB* expression is controlled by a signal cascade, PrhA-PrhI/R-PrhJ-HrpG. We constructed a mutant library of histidine kinase genes in the two-component system. The malic acid-induced *hrpB* expression was reduced in the mutant of one of the histidine kinase genes, *rsc1598*. Rsc1598 could perceive malic acid, and the signal might be transferred to a response regulator HrpG.

P8.3-003

COEXPRESSION NETWORK ANALYSIS TO UNDERSTAND THE QUORUM SENSING-DEPENDENT GENE REGULATION MECHANISM IN RALSTONIA PSEUDOSOLANACEARUM STRAIN OE1-1

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Text
Soil-borne Gram-negative bacteria, *Ralstonia solanacearum* species complex (RSSC), cause wilt diseases in a wide-range of crop plant species. For infection of a phylotype I strain of RSSC, *Ralstonia pseudosolanacearum* strain OE1-1, cell density-dependent gene regulation system, quorum sensing (QS), has an important role. Although it is known that QS affects the gene expression of OE1-1 cells as well as their behaviors, its gene regulatory manner is largely unknown. To understand the gene regulatory manner by QS, we performed gene coexpression network analysis using a series of transcriptome data. Coexpression network analysis successfully provided multiple coexpression modules with distinct gene expression patterns. Gene Ontology enrichment analysis was applied to the gene set in each module and the terms indicating specific functions were identified. The expression of the multiple modules was significantly affected by QS in both positive and negative ways, suggesting that QS has a large effect on global transcriptome change in OE1-1 cells. Also, we found that the presence of iron significantly affects the expression level of the specific modules including QS-dependent ones. These data suggest that OE1-1 cells change their behaviors during host plant infection by changing the gene expression patterns through QS-dependent gene regulation dependently on environmental conditions including iron acquisition from environments.

P8.3-004

THE TOMATO P69 SERINE PROTEASES PLAY A ROLE IN RESISTANCE TO BACTERIAL WILT

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Text

The plant intercellular place or apoplast is the main battlefield between plants and pathogens.We carried out a proteomic analysis of the tomato apoplast andidentified the serine proteases of the P69 family as specifically activated upon Ralstonia solanacearum infection in the resistant cultivar Hawaii7996.
We present the characterisation of tomato P69s in tomato defence to bacterial wilt. Transient expression of 6 P69 paralogs was performed in Nicotiana benthamiana , showing that overexpression of P69B, D and G limited multiplication of R. solanacearum, while P69C caused cell death in tomato. Heterologous production and purification of P69s showed low cleavage specificity in vitro. The P69D prodomain removal occurred in an autocatalytic and intramolecular reaction in residue(s) other than that immediately preceding the TTHT motif. Finally, generation of a P69D CRISPR loss-of function mutant in resistant tomato Hawaii 7996 rendered the plant more susceptible to R. solanacearum but not to other vascular pathogens like Fusarium oxysporum.
Our results demonstrate for the first time a key role for the P69 clade of serine proteases in plant defence.

P8.3-005

FERRIC UPTAKE REGULATORS, FUR1 AND FUR2, AFFECT THE EXPRESSION OF QUORUM SENSING-REGULATED GENES IN THE CONDITIONS WITH AND WITHOUT FERROUS IRON IN RALSTONIA PSEUDOSOLANACEARUM STRAIN OE1-1

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Text
The LysR-type transcription regulator PhcA is activated by the bacterial density-dependent quorum sensing (QS) and regulates QS-dependent genes containing virulence-related genes in the soil-borne Gram-negative β-proteobacterium *Ralstonia pseudosolanacearum* strain OE1-1. We previously identified an involvement of ferrous iron in QS-dependent phenotypes of the strain OE1-1. Furthermore, two Ferric uptake regulator sequences, Fur1 and Fur2, were found in the genome of the strain OE1-1. To elucidate the mechanisms of Fur1 and Fur2 on QS-dependent gene regulation in the conditions with and without ferrous iron, we first performed the RNA-seq to identify the transcriptome of *R. pseudosolanacearum* strains. In the condition with ferrous iron, *fur1*-deletion led to a significantly reduced expression of 267 QS-dependent genes. Among these genes, expression of 115 genes including virulence-related genes involved in production of ralfuranone, major extracellular polysaccharide EPSI and plant cell wall degradation enzymes was significantly enhanced by the *fur2*-deletion in the condition without ferrous iron. Furthermore, *fur2* expression in the condition without ferrous iron was significantly enhanced compared to that with ferrous iron. Taking results together, Fur1 and Fur2 positively and negatively regulate some QS-dependent genes in the condition with and without ferrous iron, respectively.

P8.3-006

SELECTIVE ISOLATION OF RALSTONIA SOLANACEARUM ON MSMSA IN A SAPROPHYTIC CONTEXT

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Text
For the official testing of Ralstonia solanacearum (R. sol) a high level of standardization among diagnostic laboratories already exists. Isolation of R. sol on the modified semi-selective medium from South Africa (mSMSA) is part of the official testing. Although this isolation results in highly consistent results even at low bacterial densities, it often requires more training of laboratory staff than the more common molecular techniques. The reason is that secondary bacterial infections or saprophytic bacteria on isolation plates will interfere with the development of R. sol colonies, resulting in atypical colony morphology or inhibition. Additionally, there are several physical and chemical parameters that will influence the performance of mSMSA, such as pH, brand of agar and antibiotics, quality control issues during the in-house preparation, quality control elements during the storage and expiration date, etc. Recently, a practical training on the R. sol isolation on mSMSA was organized at the Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP) in Wageningen, in the framework of the European Reference Laboratory (EURL) for Bacteria. Well-defined samples containing R. sol cells or spiked plant extracts have been used to assess the growth of R. sol (colony numbers and colony morphology) and the suppression of saprophytes, respectively on mSMSA plates prepared by the EU National Reference Laboratories (NRLs). Results will be discussed.

P8.3-007

COMPARISON OF RHIZOSPHERIC BACTERIAL COMMUNITIES OF POTATO GENOTYPES WITH DIVERSE DEFENSE RESPONSES AGAINST RALSTONIA SOLANACEARUM.

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Text

Potato (*Solanum tuberosum*) is one of the most important widespread hosts of *Ralstonia solanacearum*, the causal agent of bacterial wilt. In Uruguay, germplasm with resistance to *R. solanacearum* has been identified and advanced clones with different responses to *R. solanacearum* infection were selected and characterized. Previous results showed that plant resistance was correlated with differential bacterial colonization patterns and induced defense responses after infection. The aim of this work is to study the correlation between plant resistance and rhizosphere microbiome in selected genotypes with different responses to bacterial wilt. Plants were grown in a macrotunnel greenhouse with soil collected from a potato field. Pathogen colonization effects on rhizosphere microbiota were evaluated in healthy and infected plants. Disease progression was recorded and pathogen was quantified in rhizosphere samples. A resistant genotype showed delay in pathogen colonization and high final pathogen concentration in the rhizosphere, comparable to the susceptible genotype. These results suggest that the resistant genotype restricts rhizosphere pathogen colonization, preventing root and stem infection. Bacterial community composition is being analyzed in 76 samples comparing the sequence of V3-V4 region of 16S rARN. It is expected to identify potentially beneficial microbial groups related with resistant plants, contributing to an integrated disease control.

P8.3-008

APPLICATION OF RECOMBINANT INTERNAL CONTROL FOR INCREASING QPCR RELIABILITY IN THE QUANTITATIVE DETECTION OF RALSTONIA SOLANACEARUM SPECIES COMPLEX (RSSC) IN SOIL SAMPLES

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Text

Quantitative PCR (qPCR) was frequently used to detect soil-borne pathogens, but the

accurate quantification is compromised by poor DNA extraction and the presence of PCR inhibitors. Here, we developed a new qPCR system and an Internal Sample Process Control (ISPC) strain, RsPC, for the detection of the bacterial wilt pathogen RSSC, including *R. solanacearum*, *R. pseudosolanacearum*, and *R. syzygii*. Specific primers and TaqMan MGB probes were designed based on the analyses of 16S rDNA sequences from 603 *Ralstonia* genomes, and the RsPC was constructed by chromosome insertion of an artificial ISPC fragment in a closely related non-pathogenic strain, *R. pickettii* JCM 5969. The qPCR target sequences of RSSC and RsPC shared primers in PCR amplification, but distinguished from each another by different TaqMan probes. We tested 10 different soil samples with artificially co-spiked RsPC and *R. pseudosolanacearum* LMG 9673 at different concentrations, and found comparable recovery efficiencies (REs) of two strains in most samples, and the RE values of LMG 9673 after correction by RsPC were much closer to theoretical values. The most obvious improvement was observed in a heavy clay soil sample, in which the RE of LMG 9673 was increased by 3.0-fold. The qPCR system and ISPC strain developed in this study could be applied for the accurate detection of RSSC in soil, and similar ISPCs can be developed in future for risk analysis of soil-borne animal and plant pathogens.

P8.3-009

FIRST REPORT OF RALSTONIA PSEUDOSOLANACEARUM ON BOESENBERGIA ROTUNDA FROM THAILAND

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Text

Ralstonia pseudosolanacearum is part of the *Ralstonia solanacearum species complex* (RSSC). This group of bacteria has a very wide host range including many economically important crops, such as potato and tomato, and can cause large scale crop losses. The members of the RSSC are all quarantine organisms in the UK. Routine testing is carried out at Fera Science Ltd. to screen for the presence of R. solanacearum in latent potato tubers and river water; additionally, symptomatic samples intercepted by the UK Plant Health and Seed inspectorate are tested for the RSSC.
In October 2021 a sample of *Boesenbergia rotunda* (Chinese ginger, fingerroot) from Thailand was received at Fera showing symptoms of vascular discolouration and a milky ooze. The sample tested positive for *R. pseudosolanacearum*; the first finding on this host. Two distinct colony morphologies were observed when grown on semi-selective media and were treated as two isolates. The isolates were whole genome sequenced, and a novel *Ralstonia* phage was found to be associated with one isolate only. Host testing was carried out to assess pathogenicity, showing that the isolate with the associated phage was less virulent on eggplant and tomato. The interception of quarantine pests at the borders is vital for maintaining good biosecurity practices to prevent the entry of pathogens into countries where they are currently absent, and it is important to investigate novel methods of controlling this pathogen.

P8.3-010

VARIETY OF PATHOGENICITY OF RALSTONIA SOLANACEARUM STRAINS.

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Text

Brown rot of potato caused by Ralstonia solanacearum (Rs) (Smith) Yabuuchi et al., is one of the most important quarantine diseases of the plant. Its presence is usually associated with significant economic losses to the potato industry where the disease exists.
One of the most crucial factors responsible for the uncontrolled spread of the pathogen in the environment and during the production process is its pathogenicity.
Of the four phylotypes of the Ralstonia species listed in EPPO diagnostic protocol No. PM 7/21, each consisting of many different phylogenetic and pathogenic variants, one of the most virulent genotypes is phylotype IIB 1 (formerly known as race 3 biovar 2). This phylotype is particularly harmful because it has a relatively low growth temperature (approximately 27 °C) and often causes latent (asymptomatic) infections. It can relatively easily adapt to colder climates and is favored by the wetness of the soil. Also, the presence of the pathogen in low concentrations in potato tissue is very dangerous and allow to contributes to its rapid spread of it in the environment.
In all the above cases, it is essential to determine the virulence of pathogenicity of the pathogen, which was the purpose of the research. The obtained results allowed for the determination of the influence of the examined Ralstonia solanacearum strains, on the level of expression symptoms on the tested plants and for comparison with the obtained result of the molecular test.

Mind the Gap: Innovation and Opportunities in Seed Health testing

C1.6-1

IMPACT ON GLOBAL FOOD SECURITY AND BIOLOGICAL RELEVANCE

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Text

Seed is a globally traded agricultural product. Predictable international movement of seed is critical to ensure food security. Seed companies produce and trial seed in different countries all over the world to mitigate the risk of crop failures due to several different reasons,

including adverse weather conditions or pests. The supply of healthy seeds is essential to help assure growers of a healthy crop.
Routine seed health assays may use several different types of technology. There are basic methods such as a grow out or seed plating, in which seeds are germinated under favourable environmental conditions to encourage disease development should the seed be contaminated with a pathogen. This type of assay is considered a direct method, that is, it permits the pathogen to be observed, recovered, and confirmed as such via Koch's postulates. Given the amount of time and resources associated with direct assays, many researchers are pursuing the development and implementation of indirect assays such as ELISA and PCR.
Indirect methods provide an indication of pathogen presence as these assays detect specific proteins or nucleic acids. Indirect methods may give a positive result even when no viable pests are present. Consequently, when testing seeds with these methods, results should be interpreted carefully. Confirmatory tests based on a different biological principle may be required to confirm the presence of a viable pest in a sample and to determine associated disease risk.

C1.6-2

IDENTIFICATION AND PREVALENCE OF SEEDBORNE FUNGAL PATHOGENS ASSOCIATED WITH SOYBEAN

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Text
Seed health is critical to ensuring a healthy yield and mitigating risks associated with pathogen introduction and spread. The study identified and determined prevalent fungal pathogens associated with South African soybean seed to inform management decisions. Approximately 650 kg seed from the first season (2020) was manually separated into twenty-one symptom categories. Seed germination and fungal enumerations were conducted. Genera identified through morphology and sequencing include *Alternaria, Chaetomium, Cladosporium, Epiccocum, Fusarium, Mucor, Nigrospora, Phoma, Rhizoctonia, Sclerotinia, Stagonosporopsis* and *Trichoderma*. Prevalence was determined by fungal enumerations and germination testing from 415 and 402 seed harvested in season two (2021) and three (2022), respectively. Seedborne fungi significantly reduced seed germination in season three (3.5%) compared to season two (35.4%), suggesting weather conditions were more conducive for disease development. *S. sclerotiorum* and *Fusarium* spp. were detected in the highest frequencies across the seasons. The significant prevalence of seedborne *S. sclerotiorum* is cause for concern as seed is retained by producers for subsequent seasons, as only one fungicide is registered to control soybean stem rot in South Africa. Koch's postulates and a diversity study of 24 *Fusarium* isolates is ongoing to determine whether causal organisms of sudden death syndrome are present and differ in aggressiveness.

C1.6-3

STREAMLINING DIAGNOSTICS FOR SEEDS IMPORTED INTO AUSTRALIA

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Text

Seed is a pathway for the global spread of some pathogens that can be seedborne, and that impact crop production. The Australian Department of Agriculture, Fisheries and Forestry requires testing for specified pathogens to mitigate the risk of regulated and exotic seed borne pathogens of grains, pulses, and selected vegetable species entering Australia. While seed testing reduces the risk of introduction, seed testing can impose a significant economic impact on seed companies, associated with the cost of the high volume of seed required for testing. A flow-on effect is increased cost of seed for Australian producers. Importantly, it results in reduced availability of new genetics that improve quality and production for Australian growers and enable them to remain competitive globally, because seed companies import less into Australia. There is a need to find a balance between testing to support the biosecurity of the Australian agricultural industries and the requirement of an affordable seed supply for producers and access to new varieties. In this presentation, an overview of the regulatory seed testing required for Australia will be presented along with an overview of new research that will evaluate the application of novel metagenomic and targeted high throughput sequencing approaches to improve the efficiency, sensitivity, and cost-effectiveness of seed testing to meet the needs of Australia's biosecurity and minimise the economic impact of seed importation to industry.

C1.6-4

IDENTIFICATION, DETECTION AND MANAGEMENT OF SEEDBORNE SQUASH PATHOGENS

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Text

Squash is one of the most important vegetable crops, and it can be affected by several fungal seedborne pathogens. Samples of asymptomatic and symptomatic squash fruits (Cucurbita maxima, Cucurbita moschata) were collected from Tunisia and Italy. Following the blotter test, seedborne fungi were identified in seeds extracted from fruit samples. The most frequent fungi in Tunisia seed samples were Alternaria alternata, followed by Stagonosporopsis cucurbitacearum. For the fruits from Italy, the most frequently identified fungal species in seed samples were A. alternata, followed by Stemphylium vesicarium. Seedborne fungi were identified in all fruit samples tested, including asymptomatic fruit. Considering that S. cucurbitacearum can cause medium-high economic losses in the field, even with low seed infection, our research focused on setting up a rapid and sensitive

protocol, based droplet digital polymerase chain reaction (ddPCR). Blotter and ddPCR tests showed a high degree of correlation (R2 = 0.986, p ≤ 0.01). Our ddPCR protocol provided rapid detection and absolute quantification of S. cucurbitacearum, offering a useful support to the standard procedure. To control these fungi, the antifungal activity of seven essential oils have been studied by tests performed in vitro and in vivo conditions. Both assays showed that Cymbopogon citratus essential oil was the most effective to reduce seedborne fungi and to control transmission of S. cucurbitacearum from seeds to plantlets.

C1.6-5

FUTURE FOR HTS IN SEED HEALTH TESTING?

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Text

Tobamoviruses are a serious challenge for the production of tomato and pepper. Tobamovirus contaminated seeds are a pathway for dissemination, and several complementary methods are used to determine whether tobamoviruses are present in seeds and if this poses a risk for disease establishment. ELISA is widely used to detect tobamoviruses on seeds but this method cannot distinguish between infectious and non-infectious viruses. Only bioassays can differentiate infectious tobamovirus particles, and the efficacy of disinfection treatments is evaluated with a bioassay. Seed extract (SE) TaqMan RT-PCR assays were introduced more recently to detect ToBRFV and ToMMV. However, these assays do not discriminate between infectious and non-infectious viruses, but are very sensitive and highly specific. Naturally contaminated seed lots with tobamoviruses have been collected at Naktuinbouw since 1993. A high throughput sequencing (HTS) project using almost 100 tomato and pepper seed lots from this collection was started. The objective was to determine the presence, abundance and genetic variability of tobamoviruses. Illumina HTS was performed with purified RNA from the seed lots. In addition, a SE PCR assay and a bioassay were carried out to further characterise infection in these seed lots. In pepper and tomato seed lots, several tobamoviruses were detected using HTS, with a highly variable virus load. Pros and cons of HTS and the currently applied detection methods will be presented.

C1.6-6

MOLECULAR DETECTION OF USTILAGO NUDA IN BARLEY SEEDS AND CORRESPONDING FIELD INFECTION LEVELS

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Text

Agricultural policies aim to reduce synthetic plant protection products (PPPs), including prophylactic seed treatments. A broader use of untreated seed would help limit synthetic PPP applications. Currently, seed is certified through the visual detection of seed-borne diseases, including loose smut (*Ustilago nuda*) of barley (*Hordeum vulgare*). *U. nuda* infection is asymptomatic in seeds and plants until its teliospores develop at barley heading. PCR-based molecular detection of *U. nuda* can help streamline the certification process for untreated seed and improve its accuracy and precision. We developed a multiplex qPCR protocol that targets *U. nuda* and *H. vulgare* DNA. The new molecular assay and a visual detection method using extracted embryos were performed to analyze seed samples with varying rates of infection. The resulting disease levels were then evaluated in field. Based on the 2022 data, *U. nuda* field infections show a stronger positive correlation with the qPCR results than those from the visual analysis of embryos. The experimental results enable us to propose a threshold value of *U. nuda*/*H. vulgare* DNA to delineate healthy and diseased seeds. Future research with additional samples from practice is needed to validate the proposed threshold under diverse environmental conditions. Molecular methods in seed health testing could aid in the wider adoption of sowing untreated seed and reduce synthetic PPP application.

P1.6-001

TESTING AND TRENDS IN SEED-TRANSMITTED DISEASES OF PULSE CROPS IN MONTANA, USA

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Text

The Regional Pulse Crop Diagnostic lab (RPCDL), founded at Montana State University (USA) in 2014, conducts disease testing of pulse crops (dry peas, chickpeas, and lentils) for management of seed-transmitted diseases and to protect international trade. Testing samples through the RPCDL facilitates tracking of disease incidence and severity over time. The primary seed-transmitted disease of interest is typically Ascochyta blight (*Didymella*, *Peyronellaea* spp). Disease tracking allows the lab to do further testing for concerns such as fungicide insensitivity. For example, Ascochyta blight pathogen isolates were collected from the 2013 crop year and tested for fungicide insensitivity. Of 145 isolates, 4 chickpea (*Didymella rabiei*) and 1 dry pea (*Didymella pisi*) isolate were insensitive to pyraclostrobin; 1 chickpea isolate was insensitive to fluxapyroxad and boscalid. These seed lots were eliminated from re-planting. No insensitivity was detected in 2014, and 1 chickpea seed lot with pyraclostrobin insensitivity was identified and eliminated from re-planting in 2015. No insensitivity was detected in 2016. Persistent regional drought reduced disease levels in 2017 through 2022 and thereby negated demand for fungicide insensitivity testing. However, chickpea seed lots infested with Botrytis were noted despite dry conditions. Fungicide insensitivity testing has resumed in 2023 as high-infestation seed lots have been received.

P1.6-002

VALIDATION OF A ASCOCHYTA RABIEI DETECTION METHOD ON CHICKPEA SEEDS.

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Text

Blight of chickpea caused by Ascochyta rabiei is a not well-known disease with a significant impact on yields in both organic and conventional farming.
The AsCoLup project, funded under the CASDAR IP 2019, bringing together 15 partners aims to provide producers with knowledge on Ascochyta rabiei as well as an adapted technical itinerary in order to improve disease management, both in seeds and food production.
Pathogen detection is an important part of the technical itinerary. It contributes to the guarantee of production of healthy seeds and to the evaluation of the risk in the field.
The international validation of a seed detection method is essential for disease management.
A quantitative method on media has been chosen and the performance criteria (analytical sensitivity, analytical specificity, accuracy, repeatability and reproducibility) are studied to validate the method. A pathogenicity test is also developed to verify the pathogenicity of isolates.
The validation of this method will lead to a proposal of an ISTA method on a new pathogen/host combination.

P1.6-003

BRAZILIAN PCR TESTING IN CROP SEEDS

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Text

The PCR is an essential tool for the identification of seed pathogens from crops around the world. In Brazilian research, this theme is growing for the modernization of disease diagnostics. Then, this research has the aim of resuming information about the PCR methods from Brazilian Seed Pathology Research. For this research, on Web of Science platform were inscribed the keywords: seeds, PCR, pathogen, and Brazil. As a result, just fifty-seven papers were found about this theme. The prevalent area was Crop production (30% of the total), Dr. José da Cruz Machado (Lavras Federal University) was the most important author (6 publications). The most important pathosystems analyzed were Corynespora cassiicola, Sclerotinia sclerotium, Colletotrichum truncatum, and Phomopsis spp. (Glycine max); Stenocarpella spp. and Fusarium spp. (Zea mays), Xanthomonas spp. (Brassica spp.). Primer design and PCR methods were the important theme in this bibliometric research. The use of Pcr methods for seed pathology diagnosis is an important and few-studied theme, more research can be made to better the seed sanity in Brazil.

P1.6-004

USE OF BASIC SUBSTANCES AND POTENTIAL BASIC SUBSTANCES FOR THE CONTROL OF SEEDBORNE PATHOGENS

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Text

Seedborne pathogens represent an overly critical issue for successful agricultural production worldwide. Seed treatment with plant protection products constitutes one of the first options useful to reduce seed infection or contamination and prevent disease spread. Basic substances are active, non-toxic substances already approved and sold in the EU for other purposes, e.g., as a foodstuff or a cosmetic, but that can also have a significant role in plant protection as ecofriendly, safe, and ecological alternatives to synthetic pesticides. They are regulated in EU according to criteria presented in Article 23 of Regulation (EC) No 1107/2009. In recent years, various potential uses of already approved and potential seed treatment products were investigated for their proven activity against fungal, bacterial and viral seed-borne pathogens. The aim of this research, run within Euphresco BasicS project, is to is to collect the information on application of basic substances and potential basic substances for seed treatment and to make this large amount of published research results more manageable for consultation and use. The latest advanced research in finding the best application methods to coat seeds are also described.

This work was conducted within the framework of the Euphresco BasicS Project

Modeling and analysis to better understand and predict epidemics

C5.1-1

BAYESIAN SPATIAL EPIDEMIOLOGICAL MODELS TO ASSIST IN OUTBREAK RESPONSE. THE CASE OF XYLELLA FASTIDIOSA

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Text
Epidemiological models can assist in extracting information from disease outbreak data and improve response programs. Climatic and spatial effects on the distribution of *Xylella fastidiosa* (*Xf*) were studied in two outbreaks with different epidemiological settings (Lecce, Italy, and Alicante, Spain) using spatial Bayesian models. The climatic covariates were not related with the *Xf* distribution in the study areas, indicating that climate is unlikely to stop disease spread to adjacent areas. The probability of *Xf* presence increased with the proximity to the infested area, illustrating the strong influence of the spatial component. Though, spatial dependence in epidemiological models is often assumed directionally invariant and uniform across the study area (i.e., isotropic and stationary). These assumptions do not hold when there are elements limiting disease spread, such as geographical barriers or control measures. Hence, the effects of dispersal barriers in the *Xf* outbreak in Alicante were analyzed. A Bayesian stationary model, without barriers, was compared with non-stationary models including a continuous or discontinuous cordon sanitaire as containment barriers. The cordon sanitaire resulted in a reduced probability of *Xf* presence outside the infested area, except in the discontinuities of the cordon sanitaire with low sampling intensity. The spatial range of the stationary model provided a reference value to define the size of the buffer zone.

C5.1-2

A COMPARTMENTAL MATHEMATICAL MODEL BASED ON APHID FEEDING BEHAVIOURS ALLOWS MORE REALISTIC MODELLING OF NON-PERSISTENTLY TRANSMITTED PLANT VIRUSES

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Text
Plant viruses threaten global food security and are often transmitted by arthropod vectors. Non-persistently transmitted (NPT) plant viruses are characterised by a very short virus retention time in the vector and are transmitted almost exclusively by aphids. Compartmental models using ordinary differential equations to capture the course of an epidemic have been used in plant virus epidemiology for decades. However, the underlying model structure, in which the infective period of vectors is fixed, omits a key feature of non-persistent transmission: probing or feeding on a plant is often what causes an aphid to lose its infectivity. A recent model by Donnelly et al. (2019) captures this behaviour via a Markov chain that tracks the behaviour of individual aphids. We introduce a new compartmental model which replicates this model, while allowing the easy extensibility characteristic of compartmental models. It is comprised of linked Susceptible-Infected models for the plants and aphids, where loss of aphid infectivity is conditioned upon aphid probing and feeding behaviour, rather than occurring at a fixed rate. This additional biological realism means our model behaves differently to previous compartmental models of NPT viruses, therefore allowing us to more accurately investigate virus transmission dynamics for all NPT systems. We focus on the case of viral manipulation of host plant phenotype, which changes aphid landing and feeding behaviour to enhance virus spread.

C5.1-3

EXPLOITING SIMILARITIES WITHIN PHYLOGENETIC CLADES OF COLLETOTRICHUM SPP. TO DEVELOP A MECHANISTIC, WEATHER-DRIVEN MODEL FOR ANTHRACNOSE DISEASES

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Text

Fungi of the genus Colletotrichum cause serious pre- and post-harvest losses to several agricultural crops worldwide. A systematic literature review was conducted to retrieve the metadata on the influence of temperature on four biological processes: (mycelial growth, conidial germination, infection by conidia, and sporulation) for seven Colletotrichum clades (acutatum, graminicola, destructivum, dematium, gloeosporioides, and orbiculare) and the singleton species, C. coccodes. The metadata was first analyzed to define inter- and intra-clades similarities and differences, and then used to develop temperature-dependent equations representing the effect of temperature on the biological processes for the different clades. This clade-based approach was used to develop a mechanistic, weather-driven model for Colletotrichum anthracnose diseases, able to predict anthracnose progress during the growing season on the aerial organs of different herbaceous and fruit tree crops. The model was evaluated against the disease progress of fungi belonging to five clades on six different hosts by using data from epidemics occurred in Italy, the USA, Canada, and Japan. Results showed high concordance between model predictions and field data, with overall concordance correlation coefficient of 0.928. After further validation, the model could be used to support decision-making for crop protection in a wide range of cropping systems.

C5.1-4

EFFECTS OF PATHOGEN SEXUAL REPRODUCTION ON THE EVOLUTIONARY AND EPIDEMIOLOGICAL CONTROL PROVIDED BY DEPLOYMENT STRATEGIES FOR TWO MAJOR RESISTANCE GENES IN AGRICULTURAL LANDSCAPES

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Text

Resistant cultivars are of value for protecting crops from disease, but can be rapidly overcome by pathogens. Several strategies have been proposed to delay pathogen adaptation (evolutionary control), while maintaining effective protection (epidemiological control). Resistance genes can be *i*) combined in the same cultivar (pyramiding), *ii)* deployed in different cultivars sown in the same field (mixtures) or in different fields (mosaics), or *iii*) alternated over time (rotations). The outcomes of these strategies have been investigated principally in pathogens displaying pure clonal reproduction, but many pathogens have at least one sexual event in their annual life cycles. Sexual reproduction may promote the emergence of superpathogens adapted to all the resistance genes deployed. Here we improved the spatially explicit stochastic model *landsepi* to include pathogen sexual

reproduction, and then investigated the effect of sexual reproduction on evolutionary and epidemiological outcomes across deployment strategies for two major resistance genes. Sexual reproduction favours the establishment of a superpathogen when single mutant pathogens are present together at a sufficiently high frequency, as in mosaic and mixture strategies. However, sexual reproduction did not affect the optimal strategy recommendations for a wide range of mutation probabilities, associated fitness costs, and landscape organisations.

C5.1-5

A SPATIALLY EXPLICIT NETWORK MODEL TO ASSESS THE EFFECTIVENESS OF WITHIN- AND BETWEEN-SITE TREATMENT ON EPIDEMIC SPREAD ACROSS A LANDSCAPE

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Text

The relevance of networks for improved understanding and control of plant disease epidemics has gained prominence in plant pathology in the last two decades. Disease spread between nodes in a network has been a central topic and limited attention has been given to within-node dynamics. We develop a spatially explicitly network model to establish relative effectiveness of implementing within-node treatment (protectant or curative fungicide and roguing) and between-node treatment (quarantine, trade) on epidemic spread of an aerially dispersed pathogen. The dynamic component of the model is captured by the number of host units that are either Susceptible (S), Protected (P), Exposed (E), Infected (I), Treated (T) or Removed. Within-node dynamics modeled using rates at which, I infect others, E becomes infectious, I loses infectivity, and fungicide effectiveness parameters. Spread between nodes is modeled using a power-law dispersal kernel and the rate of trade between nodes. Surveillance time is drawn from a uniform random number and when disease is detected, a node is treated as described above. Simulations are run with the model, representing experiments with three network structures [small-world, random, and scale-free] of 200 nodes with varying sizes with three different levels of connectivity for network sizes of 200, 400 and 800. Results of the simulations will be discussed and we plan to avail the model in an open-source environment for use by interested researchers.

C5.1-6

MODELING THE AIRBORNE INOCULUM OF POLYSTIGMA AMYGDALINUM FOR IMPROVING THE CONTROL OF ALMOND RED LEAF BLOTCH

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Text

Red leaf blotch (RLB) of almond, caused by *Polystigma amygdalinum*, is one of the most important foliar diseases affecting this crop in the Mediterranean Basin and Middle East regions. The pathogen overwinters in the leaf litter, where ascospores mature in the perithecia and are released in spring, infecting new leaves. The main control strategy is the application of fungicides on a calendar basis. Thus, predicting pathogen inoculum availability is important to determine the protection period and to optimize RLB fungicide programs. A Bayesian beta-regression mixed modeling framework was assessed to model the dynamics of airborne *P. amygdalinum* ascospores considering different meteorological variables. The model with the best predictive performance included the variables ADD (accumulated degree days) and ADDwet (ADD considering precipitation and vapour pressure deficit), together with a random factor. This selected model was used to define action thresholds to delimitate the start and the end of the protection period. The performance of a fungicide program based on the proposed prediction model was commercially evaluated in comparison to the standard calendar-based program and an untreated control in terms of RLB incidence and number of fungicide sprays per season. The fungicide program based on the model was similarly effective as the calendar-based, resulting only in a 2.6% higher RLB incidence but with fewer sprays (three to four, compared with seven in the calendar).

F5.1-1

A COMBINED AEROBIOLOGICAL APPROACH ALLOWS DIFFERENTIATING THE BIOLOGY AND ENVIRONMENTAL DRIVERS OF SPORULATION OF TWO MAJOR FUNGAL PATHOGENS OF CHESTNUT

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Text

Investigating sporulation patterns is pivotal in plant disease epidemiology because it may shed light on relevant biological features of pathogens and on the environmental factors driving the release of infectious propagules. The ascomycetes *Cryphonectria parasitica* and *Gnomoniopsis castaneae* are major pathogens of chestnut (*Castanea* spp.) characterized by having both teleomorphic and anamorphic stages. In this study, we assessed and compared spatial and temporal spore deposition patterns of the two pathogens by using an aerobiological approach combining passive spore trapping, taxon-specific qPCR assays, and statistical modelling based on a novel index called Standardized Deposition Rate. Approximately 1300 samples collected at regular intervals over two years in three chestnut orchards in northern Italy were analyzed. Results showed that both species sporulate all year long, but while for *C. parasitica* peaks are seasonal and propagule loads are driven by the number of weekly rainfalls, spore deposition of *G. castaneae* increases with raising temperatures and wind gusts. Differences in the geospatial patterns of spore deposition

between the two pathogens are discussed. Our results may contribute predicting the risk of infection of these two fungal plant pathogens.

F5.1-2

AEROBIOTA COMMUNITY ASSEMBLY IN VEGETABLE PRODUCTION: ADAPTING METACOMMUNITY THEORY TO NANOPORE METABARCODING.

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Text

The intensification of agriculture has served to meet the ever-increasing food demand. However, it is also responsible for the loss of biodiversity. Among the alternatives to intensive agriculture, agroecology stands out. Its aim is to develop production systems based on the ecological performance of agrosystems, which depends in part on the assembly of the ecological communities (group of species that occurs together in space and time) and metacommunities (group of communities linked together through dispersal) that structure them. Aerobiota metacommunities represent a model system for testing metacommunity theory, as they include a variety of phylogenetically distantly related groups of organisms with different life cycles and dispersal potentials. In 2021 and 2022, aerial samples from onion fields in Québec, Canada, were sampled three times a week in six onion fields from June 1 to August 15. Targeted sequencing of the ITS region was performed on the MinION, while weekly scouting was conducted in each field to monitor plant growth and disease intensity. Using these data, the community structure of the aerobia was investigated from a temporal perspective to model how Natural (weather) and anthropogenic (pesticide treatments) perturbations impacted community composition. This research aims to develop a conceptual framework to test the hypothesis that the type and duration of a disturbance influence both the structure of the metacommunity and post-disturbance recovery.

P5.1-001

GENETIC VARIATIONS AMONG COMMERCIAL VARIETIES AND LANDRACES OF WHEAT FROM PAKISTAN

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Text

Karnal bunt caused by Tilletia indica is one of the most important disease for the international wheat trade market. In this study, epidemiological surveys were conducted in the six districts of lower Punjab, Pakistan. The causal pathogen of karnal bunt were isolated for pathogenicity trials. Wheat germplasms (199) were planted and at the 2nd leaf stage, the crop was thinned and samples were bagged, labeled, and stored for DNA isolation. At the booting stage, five boots per line were infected with spore suspension, and provided a favorable environment for disease infection. At the ripening stage, infected and non-infected

spikes were harvested separately and disease incidence was recorded. The disease incidence was found higher in commercial varieties as compared to landraces. The DNA was studied for genetic variations and trait association studies Illumina iSelect 90K wheat SNP chip was applied on the panel of 199 wheat germplasms. Based on 31,000 SNP markers, 199 wheat germplasm were grouped into two different clusters showing significant genetic variations among them. Six significant markers were identified that were associated with karnal bunt disease. Thirty-two candidate genes were also identified as resistant resources coded on six significant markers. F-box family and kinase like protein family are the genes which has also been reported previously while few novel genes linked with Karnal bunt resistance were also identified in this study.

P5.1-002

CROSS INFECTION OF BOTRYOSPHAERIA SPP. CAUSING DIEBACK IN FRUIT TREES IN CHILE

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Text

Epidemiological studies have focused on the inoculum sources that come from the same host fruit tree. Recently, Neofusicoccum australe and N. stellenboschiana have been described as causing dieback on four different fruit trees in South Africa. However, in Chile, no study has shown infection by Botryosphaeriaceae spp. obtained from different fruit tree species (hosts) induce canker and dieback symptoms in apples, blueberries, grapevines, and walnuts. Therefore, this work aims to determine the virulence of 12 isolates, including Diplodia seriata, D. mutila, Dothiorella sarmentorum, Lasiodiplodia theobromae, N. arbuti and N. parvum collected from different fruit trees with symptoms of canker and dieback. The study was carried out under greenhouse and field conditions, inoculating lignified tissues of grapevine, apple, blueberry, and walnut in central Chile. The results indicate that all the Botryosphaeriaceae species developed lesions in fruit hosts vary in severity, but the most virulent in apple, blueberry, grapevine, and walnut were N. arbuti (apple origin), N. parvum (vine, blueberry, and walnut origin) and D. mutila (walnut origin). An intermediate virulence was obtained by D. seriata (grapevine and apple origin). In contrast, L. theobromae (apple origin) had low virulence in four fruit tree hosts. This study demonstrates the cross-infection capacity of Botryosphaeriaceae obtained from different fruit tree species causing canker and dieback in several hosts.

P5.1-003

ROLE OF SEED TRANSMISSION OF MAGNAPORTHE ORYZAE PATHOTYPE TRITICUM (MOT) FOR THE EPIDEMIOLOGY OF WHEAT BLAST

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Text

Magnaporthe oryzae pathotype *Triticum* (MoT) is a dangerous fungal pathogen causing wheat blast (WB). The limited knowledge on life cycle, epidemiology and pathogenicity of MoT hamper effective disease control. The study aimed to investigate MoT infection, invasion routes, and colonization on wheat ears and seeds to assess the potential of long and short-distance seed transmission of WB. MoT was spray inoculated on susceptible Sumai 3 and resistant Milan. Incidence of MoT on Sumai 3 seeds was 100% and 20-25% on Milan. MoT sporulation rate on Sumai 3 contaminated seeds was 15 times higher than on Milan. The colonization of MoT within seed tissues was monitored by CLSM. Invasion of MoT in seeds was observed predominantly in the caryopsis germ region but also in other seed parts. In the greenhouse, no spread of MoT from infected seeds to seedlings later than GS 21 or to ears was detected, neither in Milan nor in Sumai 3. Initial blast symptoms, only found on seedlings of Sumai 3, resulted in the formation of new conidia, which may serve as inoculum source for plant-to-plant dissemination by airborne infection of plant stands in the field. Ultimately the inoculum formed on young plants may infect ears in the field and contaminate next-generation seeds. We conclude that seed transmission with MoT may disseminate the pathogen long-distance by seed trade and short-distance in the field, strengthening the importance of seed health in strategies to control any further spread of WB.

P5.1-004

POPULATION GENETICS OF GANODERMA BONINENSE, THE CAUSAL AGENT OF BASAL STEM ROT OF OIL PALM IN MALAYSIA AND INDONESIA

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Text

African oil palm (Elaeis guineensis Jacq.) is a perennial oil crop cultivated commercially in Southeast Asia since 1917. Oil palm basal stem rot (BSR) disease, which causes decay of the root bole and palm trunk and eventual death, is the most economically important disease affecting palm losses in oil palm plantations in Indonesia, Malaysia, and Papua New Guinea. SSR genotyping of G. boninense isolates collected from Malaysia and Sumatra (Indonesia) showed constant high genetic diversity and gene flow among populations. This is evidenced by the existence of three genetic clusters and different admixed populations of G. boninense across regions. Low spatial genetic differentiation of G. boninense (FST = 0.05) indicated non-restricted geographical gene dispersal, but a sign of isolation by distance was evident. Furthermore, evidence of population bottlenecks was found in the oldest oil palm plantations in Peninsular Malaysia and Sumatra isolates. The impact of the evolutionary processes on G. boninense population structure and possibly pathogenicity that differs across geographical regions will probably negate the effectiveness of the present generalised approaches to manage infected oil palms in many instances. This may seriously imperil the plantations in the future. Therefore, it is vital to develop BSR disease control taking into consideration

pathogen adaptation and environmental tolerance conferred by pathogen variability in current plantings and future replants.

P5.1-006

EPIDEMIOLOGICAL CHARACTERIZATION OF MULBERRY RUST IN BRAZIL

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Text

Mulberry rust, caused by *Cerotelium fici*, was recently detected on *Morus nigra* in Brazil. The host is an important crop for silk industry. In order to evaluate the monocyclic components of this disease, we have performed experiments under controlled conditions. Inoculum of *C. fici* (5×10^4 urediniospores mL^{-1}) was sprayed on potted mulberry plants. As a control, mulberry plants were sprayed with distilled water. Inoculated and mock-inoculated plants were kept in a dark moist chamber at 23 °C for 24 h. After this period, plants were moved to a greenhouse (25 ± 5 °C). The experimental design was completely randomized with five replicates. Pre-penetration process of *C. fici* on mulberry leaves was analyzed using a scanning electron microscope and the monocyclic components of rust were quantified over time. Twenty-four hours post-inoculation, appressoria were observed on ordinary epidermal cells, indicating direct cuticular penetration of *C. fici.* Rust latent period was 13 d, when small sporulating lesions appeared on the abaxial surface of fully expanded leaves. Mean values for lesion density, lesion size, and rust severity by the end of the experiment were 16 lesions cm^{-2}, 0.9 mm^2, and 13% leaf area diseased, respectively. The infectious period of rust was 52 d. The cumulative production of spores was, on average, 1,585 urediniospores $lesion^{-1}$. Inoculum production showed a gradual increase up to 44 days post-inoculation when 486 urediniospores were produced by a single lesion.

P5.1-007

TEMPORAL AND SPATIAL PROGRESS OF RASPBERRY LATE RUST IN TWO CROPPING SYSTEMS IN BRAZIL

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Text

Due to the increasing global demand for raspberries, its cultivation is expanding to non-traditional areas, including subtropical regions in Brazil. However, a bottleneck to production of this fruit in the country is the occurrence of late rust caused by *Aculeastrum americanum*. In order to understand whether the cropping system using plastic cover impacts on the rust behavior, the temporal and spatial progress of disease was assessed in a commercial orchard located in the municipality of Piracicaba, Brazil. The experiment was carried out in plots planted with raspberries cv. Heritage with and without plastic cover. Disease monitoring

was performed weekly between April and July 2022. The number and position of symptomatic plants as well the disease severity in the leaves were recorded throughout the evaluations. To understand the temporal dynamics of airborne *A. americanum* urediniospores, passive impactor spore traps were installed in the plots. The incidence of the disease has reached 100% of plants in both covered and uncovered plots. Disease severity and the number of urediniospores captured by traps were significantly lower in the covered plot. The dispersion index used to characterize the spatial pattern of symptomatic plants revealed that disease distribution was random in the covered plot and aggregated in uncovered plots. The results suggested that plastic covering delay the progress of late raspberries rust by reducing the dispersal of *A. americanum*.

P5.1-008

WITCHES' BROOM DISEASE OF ACID LIME (CITRUS AURANTIFOLIA L.): RESEARCH FINDINGS AND FUTURE PROSPECTS

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Text

Acid lime is a major and historical crop in Oman. Several biotic stresses affect acid lime, including witches' broom disease of lime (WBDL), which killed approximately one million lime trees since its first report in the beggining of the 1970s. The disease has also been reported in the United Arab Emirates and Iran. WBDL results in dense branching and the production of small and large number of light green to yellow leaves, and the affected trees usually collapse within 4 to 6 years of first symptom appearance. Studies have shown that 'Candidatus Phytoplasma aurantifolia' is associated with WBDL. Phylogenetic analysis of phytoplasma strains from Oman, the UAE and Iran has shown that all strains are similar. Hishimonus phycitis leafhoppers and Diaphorina citri psyllids were found to transmit phytoplasma, with lefhoppers been more efficient in transmission. The developmeht of yellow leaves was suggested to attract more H. phycitis leafhoppers, which increases the spread of phytoplasma. WBDL phytoplasma indirectly suppresses several defense genes in acid limes. The symptomatic acid lime leaves accumulate some minerals beyond the recommended levels, which was suggested to trigger the decline of lime trees. Typical WBDL symptoms were found to be suppressed under certain environmental conditions. The findings suggest that WBDL can be managed by controlling vectors, removing the symptomatic branches and growing limes in areas less conducive for disease development.

P5.1-009

MONITORING OF VENTURIA PARALIAS, A FUNGAL BIOCONTROL AGENT FOR THE INVASIVE COASTAL WEED SEA SPURGE (EUPHORBIA PARALIAS), REVEALS PATTERNS OF DISEASE ESTABLISHMENT AND SPREAD IN AUSTRALIA

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Text

Sea spurge (*Euphorbia paralias*) is a significant environmental weed of coastal ecosystems along Australia's southern coastline. Sea spurge forms dense infestations that outcompete native flora, restricts nesting habits of shorebirds and exudes a toxic latex when damaged. Host-specificity testing of *Venturia paralias*, a candidate fungal biological control agent, demonstrated that it is specific to sea spurge and that severe necrotic leaf and stem lesions are the dominant disease symptoms. The agent was approved for release in Australia to help control sea spurge in 2020. Subsequent community-led releases of the agent in parallel with releases at nine monitoring sites along the Victorian and Tasmanian coastlines have provided information on the establishment and epidemiology of the fungus. As of December 2021, community-led releases of the agent have occurred at 70 sites along the coastline, with the agent confirmed as present at more than 40% of these sites. Detailed assessments made at the nine monitoring sites at 6-12 months of the release indicated that the agent had established at all sites and begun to naturally spread to other sea spurge infestations in the vicinity (up to 250 metres). In the field, stem lesions caused by the fungus were initially observed on sea spurge and leaf lesions appeared later, which is the opposite to that observed in laboratory studies. Further spread and impact on sea spurge in relation to environmental conditions will also be presented.

P5.1-010

FACTORS INFLUENCING HULL ROT OF ALMONDS IN AUSTRALIA

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Text

Hull rot of almond is a complex disease that causes direct loss of yield due to infected rotten nuts and downgrading of in-shell nuts, as well as reducing future yield due to twig dieback and spur death caused by toxic fungal metabolites. Hull rot is commercially important in all production regions of Australia except Western Australia. Hull rot occurrence begins in January during early hull split which is the most susceptible stage for infection. Our research is focused on understanding this disease, with particular emphasis on factors that influence disease development under field conditions. Extensive surveying over two seasons found significant relationships between agronomic practices, climatic factors and disease incidence. Hull rot symptoms were significantly influenced by both irrigation and rainfall, cultivar and rootstock and fungicide used. Hull rot incidence was reduced with applications of Fluopyram/Trifloxystrobin, and Pyraclostrobin/Fluxapyroxad. Future research is continuing management strategies for hull rot. This research was part of a national project AL16005 funded by Hort Innovation using the almond research and development levy and funds from the Australian Government and in-kind contributions by Agriculture Victoria.

P5.1-011

BIOLOGICAL RESOURCE CENTERS: STRATEGIC RESOURCES FOR PLANT HEALTH.

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Text

Biological resource centers are repository were scientists can deposit their resources and make them available for future research. These often proved to be invaluable for plant health.

For instance, when the outburst of bacterial canker of kiwifruit hit New Zealand in 2010, the presence of the type strain of Pseudomonas syringae pv syringae in collection did insure a fast identification and a rapid response. Or, when an epidemic of Dickeya on pineappple broke in Australia in 2016, the analysis of strains isolated in 1956 on ginger in the same region permitted to avoid unnecessary biosecurity responses. The complete barcoding of the Pectobacterium strains held in CIRM-CFBP permitted to uncover a not-yet described species and to shed a new light on the ecology of the whole genus, thanks to the diversity of the strains available and the quality of the associated data.

The collections are the memory of our past and key for future research. However, many gaps still exist and we observe a decrease in the deposits over time. A gap in the records may hamper identification and response to a pathogen and can have important consequences for disease management or trade.

Deposit resources in a collection have a lot of personal and community benefits, resulting in sharing the necessary efforts, facilities, competences and expertise, permitting to enhance the overall quality of the preservation and to invest for the future.

P5.1-012

PATHOGENIC CHARACTERIZATION OF THREE FUSARIUM SPECIES ASSOCIATED WITH ONION (ALLIUM CEPA L.) IN BURKINA FASO

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Text

Basal rot is a widespread onion disease caused by fungi belonging to *Fusarium* genus. The disease is known to incur onion yield loss worldwide. This study aims to identify and determine the pathogenicity of *Fusarium* species associated with basal rot of onions in Burkina Faso. Thirteen *Fusarium* isolates from seven regions of Burkina Faso were identified based on morphological observations and molecular diagnosis and their pathogenicity was assessed in laboratory and Greenhouse. Onion seeds, bulbs and seedlings were used for

the pathogenicity test. For seedling test in greenhouse, a Factorial Block design with two factors (inoculated varieties and species) with five replicates was set up using pots. Results showed that, the 13 isolates belonged to *Fusarium falciforme*, *Fusarium acutatum* and *Fusarium oxysporum* species. *Fusarium. falciforme*, *F. acutatum* and *F. oxysporum* were pathogenic on onion seeds, seedlings and bulbs. *F. falciforme* caused lower seedling damping-off rate compared to that caused by *F. acutatum* and *F. oxysporum*. All these species of *Fusarium* genus induced onion bulbs rots. The results of this study confirmed the presence of these species in Burkina Faso and that they are pathogenic on onion. Therefore, it would be useful to suggest an implement sustainable management approach of these pathogens.

P5.1-013

SPORULATION AND DISPERSAL OF THE BIOLOGICAL CONTROL AGENT ASPERGILLUS FLAVUS AF36 UNDER FIELD CONDITIONS IN CALIFORNIA

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Text

Aflatoxins are potent carcinogens produced by *Aspergillus* sp. that occasionally contaminate pistachio nuts. The international markets impose restrictive limits, thus affecting producers' economies. The spread of the biological control strain AF36 (a non-toxigenic *A. flavus* strain) stands out for limiting aflatoxin contamination. Since 2017, the product AF36 Prevail®, sorghum grains coated with AF36 propagules, has been commercially used in pistachio in California, but its sporulation occasionally fails. We studied the effect of soil moisture on the % AF36-sporulated sorghum grains (S.G.) and the number of spores per grain using a sporulation index (S.I.). Under controlled conditions, S.G. was higher than 85% when soil moisture was ≥ 13%, and the S.I. was maximum at field capacity. In the field, the best AF36 sporulation occurred near the micro-sprinklers but where non-impacted by the water drops. The AF36 Prevail® loss was more pronounced in the non-tilled ground due to quick predation by arthropods. Also, the density of spores decreased markedly with the height and distance from the inoculum source, fitting well with diffusion equations. Even so, the spores of AF36 reached the canopies of the pistachios located 10 m from the inoculum source. This work has contributed to optimizing the biocontrol AF36 Prevail® application approach in tree-nut-producing areas of California in terms of retaining more inoculum in the field and treatment-cost savings.

P5.1-014

UNDERSTANDING THE EPIDEMIOLOGY AND ECOLOGY OF CARROT VIRUSES IN THE UNITED KINGDOM

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Text

In the UK, viruses severely impact carrot production causing reduction in yield, growth defects and necrosis. The main viruses of concern are carrot yellow leaf virus (CYLV) and the carrot motley dwarf complex (CMD) – the key virus of which is carrot red leaf virus (CtRLV). The main vector of these viruses is thought to be Cavariella aegopodii (willow carrot aphid), however, the peach-potato aphid, Myzus persicae, may also play a role in the transmission of these viruses. Two years of trials were carried out to determine the importance of different vector species focusing on the timing of transmission of these key viruses into carrots. Results suggest that a focus on early season vector control strategies may be a better approach to manage the transmission of viruses in UK carrot crops by comparison to prolonged full-season treatment. However, the sources of these viruses and their epidemiology remain unclear.

To better understand the sources of these viruses in UK carrots, baseline surveillance has been conducted focused on crops and associated weeds using ecological sampling approaches supported by high throughput sequencing. This has revealed previously known viruses of Apiaceae spp.. Some of these viruses, such as CtRLV, appear to form phylogroups associated with either crop or non-crop hosts. The factors influencing these host associations, such as vector influence and agronomy, will be discussed.

P5.1-015

STUDY OF CHILLI ANTHRACNOSE DISEASE; A POTENTIAL THREAT TO CHILLI CROP IN MAJOR CHILLI PRODUCING AREAS OF PUNJAB, PAKISTAN

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Text

Chilli anthracnose is a widely distributed and economically important disease which affects the crop at pre and post-harvest crop stages. The disease is caused by *Colletotrichum capsici* and *C. gloeosporoides* which produce small, circular black spots with concentric rings of acervuli on fruit skin which turn the fruit black. The field survey was conducted in five major chilli-growing districts of Punjab province viz., Rawalpindi, Kasur, Vehari, Okara, Multan and Bahawal Nagar to access the disease incidence and severity. The study revealed variations in mean disease incidence and severity levels in the five visited districts. The mean disease incidence was highest in the Kasur district (35.1%) followed by 24.8% in the Vehari district. Of the five districts, the minimum mean disease incidence was observed in the Rawalpindi district (09%). The maximum disease severity (24.6%), was measured in terms of fruit area infected from the Kasur district followed by 17.3% from the Vehari district and minimum severity was observed at 12% in the Rawalpindi district. It reveals the predominance presence of anthracnose disease as a major constraint to chilli cultivation in

Punjab, Pakistan. The disease is also been reported in other chilli-producing countries of the world and required joint efforts towards the formulation and adaptation of joint research for devising the effective management strategies to reduce the losses.

P5.1-016

POPULATION GENETIC RELATIONSHIPS OF WHEAT PUCCINIA TRITICINA BETWEEN YUNNAN-GUIZHOU AND NORTHWEST, CENTRAL AND EASTERN CHINA

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Text

Wheat leaf rust caused by *Puccinia triticina* (*Pt*) is an important fungal disease of cereals in the world, which frequently occurs in the southwest China as well as in the Huang-Huai-Hai wheat regions, and gradually becoming serious in the northwest China in recent years. Leaf rust in Guizhou is increasing every year due to its unique geography, but there have been few studies on the population structure of leaf rust in Guizhou in the past. Leaf rust in Yunnan is easily endemic and has an early onset. However, little research has been done in the past on population genetic relationships between Yunnan and most other regions. In this study, 246 *Puccinia triticina* isolates were collected from eight provinces including Yunnan, Guizhou, Xinjiang, Shaanxi, Gansu, Hubei, Henan, and Shandong in 2021. The population genetic structure and genetic diversity as well as the relationship between ecological factors and genetic diversity were analyzed by SSR molecular markers to infer the mycological relationships and exchanges of the *Pt* populations between different regions and Guizhou and Yunnan. Our study found that wheat *Pt* in Yunnan and Guizhou had the ability to spread to central (Hubei) and eastern (Shandong) China. Similarly, *Pt* in Shaanxi and Gansu tended to spread to central (Hubei) and eastern (Shandong) China. Lastly, *Pt* in Xinjiang showed moderate genetic divergence from other populations.

P5.1-017

MONITORING SPORE DISPERSAL AND EARLY INFECTIONS OF DIPLOCARPON CORONARIAE CAUSING APPLE BLOTCH USING SPORE TRAPS AND A NEW QPCR METHOD

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Text

Apple blotch (AB) is a major disease of apple in Asia and recently emerged in Europe and

the USA. It is caused by the fungus Diplocarpon coronariae (Dc) (formerly: Marssonina coronaria; teleomorph: Diplocarpon mali) and leads to severe defoliation of apple trees in late summer resulting in reduced yield and fruit quality. To develop effective disease management strategies, a sound knowledge of the pathogen's biology is crucial. Data on the early phase of disease development is scarce: no data on spore dispersal in Europe is available. We developed a highly sensitive TaqMan qPCR method to quantify Dc conidia in spore trap samples. We monitored temporal and spatial dispersal of conidia of Dc, and progress of AB in spring and early summer in an extensively managed apple orchard in Switzerland in 2019 and 2020. Our results show that Dc overwinters in leaf litter and spore dispersal and primary infections occur in late April and early May. We provide the first results describing early-season dispersal of conidia of Dc, which, combined with the observed disease progress, helps to understand the disease dynamics and will be a basis for improved disease forecast models. Using the new qPCR method, we detected Dc in buds, on bark, and on fruit mummies, suggesting that several apple tissues may serve as overwintering habitats for the fungus, in addition to fallen leaves.

P5.1-018

MORPHOLOGICAL AND PATHOGENIC VARIABILITY OF AUSTROPUCCINIA PSIDII FROM GUAVA AND ROSE APPLE

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Text

Myrtle rust caused by *Austropuccinia psidii* has been the subject of several recent studies due to its unusual polyphagous lifestyle. The high pathogenic and genetic variability of the pathogen was already reported, indicating that the complexity of the species is higher than currently known. We aimed to evaluate morphological and epidemiological aspects of *A. psidii* specificity to guava (*Psidium guajava*) and rose apple (*Syzygium jambos*). Suspensions of isolates GM1 (from *P. guajava*) and JM1 (from *S. jambos*) (2×10^4 urediniospores mL^{-1}) were sprayed on *S. jambos* leaves. The density of the lesions (number of lesions per cm2) was quantified, and the germination of urediniospores and the formation of appressoria of both isolates was evaluated in a scanning electron microscope. The percentage of germination on leaves was 97%, with 93% of appressoria for JM1 and 93% with 55% of appressoria for GM1. A higher density of sporulating lesions was observed for *S. jambos* plants inoculated with JM1 when compared with GM1, which caused small necrotic areas without sporulation. The mean densities of lesions were 38.7 and 11.6 lesion per cm2 for JM1 and GM1 isolates, respectively. These results indicate that there is pathogenic specialization within *A. psidii* isolates, which will be used in further studies regarding the genetic complexity of this fungus.

P5.1-019

CURRENT EPIDEMIOLOGICAL SITUATION OF MEALYBUG WILT OF PINEAPPLE DISEASE IN ECUADOR

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Text

In this study, we analyze the current epidemiological situation of mealybug wilt of pineapple (MWP) disease in Ecuador. Ninety symptomatic and asymptomatic leaves samples from MD2 hybrid and cultivar “Criolla” were collected for viral detection in a pineapple plantation located in Santo Domingo of the Tsachilas, the largest pineapple producing province in the country. MD2 hybrid showed several tip dieback stages, while cultivar “Criolla” did not show typical MWP symptoms. To determine virus prevalence, total RNA extraction was carried out followed by reverse transcription - polymerase chain reaction (RT-PCR). Samples were tested for eleven viruses, including the ampeloviruses pineapple mealybug wilt-associated virus 1 (PMWaV-1), PMWaV-2, PMWaV-3, PMWaV-5 and PMWaV-6; the sadwaviruses pineapple secovirus A (PSV-A), PSV-B, PSV-C and PSV-D; and two badnaviruses, pineapple bacilliform CO virus (PBCOV) and PBERV. The virus identity was confirmed by cloning, Sanger sequencing and blast. The presence of mealybugs (Dysmicoccus spp.) was observed in all the symptomatic pineapple plants sampled in this study. The RT-PCR results revealed the presence of PMWaV-1, PMWaV-2, PMWaV-3, PSV-A, PSV-B and PBCOV in MD2 hybrid. These viruses, except PMWaV-2, were also detected in asymptomatic “Criolla” samples, suggesting that plant genotype is related with virus-induced symptom expression in pinneaple. These findings coincide with those previously reported in Hawaii and Australia.

P5.1-020

IMPACT OF CULTIVAR RESISTANCE ON CERCOSPORA BETICOLA EPIDEMIOLOGY ON SUGAR BEET

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Text

Cercospora leaf spot (CLS) is the most destructive foliar disease on sugar beet caused by the fungal pathogen Cercospora beticola. The emergence of fungicide-resistant populations highlights the importance of developing and cultivating resistant cultivars. Understanding the interactions between the cultivar resistance and C.beticola is essential in CLS management. A field trials were designed in 2022 and 2023 to investigate the interactions from an epidemiological aspect. We aimed to describe the relationship between the cultivar resistance and the spore flight of C.beticola. The trials were conducted in two geographical locations as a completely randomized block design with four cultivars containing different resistant properties in triplicate. In the trial in 2022, spore flight during the vegetation period was examined by a pre-developed method using spore traps and TaqMan real-time PCR assay. Disease development on each cultivar was also monitored during the same period. We observed a delayed disease incidence and decreased disease severity in highly resistant cultivars. Results of the real-time PCR shows a similar tendency of reduced spore quantity sampled from the highly resistant cultivars.The results indicate that the highly resistant

cultivars produce fewer secondary aerial spores, which further decreased the disease development. The trial will be repeated in 2023 to gain further insight into the interaction between cultivar resistance and CLS epidemiology.

P5.1-021

PARTIAL RESISTANCE TO MYRTLE RUST ON GUAVA CV. SUPREMA EXPRESSED BY REDUCTION OF UREDINIOSPORE PRODUCTION OF AUSTROPUCCINIA PSIDII

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Text

Myrtle rust (*Austropuccinia psidii*) is the most important fungal disease on guavas in Brazil and cultivars with partial resistance to the disease should be used as an alternative to the consolidated cv. 'Paluma', which is susceptible to the disease. While infection and colonization of guava leaves by *A. psidii* are well characterized in cv. 'Suprema', components of partial resistance related to inoculum production have not yet been evaluated. The objective of this study was to evaluate the infectious period and the urediniospore production of *A. psidii* in Paluma and Suprema. Young leaves of potted Paluma and Suprema plants were inoculated with an *A. psidii* suspension (2.5×10^4 urediniospores mL^{-1}). From one week after symptoms appeared, the inoculum produced on leaves was weekly collected using a glass nozzle and a suction pump. The *A. psidii* urediniospore concentration was determined using a Neubauer chamber. The disease infectious period was approximately 35 days in both cultivars. The *A. psidii* urediniospores weekly production was 2.5 to 14 times higher in Paluma than in Suprema. Considering the complete infectious period, each rust lesion in Paluma produced between 2,294 and 10,273 urediniospores, while in Suprema only 580 to 1,517 urediniospores were produced per lesion. The lower inoculum production in Suprema may act by reducing the rate of disease increase (*r*) and slowing down the epidemic in the field. Histopathological analyses of diseased leaves are underway.

P5.1-022

LARGE-SCALE ECOLOGICAL SURVEYS ACROSS ARABLE LANDSCAPE TO DESCRIBE THE EPIDEMIOLOGY OF VECTOR-BORNE PHYTOBACTERIUM CANDIDATUS LIBERIBACTER SOLANACEARUM IN THE UK.

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Text

Candidatus Liberibacter solanacearum (Lso) is a non-culturable, phloem-limited and vector-borne phytobacterium transmitted by species belonging to the superfamily Psylloidea (Hemiptera). Five haplotypes of Lso have thus far been described that are known to cause widespread losses across the globe in commercially important Solanceae and Apiaceae crops. In apiaceae crops damaging outbreaks are regularly occurring across the Middle East and mainland European countries. Lso and some of its vectors have also been recorded in the UK in association with arable areas utilised for important apiaceous crops (carrots and parsnips); however, there have not yet been reports of disease on the UK apiaceous crops production. Lso is an obligate pathogen so studies on its complex epidemiology and population dynamics rely on molecular testing of plants and vectors from the field. In this study we completed a large-scale ecological survey to describe the epidemiology and potential management of Lso in the UK with the aims: a) to investigate the incidence and distribution of Lso in carrot and parsnip fields, b) to determine the UK-wide diversity and potential distribution of Lso vector species, and c) to investigate the potential role played by wild non-crop Apiaceae (wild hosts) that are present in and around apiaceous crops in the UK.

P5.1-023

INVENTORY OF LEAF DISEASES OF PEANUT IN BURKINA FASO AND EPIDEMIOLOGICAL STUDY OF ASSOCIATED VIRUSES

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Text

Peanut is a major oil-seed crop in the world. It contributes to food security and generates income for farmers in Africa. Unfortunately, this crops is subject to numerous leaf diseases which have been reported in West Africa. In recent years, there is a resurgence of leaf diseases on peanut crops in Burkina Faso. They induce specific symptoms often associated with plant stunting and yield losses. Preliminary data indicated the occurrence of fungi and a complex of virus species. However, these data remained old and not up to date. The epidemiology of these diseases remains poorly understood, which hampers the design of appropriate control measures to reduce yield losses. The research planned in the thesis aims to inventory peanut leaf diseases and identify the main epidemiological parameters of diseases caused by virus in Burkina Faso. More than a thousand peanut leaf samples were collected in several regions of Burkina Faso during year 2022. During the surveys, the common diseases were rosette, peanut stripe, peanut clump, peanut stunt, peanut yellow spot diseases. The same diseases were reported by farmers when interviewed. Base on molecular diagnosis, the next step of this study will contribute to generate information on virus species and hosts. Lastly, available peanut germplasm will be screened to identify tolerant or resistant accessions to be included in integrated disease management strategies.

Keywords: Arachis hypogaea, diseases, epidemiology, virus, Burkina Faso.

P5.1-024

DAMAGE TO GUAVA PLANTS CAUSED BY MULTIPLE INFECTIONS OF AUSTROPUCCINIA PSIDII

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Text

Rusts are polycyclic diseases which can cause polyetic damage to perennial plants. This type of damage has not yet been quantified for myrtle rust, caused by Austropuccinia psidii. Therefore, this work aimed to simulate, in a greenhouse, the polycyclic process that occurs in the field and to assess its impact on the development of 'Paluma' guava plants. For this purpose, guava plants were inoculated with 2 x 104 urediniospores.mL-1 suspensions of A. psidii for three times, 14-days apart. Humid chambers were provided for 24 h after each inoculation. As control treatment, guava plants were sprinkled with water. The inoculations were performed on the three youngest pair of leaves. Disease severity, leaf area, the length of the main stem and internodes and the total number of leaves were assessed weekly. The dry mass of leaves, shoots and roots was quantified by the end of the experiment. Inoculated plants showed reduced leaf area and stem length (p<0.05) in relation to healthy plants. The shoot growth of inoculated plants was reduced by 30% as compared to healthy plants. Disease severity reached 40% of the leaf area and symptoms of leaf curling and leaf wrinkling were observed before leaf drop. A severe defoliation occurred only in inoculated plants. There was a significant reduction (p<0.05) in the dry mass of plants submitted to successive inoculations with A. psidii. The successive inoculations of A. psidii cause intense reduction on the development of ‘Paluma’ guava plants.

P5.1-025

RESEARCH ON METEOROLOGICAL FACTORS RELATED TO THE INCIDENCE OF RICE FALSE SMUT

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Text

Rice false smut caused by *Ustilaginoidea virens* is the most crucial grain disease in rice production worldwide. The disease has been considered a minor disease that occurs scatteringly in certain regions under certain climate conditions. However, rice false smut could significantly reduce the quantity and quality of rice grains. To make a disease forecasting system for the disease, we aimed to find out the factors contributing to the disease by understanding the relationship between its incidence and weather factors. The primary inoculum (ascospores) invades rice flowers at the booting stage. Therefore, we analyzed the correlations during the booting, flowering, and both stages. First, in the booting stage, the average relative humidity had a statistically significant positive correlation (Pearson’s r = 0.705, p < 0.05). The disease incidence decreased as average sunshine hours increased, although this correlation was non-significant. Second, no significant correlations were found between all factors and the disease incidence in the flowering stage. Lastly, from the booting to flowering stages, the average relative humidity had a significant positive correlation (Pearson’s r = 0.644, p < 0.05). These results suggest that the influence

of weather factors in the booting stage may be critical. For successful and accurate predictions, we keep conducting additional annual investigations on the host, pathogen, and environment.

P5.1-026

GENE DRIFT, SEXUAL REPRODUCTION, AND SEXUAL RECOMBINATION OF PUCCINIA STRIIFORMIS F. SP. TRITICI AND PUCCINIA STRIIFORMIS F. SP. HORDEI

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Text

Stripe rust on wheat and barley caused by *P. striiformis* f. sp. *tritici* (*Pst*) and P. striiformis f. sp. *hordei* (*Psh*), respectively. Whereas, *Pst* can attacked barley. To understand relationship between both rusts, 260 isolates were collected from wheat (120) and barley (140) from 2018-2020, identified on wheat and barley genotypes, and genotyped by 26 KASP-SNP markers. A cross between *Pst* and *Psh* was made to determine sexual recombination. As a result, massive multi-locus genotypes (MLGs), high virulence diversity was detected in wheat, barley, mixed (virulent to barley and wheat) populations. Common MLGs were detected between or among populations. Highly genotypic diversity was detected in the three populations, and low linkage disequilibrium were found in the most sampling sites of both crops, indicating that two stripe rust populations were sexual. Phenotype and the population structure support the wheat and barley forms were separate. However, clustering and common MLGs exhibited a similar lineage in the mixed population and other both populations, displaying gene drifts among these populations. Many isolates in mixed population originated from sampling sites where sexual reproduction occurred. A F_2 progeny were established from a cross between a *Psh* isolate and a *Pst* isolate on *B. aggregata* seedlings, showing avirulence / virulence segregation to 8 *Yr* loci. The results make an insight into evolution of *Pst* and *Psh* in mixed wheat and barley growing regions.

P5.1-027

ASCOSPORE RELEASE BY NEONECTRIA DITISSIMA IN NORWAY.

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Text

European canker, caused by the ascomycete *Neonectria ditissima*, is a significant threat to the Norwegian apple industry. A major part of ascospores of *N. ditissima* are dispersed in air following rain and moist periods, while conidia, and occasionally also ascospores, are splash dispersed during rain. Relative importance and timing of ascospore release may differ between geographical locations. It has not been clear when ascospores are released in

Norway, thus limiting the understanding of the pathogen biology and disease development, and the potential deployment of management options. Timing of ascospore release in two Norwegian apple production regions and its associations with weather parameters were studied for three years. Burkard volumetric spore traps were used to capture ascospores, and weather stations provided hourly data of temperature, rainfall, and other factors. Ascospores were captured year-round. Peak periods of ascospore production occurred in late spring (May-June) and to a lesser extent in the autumn (Sept.-Oct.). The highest numbers of ascospores per day were captured when temperatures were between 5°C and 15°C and rainfall <10 mm. Ascospores were captured after up to 30 days without rainfall. The importance of the fact that ascospores potentially are available year-round in apple orchards will be discussed in relation to management options.

P5.1-028

GROUNDWATER AS A RESERVOIR FOR PLANT PATHOGENIC BACTERIA: THE CASE OF THE PSEUDOMONAS SYRINGAE COMPLEX IN THE ALLUVIAL AQUIFER OF AVIGNON

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Text

During the last decades, the use of groundwater for irrigation has increased following agriculture intensification and climate change, especially in southern Europe. However, there is a significant lack of information on the presence of plant pathogens in aquifers the current knowledge being to consider the risk negligible.
In this work, we report evidence for the presence of bacteria from the Pseudomonas syringae (Ps) complex an archetypical phytopathogenic bacterium, at various places and dates in the groundwater of the Avignon region, an intensively irrigated area in the southeast of France. The concentration of Ps was variable and inversely correlated with water conductivity explaining 27% of the variability. The mean abundance of Ps was 100 times lower than in the River Durance connected with the aquifer but surprisingly, their genetic structure was more homogeneous than in the river. Moreover, most strains (98%) from groundwater were potentially pathogenic on plants according to lab tests, while in the river only 66% were pathogenic. Determinants of this low diversity and prominence of pathogenic strains in groundwater remain to be identified.
We conclude that aquifers are potential reservoirs of plant pathogens. More surveys are needed, notably to understand the real impact on crops during irrigation. These results could be included in prediction models and new approaches to disease forecasting and surveillance and could lead to the adaptation of agricultural practices.

P5.1-029

EPIDEMIOLOGY AND GENETICS OF THE WHEAT YELLOW RUST FUNGUS

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Text

Yellow rust (stripe rust) caused by the biotrophic fungal pathogen Puccinia striiformis f. sp. tritici is one of the most important and destructive wheat diseases globally. A recent study at Aarhus University, Denmark, discovered that several wheat varieties acquired significant resistance to yellow rust after a prolonged cold treatment. The aim of this study is to investigate the genetics of virulence in yellow rust and the durability of cold-induced resistance in wheat when challenged with yellow rust races of different origin.
Unique sexually derived progeny isolates from three dominant genetic groups in Europe are being both genotyped using 19 SSR markers and virulence phenotyped on a differential set of wheat lines carrying well-characterized yellow rust R-genes. Progeny isolates will be selected to further investigate the durability of cold-induced resistance in wheat by conducting virulence phenotyping on newly bred wheat cultivars with cold-induced resistance. Subsequently, histological analysis of plant-pathogen interactions will be carried out to determine plant defence responses and pathogen development. The results of these studies will provide improved knowledge on how yellow rust virulence is inherited and enable assessment of the potential of cold-induced resistance in wheat against yellow rust. This will contribute to the development of optimized breeding programs for resistant wheat varieties and a better understanding of plant-pathogen interactions.

P5.1-030

POTENTIAL IMPACTS OF CLIMATE CHANGE ON IMPORTANT WHEAT AND MAIZE DISEASES IN EGYPT

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Text

Yellow rust (YR, *Puccinia striiformis* f. sp. *tritici*) and late wilt (LW, *Magnaporthiopsis maydis*) are damaging diseases on winter wheat and summer maize, respectively, in Egypt. Assessing impacts of climate change on these diseases is necessary for developing adaptation measures and food security. YR severity was recorded in late February/early March on six wheat cultivars in 2013-2020 in seven governorates, while LW incidence was recorded in 2005-2019 in 13 governorates. Climate change scenarios were created with two climate models under RCP4.5 and RCP8.5 in four future periods. Best regression models were determined by step-wise regression on monthly rainfall and average monthly minimal, maximal and mean temperatures in December to February for YR for a very susceptible cv. Gemmeiza-11 and a moderately susceptible cv. Misr-1, and in June to August for maize LW. For YR, there was little disease before 2016 but severity started to increase since then. For LW, incidence was greater in Lower than in Upper Egypt (5-20% versus 3 to 15%). Initial

results in Al-Behera governorate showed that YR severity was predicted at 100% for cv. Gemmeiza-11 under all climate change scenarios but it will increase from 30 to >60% for cv. Misr-1 in future climatic conditions. However, a small increase in LW incidence was predicted (<3%). This suggests a greater impact of climate change on winter diseases and thus Egypt should develop control strategies for this wheat disease.

P5.1-031

DETECTION OF PHYTOPHTHORA PALMIVORA CAUSATIVE AGENT OF THE DISEASE BUD ROT IN SOIL AND IRRIGATION WATER IN AN OIL PALM PLANTATION IN COLOMBIA

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Text

Oil palm Bud Rot (BR) caused by *Phytophthora palmivora* is the most important disease in Colombia due to the more than 70.000 hectares affected in the Central and Southwestern zone and the almost 15.000 hectares that are currently being affected in the Northern zone of the country,. Understanding the frequency of appearance of the pathogen in areas where the disease is advancing is valuable information for producers to take the most appropriate measures to help mitigate the impact of this pathology. In order to verify the presence of the pathogen, a sampling was carried out that involved water sources such as rivers and soil from plots with a low incidence of the disease. To capture the pathogen, leaflets from healthy palms and fruits were used as bait traps. For the latter, three points of the river were monitored at three different depths of 10, 50, and 100 cm. In soil, a total of 528 samples from 54 sites were analyzed, where 80% of the samples analyzed were positive for *Phytophthora*, a comparison of the river monitoring points, where it was only possible to verify the presence of the pathogen between 20 and 33.3% of the bait traps analyzed. The presence of the microorganism in this samples taken indicates a constant source of inoculum and possibly an increase in new cases of BR from plantations that use river water in their irrigation systems.

P5.1-032

EFFECT OF THE TRUNK SHAKER HARVESTING ON THE DISPERSION OF VENTURIA OLEAGINEA SPORES, THE CAUSAL AGENT OF OLIVE SCAB

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Text

Traditional olive oil orchards are usually harvested by trunk shakers, which can cause the

dispersion of *Venturia oleaginea* spores, the causal agent of olive Scab, but it has not been studied. Six olives of the susceptible cv. Picual and six trees of the resistant cv. Frantoio were used for evaluating the latter hypothesis. Thus, we established eight spore trapping points per tree in two opposite lines: under the tree canopy, at 1, 3, and 6 m from the trunk. At each point, three slides, impregnated with a silicone-3%cyclohexane solution, and two Petri dishes with selective media. The olives were then vibrated for 10-12 s with a trunk shaker (AgromelcaTM) adapted to a John Deer tractor. Tree vibration energy was determined using accelerometers. Two non-shaked olives were used as control. The density of captured spore was maximum (38 spores×cm²) under the tree canopy and decreased exponentially with distance (at 6 m, it was 6 spores×cm²). Nineteen percent of the *V. oleaginea* spores were trapped attached to their conidiophore. Also, more than 20 epiphytic fungal species were isolated, with the genera *Alternaria*, *Aspergillus*, and *Penicillium* being the most frequent. It should be noted that pathogen some spores (0.1 spore×cm²) were also caught on non-shaked trees. The data reveal that trunk shakers cause extraordinary dispersion of pathogen spores. We are currently conducting new trials under controlled and field conditions to investigate this pathogen's spore dynamics further.

P5.1-033

RELATIVE CONTRIBUTION OF LOCAL AND LANDSCAPE FACTORS ON THE CO-OCCURRENCE, DIVERSITY AND SEVERITY OF WHEAT DISEASES

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Text

Several pathogens can co-occur in arable fields or co-infect the same plant. To be efficient, control strategies need to consider the multiple pathogens that can affect a given crop. The design of such strategies require a better understanding of how local management (e.g. crop variety), landscape characteristics (e.g. amount of the same crop around) and their interactive effects affect the incidence and severity of multiple diseases in a field. Spatiotemporal field surveys considering multiple diseases simultaneously remain however rare, limiting the development of agroecological solutions.

Taking wheat diseases as a case study, we selected 56 landscape units of 1-km radius distributed along gradients of amount of wheat (from 11% to 48%) and of organic farming (from 0.1% to 76%) in the long-term research platform "Zone Atelier Plaine & Val de Sèvre" (South-West of France) in 2021 and 2022. In each central wheat field within a landscape unit, we carried out symptom observations in a 4 × 6 grid by recording the incidence and severity of wheat leaf, ear, and stem base diseases at three growth stages during the growing season.

We found that: (i) a large diversity of pathogens co-occur in a given wheat field (pathogen-specific facilitative, neutral, suppressive effects); (ii) both a low amount of wheat and a high amount of organic farming in the surrounding landscape increase the number of diseases that are present but decrease the severity of plant disease epidemics.

P5.1-034

EPIDEMIOLOGY OF CACAO MILD MOSAIC VIRUS (CAMMV)

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Text

Cacao mild mosaic virus (CaMMV) is considered an emerging pathogen of *Theobroma cacao* that reduces yield and causes branch dieback, posing a risk to the $12 billion global cacao harvest and to the millions of small holder farmers that depend on the crop for their livelihoods. In the past few years, CaMMV has been detected in the USA, Brazil, England, and Indonesia. Two widely known transmission routes are mealybugs and the use of infected material during grafting. Research has shown that the virus can be transmitted even when symptoms are absent. More recently, we discovered up to 68% of seedlings grown from seeds of infected mother trees were also infected, thus adding an additional transmission route. This is significant because although the importance of screening budwood for viruses is understood, trees whose seeds are propagated for rootstock are not routinely screened. It also means that the movement of whole pods can introduce the pathogen to new locations. Current research is also looking for alternative hosts of the pathogen that could serve as inoculum reservoirs. Relatives of *T. cacao*, such as *Ceiba* spp. and *Ochroma* spp. are present in regions where the crop is grown commercially and could serve as CaMMV reservoirs. Infected plants cannot be cured, so preventing transmission and removing inoculum reservoirs are the most effective ways to combat it. CaMMV epidemiology and its implication on germplasm conservation and commercial production will also be discussed.

P5.1-035

ANALYSIS OF LITERATURE DATA MAY INCREASE THE EPIDEMIOLOGICAL KNOWLEDGE ON GRAPEVINE TRUNK DISEASES

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Text

Grapevine trunk diseases (GTDs) are serious threats in all viticultural areas of the world, and their management is complex and frequently inadequate. Wounds are considered the entry points for fungi causing GTDs, and particularly pruning wounds. Quantitative analyses of published information were performed (i) to determine the effects of temperature on mycelial growth and the effects of temperature and moisture duration on spore germination, and (ii) to identify the factors that most affect the length of pruning wound susceptibility. The mycelial growth of the fungi causing Botryosphaeria dieback (BD) and the Esca complex (EC) responded similarly to temperature, and preferred higher temperatures than those causing Eutypa dieback (ED) (with optimal temperature of 25.3, 26.5°C, and 23.3°C, 27 respectively).

At any temperature, the minimal duration of the moist period required for 50% spore germination was shorter for BD (3.0 h) than for EC (17.2 h) or ED (15.5 h). Concerning infection through pruning wounds, infection incidence was higher for fungi associated with BD than those associated with ED or EC, and wound susceptibility decreased faster for ED than for other GTD agents. Grapevine variety and pruning season also affected the wound susceptibility period, with Sauvignon Blanc showing longer susceptibility than other varieties. These results increase our understanding of GTD epidemiology and should help growers to control infections.

P5.1-036

RE-ASSESSING THE SUSCEPTIBILITY PERIOD OF CITRUS FRUIT TO PHYLLOSTICTA CITRICARPA INFECTION IN SOUTH AFRICAN ORCHARDS

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Text

The severity of citrus black spot (CBS), caused by *Phyllosticta citricarpa*, depends on several factors including the age of the fruit at the time of infection. Fruit have been reported to become resistant to CBS infection with maturity, but recent research indicate that fruit is susceptible to infection for longer periods than previously assumed. To conclusively demonstrate ontogenic resistance development of citrus fruit to *P. citricarpa* infection, fruit in commercial Valencia, Nova and Empress orchards were inoculated on a monthly basis with different concentrations (10^1, 10^3 and 10^5 conidia/mL) of *P. citricarpa* suspensions or exposed to natural pathogen infection at different times through a staggered fungicide spray program. Significant increases in CBS incidence and severity were observed between November and January on inoculated Valencia oranges and Nova mandarins. Although orange fruit were still susceptible after January, CBS incidence and severity were very low and comparable to the un-inoculated control in the inoculation trials. These observations of ontogenic resistance were supported in the 2017-2018 staggered spray trials: leaving orange trees unprotected after January did not significantly increase the incidence and severity of CBS. Findings from the mandarin spray trials were inconclusive due to low disease pressure in both seasons.

P5.1-037

SYMPTOMS IN IMMATURE AND RIPE APPLE FRUIT CAUSED BY COLLETOTRICHUM SPECIES ISOLATED FROM GLOMERELLA LEAF SPOT

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Text
Colletotrichum isolates from Glomerella leaf spot (GLS) symptoms can also cause bitter rot (BR) and small lesions spot, called here as Colletotrichum fruit spot (CFS). We investigate 5 *Colletotrichum* species, obtained from GLS symptoms on leaves, on causing diseases in immature and ripe apple unwounded fruit, assessing symptoms development. *C. chrysophilum, C. nymphaeae, C. paranaense, C. melonis* and *C. siamense* were inoculated in immature 'Gala' (Ø = 5.5 cm) and 'Eva' (Ø = 4.8 cm) fruit in the field (2016/17 season). Subsequently, *C. chrysophilum* and *C. nymphaeae* were inoculated in different fruit sizes (Ø = 2.4–6.3 cm) in the laboratory and in the field (2017/18 and 2021/22 seasons). Gala and Eva cultivars differed regarding their susceptibility to Colletotrichum fruit spot (CFS). The CFS lesions of inoculated fruits did not evolve to BR after harvest, even under optimal environmental conditions for the pathogen up to the sixth week of incubation. Gala was susceptible to *C. chrysophilum, C. nymphaeae, C. paranaense, C. melonis* and *C. siamense*, showing symptoms of CFS in immature fruit inoculated in the field, and for 'Eva', the incidence varied between species. Only CFS symptoms were observed in both cultivars inoculated in the field, at harvest. CFS incidence in 'Gala' reached 50% for all species and fruit sizes. For 'Eva', *C. melonis* caused CSF in the 2016/17 season (Ø = 4.8 cm) and, C. chrysophilum and *C. nymphaeae* in the 2021/22 (smallest fruit sizes only).

P5.1-038

EVALUATING THE CLIMATIC SUITABILITY OF THE MEDITERRANEAN BASIN FOR CITRUS BLACK SPOT (PHYLLOSTICTA CITRICARPA) THROUGH A GENERIC INFECTION MODEL

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Text
Citrus black spot (CBS), caused by *Phyllosticta citricarpa*, was reported for the first time in Tunisia in 2019. Previous studies indicated that this pathogen was unable to develop the disease in Mediterranean climates, while others suggested the contrary. In this study, a generic model was used to evaluate the suitability of the climates in the Mediterranean Basin for CBS development by simulating potential infections by ascospores and pycnidiospores of *P. citricarpa*. The model was implemented for the citrus-growing regions in the Mediterranean Basin and locations where CBS is present worldwide, using hourly climatic data at high spatial resolution (~9 km) retrieved from the ERA5-Land dataset. Two simulation scenarios were considered, in the first one the model parameters were set with values from literature and in the second estimated by a Bayesian inferential process. The results indicated that ascospore and pycnidiospore infections would be mainly concentrated in autumn, and also in spring for pycnidiospores. In contrast to previous studies, the model consistently estimated that the percentage of favourable hours for pycnidiospore infections was higher than for ascospores. The values simulated for Tunisia and several CBS-affected locations in South Africa were similar to those in citrus-growing regions in Europe and Northern Africa, where the disease has not been reported. These results confirm the climatic suitability of the Mediterranean Basin for CBS development.

P5.1-040

MODELING THE SPATIAL SPREAD OF YAM MOSAIC VIRUS (YMV) IN SEED YAM FIELDS IN NIGERIA

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Text

Yam (*Dioscorea* spp.) production in West Africa is constrained by the yam mosaic virus (YMV). YMV-resistant yams are unavailable, but the recent introduction of rapid clean seed propagation technologies promises to control the yield losses. However, rapid YMV reinfection to clean yam seedlings in the field undermines the effectiveness of new technologies. This study between 2016 and 2020 determined the factors contributing to the spread of YMV in field trials conducted in Ibadan, Nigeria. Yellow water traps were set to monitor aphid diversity and movement. Seedlings were assessed monthly for YMV infection. The study revealed no significant difference ($P>0.05$) in the number of aphids trapped at different positions within trials. The overall distribution of aphids demonstrated a biphasic pattern marked by an initial increase in the aphid population followed by a rapid decline. Eight aphid species were trapped, of which *Aphis spiraecola* and *Pentalonia* spp., were dominant. YMV incidence was highest in 2019 (46.4±3.3%) and lowest in 2016/17 (7.9±4.5%), and we found evidence showing clustering of YMV-infected seedlings at the edge of trial fields. Results demonstrated the role of non-yam colonizing aphids in YMV spread, the correlation between symptom severity, tuber yield, and seedling establishment, and the importance of integrated management, especially positive control, to manage YMV reinfection to clean plants in Nigeria. We will present the findings in this presentation.

P5.1-041

SURVEILLANCE AND MITIGATION STRATEGIES FOR WHEAT BASED ON CROP LANDSCAPES, TRADE NETWORKS, AND THE ECOLOGICAL NICHES OF 100 PATHOGENS

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Text

Wheat, a major crop for global food security, is threatened by diverse pathogen species. Some wheat pathogens such as rust fungi have been the focus of extensive studies to optimize mitigation. Here we evaluate more general strategies for surveillance and mitigation of a range of wheat pathogens. Effective management of an increasing burden of diseases requires geographic prioritization to guide epidemic risk-reducing efforts by global and

national stakeholders. Our first objective is to provide candidate priority locations for epidemic surveillance based on global risk analysis of wheat cropland and trade networks. For example, locations with high cropland connectivity in Kansas, Nebraska and North Dakota in the USA, and in each wheat production region, are identified as likely important for pathogen spread. Pathogen introduction risk via wheat trade networks, if there is inadequate phytosanitary testing, is higher in countries such as the United Arab Emirates, the Netherlands, and the USA. Our second objective is to provide a global biogeographic analysis of 100 economically important wheat pathogens based on their reported geographic distribution. Although pathogen richness peaks in countries with high wheat cropland extent, early epidemic emergence events have been more frequent in the native range of wheat than elsewhere. These findings provide starting points for building global epidemic surveillance and mitigation systems to support sustainable wheat production.

P5.1-042

EXPLORING THE DIVERSITY AND PREVALENCE OF PSEUDOMONAS SYRINGAE IN SWEET CHERRY ORCHARDS OF NEW ZEALAND

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Text

Bacterial canker of cherry, caused by *Pseudomonas syringae* pathovars, is a major constraint to cherry growing in New Zealand and particularly in Central Otago, the primary growing area for cherries. To gain a better understanding of the disease's epidemiology, *Pseudomonas* spp. isolates were collected from symptomatic and asymptomatic cherry tissue from 23 commercial Central Otago cherry orchards in 2015. Isolates were classified into different taxonomic groups using phylogeny based on the *gltA* gene sequence for all strains (250) and Multi Locus Sequence Analysis (MLSA) of four housekeeping genes for 35 strains. The two main taxonomic groups were *P. syringae* pv. *syringae* (Pss) and *P. syringae* pv. *morsprunorum* race 1 (Psm1), in Phylogroup 2 (PG2) and Phylogroup 3 (PG3), respectively. The third group comprised non-pathogenic strains classified as *Pseudomonas* spp. Strains of Psm1 formed a monophyletic group, representing an almost clonal population. There was more variation detected within strains of Pss, although they were restricted to group PG2b. Non-pathogenic P. spp. and pathogenic Pss and Psm1 strains coexisted in the same orchard. It was concluded that Pss is the predominant pathovar in Central Otago. This is the first detailed study of the *P. syringae* species complex in cherry orchards in New Zealand and provides the basis for future epidemiology studies.

P5.1-043

COMPLEMENTARY APPROACHES TO QUANTIFY AND CHARACTERIZE INOCULA DYNAMICS AND LEAF INFECTION AT PLOT LEVEL: CASE OF BLACK LEAF STREAK DISEASE

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Text

Black Leaf Streak Disease is a major leaf disease of banana caused by the airborne ascomycete Pseudocercospora fijiensis. A better understanding of the disease epidemiology would help to find alternatives to fungicides. According to empirical knowledge, plant infection is mainly due to the inoculum from outside the plot (external inoculum, supposedly ascospores) whereas the inoculum within the plot (internal inoculum, supposedly mainly conidia) plays a minor role. We propose to implement two complementary and original experimental approaches to (i) characterize and quantify the external and internal inocula of a plot, and (ii) to identify which leaves are infected by each inoculum. The study is carried out in Guadeloupe, on two experimental plots planted either with a susceptible or a partially resistant cultivar to reduce ascospore production. For the first aim, we install six Burkard multi-vial cyclone samplers above and under canopy to catch respectively the external and internal inocula. Then, we quantify conidia by microscopy and ascospores with quantitative PCR. For the second aim, we describe the leaf infection due to the external and internal inocula by comparing with image analysis the number of lesions produced on leaves, protected or not from the inocula with spore proof nets. The inocula and infection dynamics will be related to the cultivar and the microclimate. This study is the first contribution to understand the role of each inoculum in the leaves' infection.

P5.1-044

GRAPEVINE TRUNK DISEASE PATHOGENS IN ROOTSTOCK MOTHER VINES: A POTENTIAL THREAT TO THE SOUTH AFRICAN GRAPEVINE INDUSTRY

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Text

Rootstock mother vines are known as sources of grapevine trunk disease (GTD) pathogen inoculum. Vines become infected through pruning wounds and spread to canes which are used as propagation material. The risks associated with ageing mother plants are unknown as most of these infected canes appear visually healthy. The aim of the study was to characterize GTD pathogens from mother vines and one-year-old canes harvested from these vines. Fungal isolations were made from 1900 mother vines of different ages and 2050 one-year-old canes. Isolates were identified based on morphology, species-specific PCRs and amplifying and sequencing relevant gene regions of representative isolates. From mother vines, *Phaeomoniella chlamydospora* occurred at the highest incidence (25.9%), followed by Botryosphaeriaceae spp. (18.6%; predominantly D*iplodia seriata*), Basidiomycetes (12.4%; predominantly *Fomitiporia* sp.) and *Phaeoacremonium* (5.9%; predominantly *P. minimum*). All major GTD pathogens occurred in mother vines as young as 4-years-old, including wood rotting Basidiomycetes. This is of great concern since wood rotting fungi could drastically reduce the productive lifespan of mother vines. A total of 4.0%

of one-year-old canes harboured GTD pathogens of which Botryosphaeriaceae species were predominant (3.4%), followed by *Diaporthe* species (0.3%) and *Phaeomoniella chlamydospora* (0.2%). The productive lifespan of rootstock mother plants must be re-evaluated and not merely be based on age.

P5.1-045

EPIDEMIOLOGICAL, EVOLUTIONARY AND ECONOMIC OUTCOMES ASSOCIATED TO THE COEXISTENCE OF MONOGENIC AND PYRAMIDED RESISTANT CULTIVARS IN AGRICULTURAL LANDSCAPES: A CASE-STUDY WITH THE MANAGEMENT OF DOWNY MILDEW IN WINE GROWING AREAS.

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Text

Downy mildew represents a real threat for grapevines in all vine-growing areas of the world, leading to significant yield losses and massive recourse of fungicides. Over the past years, breeders have been engaged in breeding programs for resistance to grapevine downy mildew, resulting in the creation of several resistant varieties. At present, growers can plant monogenic (with mainly the resistance factors Rpv1, Rpv3 but also Rpv10 and Rpv12) or pyramided cultivars (mainly cumulating Rpv1 and Rpv3). Currently, the resistance factors Rpv1 and Rpv3 start to be deployed in France. These two resistance factors can be deployed in: (i) monogenic cultivars sown in the same field (mixture strategy), (ii) monogenic cultivars sown in different fields (mosaic strategy), (iii) pyramided cultivars (pyramid strategy) and (iv) in hybrid strategies that combine the three previous basic strategies. Here, we used the spatially explicit stochastic model *landsepi* to investigate the epidemiological, evolutionary and economic outcomes associated to these deployment strategies. Our results particularly highlight the risks for resistance durability associated to the coexistence of monogenic and pyramided cultivars in the same landscape. Finally, we discuss how the model *landsepi* has been used to design deployment scenarios and discuss their outcomes with the staff of a cooperative cellar growing nearly 2000 ha of grapevine in South-western France.

P5.1-046

COLLETOTRICHUM POPULATION STRUCTURE IN OLIVE ORCHARDS FROM THE REGION OF PREVEZA, GREECE AND EFFECT ON OLIVE OIL QUALITY

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Text

Anthracnose disease in olive orchards has acquired pandemic characteristics worldwide with significant yield losses and olive oil deterioration. In the present study, the population structure of Colletotrichum species of two orchards in Preveza, Greece was investigated during the period 2021 - 2023. Additionally, the effect of the intensity of olive fruit infestation by Colletotrichum species complex on quality and organoleptic characteristics in extracted oil was evaluated. Olive droops were collected from the olive groves based on symptom incidence, common harvesting practices and disease management. Preliminary sequencing analysis of the ITS (internal transcribed spacer) region of fungi isolated from olive fruits collected in 2021 indicated the presence of C. godetiae and other Colletotrichum spp. in the same groove. Olive oil extracted from these olive fruits by cold pressing was of lower category and quality. They exhibited higher acidity and peroxide value, and lower levels of phenolics, antioxidant activity and organoleptic quality. A further epidemiological study is under way in 2022- 2023, with additional isolation of Colletotrichum spp., their evaluation of sensitivity to fungicides, but also quantification of the pathogen biomass in olive fruit, petioles, stems and alternative hosts. Correlation of results with olive oil quality and local climatic conditions will lead to the development of an integrated protocol for disease management.

P5.1-047

TRACKING ASIAN SOYBEAN RUST IN BRAZIL

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Text

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, is one of the most severe diseases of soybean. The fungus survives year-round in Brazil and regulatory measures were adopted to reduce the inoculum between crop seasons (soybean-free period). In 2004, a website (consorcioantiferrugem.net) was developed to track the disease. After 2010, the information added to the website focused on the first reports of ASR during the crop season, to warn farmers of the fungus´s presence. The information is available in an App that releases warning messages. From 2010 to 2023, average reports before R5 stage (beginning seed) in commercial areas were 23%, and after R5, 77%. Reports on the website show a later onset of the disease, with less damage potential. Since 2005, the soybean cultivars cycle has been reduced in Brazil, and early soybean sowing allowed a significant increase in the sowing of successive crops (e.g., soybean-corn, soybean-cotton). Soybean is sown in Brazil after September and the first ASR reports on the website start in November (2%) and December (11%). Harvest starts in January, showing that areas sowed earlier

escape the disease. The reports increase in January (38%) when the first areas are harvested. Soybean rust in Brazil is a threat to areas sowed late in the season. Data from the website gathered from 2010 to 2023 shows a predominance of SBR first occurrence after R5 and the first reports from November and December, with a significant increase in January.

P5.1-048

ECONOMIC INEFFICIENCIES IN PRIVATE MANAGEMENT OF EPIDEMICS SPREADING BETWEEN FARMS

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Text

Most plant disease epidemics spread both within and between farms. However, in the absence of collective action, each farmer generally takes disease control decisions based on personal costs and benefits. It is important to identify under which conditions the combination of such private control decisions can have synergistic or antagonistic effects, and can lead to collective economic inefficiencies. We used the game theory framework to investigate these questions, considering a simplified two-period game where two farmers decide whether or not to control an epidemic on their farm. Taking the example of sharka epidemics, caused by plum pox virus in Prunus orchards, we characterized the game and its outcomes according to initial epidemic conditions and focused on those likely to produce economic inefficiencies. Our results show that depending on the initial infection levels, a broad range of games may arise, some of which involving synergistic or antagonistic control decisions. This means that the nature of strategic interactions between famers may change depending on the state of the epidemic. After a thorough characterization of the epidemic conditions for which private management produces collective economic inefficiencies, we investigated the expected effect or different public policy incentives aiming to reduce such inefficiencies.

P5.1-049

MODEL-BASED CHARACTERIZATION OF INTERACTIONS BETWEEN PLUM POX VIRUS STRAINS FROM A FIELD SURVEY

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Text

Multiple pathogen strains often co-circulate within the same host population and may lead to multiply infected individuals. However, the characterization of the interactions between strains and of their epidemiological impact is still a burgeoning research field. Here we show how modeling multi-strain epidemiological dynamics provides a more reliable assessment of strain interactions in the field than statistical tests of independence. The constructed model, which accounts for orchard age and all possible strain combinations, was applied to test for

interactions between three virus strains under the assumption of endemicity. The Serbian survey data used for the inference showed that sharka, caused by the plum pox virus (PPV), is widespread in the 91 sampled plum orchards and that three strains (M, D, and Rec) are present even as double and triple infections in the field. The observed co-infection frequencies were compared with their predicted frequency under the neutral/null hypothesis that there is no direct or indirect interaction between strains. We showed that D+Rec co-infections were less frequent than expected in the neutral case but also, in contrast with model predictions, that D+Rec coinfection frequency decreased with the orchard age. Our results raise biological and epidemiological questions on the Rec strain of PPV and emphasizes the importance of coupling models and data to understand and predict dynamics in complex epidemiological systems.

P5.1-050

GLOMERELLA LEAF SPOT IN APPLE ORCHARDS OF SOUTH TYROL (ITALY) AND THE DEVELOPMENT OF CONTROL STRATEGIES

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Text

Glomerella leaf spot (GLS), caused by several *Colletotrichum* species, is an emerging disease of apple worldwide. So far, GLS was restricted to apple growing areas with humid, subtropical climate, and was not reported from Europe. But, extreme weather conditions, including heavy rainfall and warm temperatures in summer 2020, led to an unknown symptomatology in South Tyrolean (Italy) apple orchards: necrotic lesions and chlorosis developed on leaves, premature leaf dropping was observed. Within a few days, circular, brownish spots appeared on above 90 % of apples in affected orchards. Fungal isolates were obtained from symptomatic leaves and fruit spots, morphological analysis was performed as well as multi-locus sequence analysis based on the eight gene loci: the ITS region; actin (ACT), DNA-lyase (APN2), calmodulin (CAL), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glutamine synthetase (GS), beta-tubulin (TUB2), and partial mating type gene (Mat1-2). *Colletotrichum chrysophilum* was identified as causal agent of GLS in South Tyrol. Pathogenicity assays on fruit and apple seedlings were established, and Koch’s postulates were confirmed. These results constitute the foundation for the development of in vitro and in vivo screenings of biologicals as well as of active substances. Together with agronomical measurements, these findings shall contribute to targeted plant protection and containment strategies for the largest contiguous apple growing area in Europe.

Molecular aspects of plant-fungal interactions Part 1: Effectors

C3.1-1

TOWARDS UNDERSTANDING HOW MAGNAPORTHE ORYZAE CO-OPTS PLANT ENDOCYTOSIS FOR TRANSLOCATION OF CYTOPLASMIC EFFECTORS

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Text

Fungal and oomycete pathogens deliver effector proteins directly into plant cells to suppress the plant immune system, reprogram cellular processes and enable pathogens to rapidly invade and proliferate within plant tissue. To date, little is known about the mechanism by which these pathogens translocate effector proteins across the plasma membrane into the plant cytoplasm. The M. oryzae-rice pathosystem possesses advantages for the study of effector cell biology due to the specific localization of cytoplasmic effectors within the specialized biotrophic interfacial complex (BIC) before translocation. We have shown that cytoplasmic effectors within BICs are packaged into dynamic, vesicle-like membranous effector compartments (MECs) that are occasionally observed in the host cell cytoplasm. Live cell imaging with fluorescently labeled proteins in rice revealed that these MECs colocalize with the plant plasma membrane and with CLATHRIN LIGHT CHAIN 1, a component of clathrin-mediated endocytosis (CME). By contrast, early- and late-endosome markers Ara6 and Ara7 did not colocalize with MECs in BICs. Inhibiting CME using virus-induced gene silencing and chemical treatments resulted in cytoplasmic effectors in swollen BICs lacking MECs. We also identified the first plant plasma membrane-associated effector, Bas83, that appears to play a role in recruiting plant membrane for the endocytic machinery at the BIC. Silencing of the BAS83 gene suggests a potential role of this effector in fungal virulence. Colocalization assays between fluorescently-labeled effectors and rice LifeAct showed accumulation of plant actin at BICs, suggesting possible short-distance transport of MECs. Since endosomes normally deliver cargos to other compartments and not to the cytoplasm, and MECs do not colocalize with early-and late endosome markers, it is unclear if effectors enter through the normal endosome system. A major question remains about how cytoplasmic effectors are released from MECs into rice cell cytoplasm. One possible route is lipid degradation in the MEC via fungal lipases. In fact, we identified a BIC and MEC localized lipase-like effector that may be associated with effector content release from MECs into rice cell cytoplasm. Taken together, this study provides evidence that cytoplasmic effector translocation is mediated by CME in BICs and suggests a role for M. oryzae effectors in coopting plant endocytosis.

C3.1-2

EVOLUTION OF STRUCTURALLY CONSERVED EFFECTOR FAMILIES IN ASCOMYCETE FUNGI

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Text
The range of hosts that parasites can infect is a key determinant of the emergence and spread of disease. In fungal pathogens, host range varies from a single host genotype (specialists) to hundreds of unrelated species (generalists). Pathogen small-secreted protein effectors play a major role in manipulating plants to facilitate disease. Studies of host adaptation in plant pathogen specialists revealed strong diversifying selection in effector genes, associated with tradeoffs on effector activity in diverse plant species. Tradeoffs on effector activity may easily account for evolution by host switching but not for host range expansion and the evolution of generalists. To search for candidate effectors associated with a generalist lifestyle, we analyzed the predicted structure of small-secreted proteins lacking functional annotation (orphans) across twenty Ascomycete genomes. I will present evidence for the existence of a limited repertoire of conserved general-purpose effectors. Insights into their macroevolution will be discussed. These findings point towards molecular mechanisms and evolutionary patterns favoring parasitism on multiple hosts.

C3.1-3

ENTERING VIA THE FRONT DOOR: CYTOPLASMIC EFFECTORS TAKEN INTO HOST CELLS VIA ENDOCYTOSIS

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Text
Phytophthora plant pathogens threaten food security, forestry and ecosystems. The delivery of effectors into host plant cells to modulate plant defence is essential for infection success. The RxLR class is the best characterised group of cytoplasmic effectors. How they are translocated is still being unravelled and a key question is: how do they enter host plant cells after secretion?
Tagged forms of key endocytosis components, clathrin and Ara6, expressed in infected plant cells, associate with vesicles around P. infestans haustoria. Silencing expression of clathrin or Ara6 in Nicotiana benthamiana reduced P. infestans infection and reduced translocation of tagged RxLR effectors into infected cells. In contrast, silencing PP1c isoforms, susceptibility factors that are not required for endocytosis, reduced infection but did not attenuate RxLR effector uptake.
After endosome enrichment tagged RxLR effector Pi04314-RFP co-localised in sucrose gradients with clathrin-labelled vesicles. Immunoprecipitation of clathrin- or Ara6-associated vesicles co-immunoprecipitated RxLR effectors Pi04314-RFP and Avrblb1-RFP but not the apoplastic effector PiSCR74-RFP from infections with transgenic P. infestans lines secreting these fusions. Proteomic analyses of proteins detected in immunoprecipitated Ara6-labelled endosomes from infection revealed enrichment of multiple RxLR, but no apoplastic effectors. Our data show that uptake of P. infestans RxLR effectors into plant cells occurs via CME.

C3.1-4

PARASTAGONOSPORA NODORUM USES DIVERSE EFFECTOR FUNCTIONS TO FACILITATE THE COLONIZATION OF WHEAT

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Text
To facilitate the completion of its pathogenic life cycle on wheat, *Parastagonospora nodorum* secretes multiple necrotrophic effectors (NEs), which are defined as effectors that trigger programmed cell death (PCD). To induce PCD, NEs target wheat susceptibility genes resulting in the initiation of a defense response (DR), including PCD. Typically, initiation of DR pathways would result in resistance, however, *P. nodorum* uses the triggering of the DR to its advantage. Five NEs have been functionally validated and characterized, and at least eight host targets have been validated, with some NEs having multiple host sensitivity genes. In addition to NE activity, two of the five NEs have secondary effector functions, including chitin binding/chitinase protection (SnTox1) and inhibition of PR1 protein activity (SnTox3). We used confocal microscopy to show that in addition to its NE function, SnTox5 also showed hallmarks of an additional effector function where it facilitated the colonization of the mesophyll, even in the absence of its PCD-inducing target *Snn5*. Subsequent RNA-Seq analysis comparing a *P. nodorum* wild type and an *SnTox5*-disrupted strain showed that SnTox5 is involved in the regulation of wheat DR genes as well as several transcription factors previously shown to regulate DR pathways in other plant-pathogen interactions. This result shows that in addition to its NE activity, SnTox5 is likely modulating the DR to facilitate the colonization of wheat.

C3.1-5

LESSONS TO LEARN FROM A GALL-INDUCING FUNGUS

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Text
Smut fungi form a large group among the basidiomycetes and are biotrophic specialists in infecting a diverse set of mainly grasses, among them important crops like sorghum, millet, barley and maize. The maize smut fungus Ustilago maydis serves as an important model for smuts fungi and induces prominent galls on all aerial parts of its host, reflecting a metabolic and developmental reprogramming of the plant. This massive manipulation of the host is achieved with the help of fungal secreted molecules, so called effectors. In a systematic approach we screened in the past decade hundreds of putative effector proteins to identify their specific place of action and their functions on the plant side. Here I will present our current molecular understanding of the fungal effectome and the biotrophic interaction between the fungus and its host plant maize.

C3.1-6

PLANT PATHOGENS MANIPULATE HOST MICROBIOTA TO PROMOTE DISEASE DEVELOPMENT

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Text

Beneficial plant-associated microbes are found in and on all organs of the plant and help to mitigate (a)biotic stresses. Moreover, plants are able to shape their microbiota and specifically attract beneficial microbes to suppress pathogen attack. Hence, the plant's microbiome can be considered an inherent, exogenous layer that complements its endogenous innate immune system. Microbes typically secrete a plethora of molecules into their environment to promote niche colonization. Especially soil-dwelling microbes are well-known producers of antimicrobials that are exploited to outcompete microbial co-inhabitants in the soil. Plant pathogenic microbes similarly secrete a diversity of molecules into their environment for niche establishment. Upon plant colonization, microbial pathogens secrete so-called effector proteins that promote disease development. While such effectors are typically considered to exclusively act through direct host manipulation, for instance through the suppression of host immune responses, increasing evidence demonstrates that pathogenic fungi exploit effector proteins with selective antimicrobial properties to promote host colonization through the manipulation of beneficial host microbiota. Given that effector-mediated microbiota manipulation may have evolved in fungal ancestors that encountered microbial competition before symbiosis with land plants evolved, we propose that effector-mediated microbiota manipulation is fundamental to fungal biology.

F3.1-1

NOVEL NUCLEAR LOCALIZATION SEQUENCE OF MOHTR1, A NUCLEAR EFFECTOR OF THE RICE BLAST FUNGUS, IS CRUCIAL FOR FUNGAL PATHOGENICITY AND PLANT IMMUNE-RESPONSE BY TRANSCRIPTIONAL REPROGRAMMING

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Text

Plant pathogenic effector is secreted into the host and modulates the host immune system. Nuclear effectors translocated in the host nuclei and interact with proteins and DNA to regulate various defense mechanisms. Nuclear localization sequence (NLS) is the most well-known factor for nuclear transportation. However, the molecular mechanism of NLS-associated vehicles and the roles of NLS in transcriptional reprogramming still need to be understood. We previously reported that MoHTR1, a nuclear effector of the rice blast fungus, *Magnaporthe oryzae*, is translocated to rice nuclei but not to fungal nuclei. MoHTR1 was

localized in the plant nucleus by interacting with rice importin alpha. We found one NLS (PGRSKKE) and further identified that RxKK residues were necessary for the nuclear localization of MoHTR1. MoHTR1 NLS altered the localization of cytoplasmic effectors of *M. oryzae* in the host. Furthermore, nuclear effector candidates which have RxKK sequence also localized in rice nuclei. SUMOylation, post-translational modification, was involved in the secretion and translocation of MoHTR1 to biotrophic interfacial complexes and host nuclei. In addition, MoHTR1 NLS was essential for the pathogenicity of *M. oryzae* by reprograming defense-related genes and host target gene candidates. Our findings will provide unprecedented details on the roles of plant-specific NLS on nuclear effector in pathogen-host interactions.

F3.1-2

ALLELE-SPECIFIC RECOGNITION OF THE MAX EFFECTOR AVRRVI6 BY RVI6 RESISTANCE PROTEIN IN THE APPLE–VENTURIA INAEQUALIS PATHOSYSTEM

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Text
Apple scab caused by the pathogenic fungi *Venturia inaequalis* is the most common disease in apple orchards. Resistance genes such as *Rvi6* have been introduced in apple varieties to reduce the use of fungicides in the management strategies of this disease. However, in the 90's, we observed a breakdown of *Rvi6* resistance and the emergence of *V. inaequalis* virulent stains on trees carrying *Rvi6*. Using genomic analysis, we identified *AvrRvi6*, the first fungal avirulent gene responsible for a gene-for-gene interaction in the Apple-*V. inaequalis* pathosystem. *AvrRvi6* codes for a small secreted protein with structure similar to the MAX effector family. On the plant side, Rvi6 is a receptor-like protein (RLP), similar to RXEG1 and predicted to localize at the plasma membrane. We observed that fungal virulent strains have mutations in the coding or regulatory sequences of AvrRvi6 which we hypothesize are responsible for the escape of Rvi6 immunity. Using transient expression in *Nicotiana benthamiana* we were able to replicate the recognition of AvrRvi6 by Rvi6 resulting in a strong cell death phenotype. This *N. benthamiana* assay allowed us to test the AvrRvi6 alleles and identify essential residues for Rvi6 recognition. We also investigated the protein-protein interactions between AvrRvi6 and Rvi6 and the subcellular localization of this allele-specific immune recognition.

P3.1-001

The infection cushion, a key organ of virulence for Botrytis cinerea

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Text
A collection of Botrytis cinerea ATMT-strains revealed that random mutants exhibiting total loss of virulence toward different host plants share a common profile including impaired secretion of hydrolytic enzymes and severe deficiency in mature infection cushion (IC). IC is a multicompound appressorium differentiated by an epiphytic mycelium to penetrate a plant host. Transcriptomic and proteomic analysis of the mature IC highlighted high secretion of ROS and proteins involved in virulence such as plant cell-death inducing proteins. These results support a role for the IC in stimulating plant immunity and inducing necrotrophy of the pathogen. But surprisingly, effectors suppressing the plant chitin-triggered immunity were also induced in the IC. Chitin deacetylases genes (cda) are up-regulated and the conversion of chitin into chitosan was confirmed by differential staining of the IC cell wall. Cda mutants show a reduced pathogenicity compared to the wild-type strain and stimulate plant immunity. A LysM effector accumulated by the IC can bind the chitin in the fungus cell wall and protects hyphae against degradation by external chitinases. It is also able to sequester chitooligosaccharides and to prevent them from inducing ROS production in A. thaliana. Deletion strains of the LysM gene show a delay in infection initiation. It is hypothesized that the infection cushion must hide from the plant during the asymptomatic phase and then induces necrotrophy.

P3.1-002

NPS2, ENCODING A NON-RIBOSOMAL PEPTIDE SYNTHETASE, IS A VIRULENCE FACTOR OF THE MAIZE ANTHRACNOSE FUNGUS C. GRAMINICOLA

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Text
Iron is an important nutrient that plays a prominent role in diverse biological functions in all organisms, including fungi. Due to its poor solubility, iron is a limiting factor in growth and development. On the other hand, an excess of iron leads to toxicity through the formation of hydroxyl radicals. In the maize anthracnose fungus *Colletotrichum graminicola*,the non-ribosomal peptide synthetase Nps2 is responsible for the synthesis of the intracellular siderophore ferricrocin and thus indispensable for iron scavenging and for prevention of iron toxicity. Also, in Magnaporthe oryzae and Alternari alternata Nps2 is required for the biosynthesis of ferricrocin as an intracellular storage siderophore. Since the exact role of Nps2 in C. graminicola is yet unknown, we generated *NPS2* deletion and green fluorescent protein (GFP) promoter fusions strains to elucidate the role of NPS2 in intracellular siderophores biosynthesis and control of intracellular iron homeostasis under iron deficiency and iron surplus conditions. Our findings to date show that expression of *NPS2* occurs at each stage of pathogenesis. Although the differences in growth and development between WT and *Δnps2* strains appear to be marginal under standard conditions, the deletion mutants show higher susceptibility to oxidative stress. In addition, infection experiments on maize leaves, qPCR analyses, and quantification of infection structures revealed that NPS2 is

required for full virulence of *C. graminicola*.

P3.1-003

VIRULENCE PROFILE OF COLLETOTRICHUM FALCATUM WENT ISOLATES PREVAILING IN PAKISTAN

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Text

Sugarcane grows well in the tropical and subtropical climates of Pakistan. Diseases, particularly, red rot caused by Colletotrichum falcatum, is the major factor for country's low sugarcane yields of only 60.31 tons/ha. Losses estimated at 10-77% in cane yield and 4-74% in sugar recovery have been reported. C. falcatum is highly variable pathogenically making it extremely difficult to obtain stable resistant varieties. Hence, detail studies were initiated to identify the number of strains present in Pakistan. The pure cultures (n=12) were prepared from naturally infected sugarcane stalks, surveyed on 09 commercially grown varieties from 28 different localities during 2020-21 cropping season. The cultures were identified as C. falcatum based on spore morphology and were divided into under three distinct molecular groups as Group I, II, and III. Among the isolates, 5 proved to be virulent with light colored and abundant sporulating mycelium, 2 were moderately virulent with light colored and moderate sporulating type and 5 were least virulent with dark colored and least sporulating mycelium. The isolates showing variable virulence were characterized at molecular level by 18S rRNA/ITS gene analysis. The PCR conditions were optimized for the amplification of 600-700 bp 18SrRNA/ITS genes. The genes were sent to commercial organization for sequencing and analysis at NCBI/gene bank using the blast n homology tool to identify the isolates at molecular level.

P3.1-004

GENETIC TRANSFORMATION AND EXPRESSION OF DSRED AND EGFP IN ASCOCHYTA PISI TO CHARACTERIZE ASCOCHYTA BLIGHT DISEASE PROGRESSION IN PEA (PISUM SATIVUM L.)

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Text

The Plant immune system is made up of a complex response network that involves several lines of defense to fight invading pathogens. In this study interaction between Ascochyta pisi fungus and pea genotypes was explored to investigate the progression of ascochyta blight (AB) in pea. Here we developed genetics transformation system for A. pisi by constructing a new binary vector, pBIF-DsRed and pBIF-EGFP, for the constitutive expression of the red fluorescent protein (DsRed) and green fluorescent protein (EGFP) used as a highly efficient vital marker to study the developmental changes in A. pisi during AB disease progression. The initial infection stages were similar in both the resistant and susceptible accessions where A. pisi uses infection structures such as germ tubes and appressoria to gain entry into the host while the host uses defense mechanisms to prevent pathogen entry. The pathogen attempted to penetrate and colonize in radly enter in resistant, but further fungal advancement appeared to be halted, and A. pisi did not enter the mesophyll. But successful entry and colonization in susceptible, coincided with structural changes in A. Pisi. Pycnidia-bearing spores appeared 3-14 days post-inoculation. The use of fluorescent proteins in plant pathogenic fungi together with confocal laser scanning microscopy, provide a valuable tool to study the intracellular dynamics, colonization strategy, and infection mechanisms during plant-pathogen interaction.

P3.1-005

IDENTIFYING EFFECTORS FROM THE FUNGAL PATHOGEN ZYMOSEPTORIA TRITICI THAT CAN OVERCOME RESISTANCE IN COMMERCIAL WHEAT CULTIVARS

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Text

Zymoseptoria tritici causes the major fungal wheat disease Septoria tritici blotch (STB), which is the most economically destructive disease of wheat worldwide threatening global food security. Disease management is obtained through fungicide application and breeding for resistance. Control of STB by applying fungicides has resulted in the frequent development of fungicide-resistant strains over the last decades. Therefore, crop protection to STB is more and more dependent on resistant cultivars. To deploy these cultivars effectively, we require knowledge on the molecular mechanisms employed by Z. tritici to overcome host resistance in wheat. Hence, identification of isolates that can overcome resistance and identification of the virulence genes (effectors) responsible is a crucial factor in designing new effective approaches for STB management. We aim to (1) identify Z. tritici virulence factors, contributing to the aggressiveness of Z. tritici on commercial wheat cultivars and (2) perform functional characterization of the (a)-virulence factor(s) that are key elements in the Z. tritici-wheat interaction. To accomplish these goals, we are employing, a Genome-Wide Association Study followed by functional genomics. This will allow the future informed deployment of resistant wheat cultivars and the ability to monitor for virulent Z. tritici isolates in the field.

P3.1-006

ANALYSIS OF FACTORS INVOLVED IN GROWTH INHIBITION AND BLACKENING OF RICE ROOTS INFECTED WITH PYRICULARIA GRISEA

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Text

Pyricularia spp. are pathogenic filamentous fungi that cause blast disease mainly in various monocotyledonous plants. *Pyricularia* isolates consist of pathotypes that show strict host specificity at the plant genus level in above-ground parts such as leaves. However, we reported that the host specificity of *Pyricularia* fungi was less strict in the underground parts. We found that *P. grisea* isolated from *Digitaria*, Dig4-1, caused strong growth inhibition and blackening lesion on rice roots. It was known that Dig4-1 secreted a phytotoxin, Pyrichalasin H belonging to cytochalasans. We constructed a mutant lacking the PyiB gene involved in Pyrichalasin H biosynthesis. The resultant mutant ?pyiB showed a reduction in root growth inhibition, suggesting that Pyrichalasin H is one of the factors inhibiting root growth. While most *Pyricularia* isolates showed a browning lesion on rice roots, Dig4-1 induced a blackening lesion. Cytological observation of blackening lesions revealed that infection hyphae were intensively localized at the outer layer of the root tissue. In addition, infected root cells were filled with hyphae. ROS generation was relatively lower at blackening lesions than browning lesions. RNA-seq analysis revealed that expression levels of defense-related genes were low at blackening lesions than browning lesions. These results suggest that the Digitaria pathotype is more virulent on rice roots than other *Pyricularia* pathotypes.

P3.1-007

MELALEUCA QUINQUENERVIA; TOWARDS A MODEL FOR MYRTLE RUST RESEARCH

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Text

Myrtle rust caused by the biotrophic fungal pathogen *Austropuccinia psidii* infects hundreds of species in the family Myrtaceae, with variability in response to the pathogen identified both within and between species. As a result, studies investigating resistance to *A. psidii* have been conducted in a range of Myrtaceae species. While important to understand the extent of species susceptible to the disease, no model has been established for the study of *A. psidii*. *Melaleuca quinquenervia* is a keystone paperbark species broadly distributed across the east coast of Australia and displays a variable response to *A. psidii*. It can be propagated from cuttings, seed can be collected year-round, seedlings establish rapidly, a high-quality genome has been generated, and transcriptomic analysis conducted. These qualities provide the opportunity to utilise this species as a model system for the study of resistance to *A. psidii*. Using our chromosome-level phased genome, we have annotated genes encoding the Nucleotide-binding Leucine-rich Repeat (NLR) domain intracellular receptors within a resistant *M. quinquenervia*, an important family of plant resistance genes. Transcriptomic analysis of the progeny from this tree sheds light on the mechanisms and pathways for resistance to *A. psidii*. Importantly, these analyses provide a framework for identification of

resistance genes and pathways in other *Melaleuca* species and a proof of concept for this species as a model.

P3.1-008

THE PERFECT DAWN: THE ROLE OF EARLY MORNING LIGHTING ON IMMUNITY

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Text
Botrytis cinerea (grey mould) is estimated to cost up to a global $10 billion per year in crop losses and management. Many of its hosts are greenhouse crops (e.g. tomato, lettuce, strawberry, grape). These controlled greenhouse environments provide the warmth and humidity required for rapid fungal disease spread. Moreover, many indoor farms use supplementary artificial lighting, or are fully dependent on artificial lights.

Recent work has identified a light-dependent Dawn Burst Transcriptional Network in *Arabidopsis thaliana*, which is independent of the circadian clock (1). We have found that the Dawn Burst hub genes (HY5, HYH, BBX31) influence resistance to *B. cinerea* in a time-dependent manner. This suggests that modifying early morning lighting (i.e. dawn) may enhance disease resistance in an indoor farm setting.

However, these experiments were carried out under unnatural lighting conditions and so may not necessarily be commercially applicable. Here, we simulate more realistic dawns to investigate the effect of dynamic morning light on lettuce (*Lactuca sativa*) and Arabidopsis, and their response to *B. cinerea* infection. This enables us to gain a more nuanced understanding of the role of the Dawn Burst Network on immunity, as well as potentially developing commercially applicable lighting regimes.

1. Balcerowicz et al. 2021. https://doi.org/10.1016/j.molp.2021.03.019

P3.1-009

FOREST TREE MYCOBIOME: IMPACT ON HETEROBASIDION PATHOGENESIS AND PLANT HEALTH

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Text
Microorganisms constitute an integral component of all terrestrial ecosystems and plays an important role in maintaining the host fitness. In fact, all macroorganisms live in close association with a diverse range of microbial symbionts. Despite a significant progress in our understanding of the plant microbiome, very little is known about their effect on host genetics

as well as on other tree microbiomes. In this study, we analysed the mycobiome of asymptomatic and symptomatic Norway spruce trees naturally infected by Heterobasidion spp. Our results demonstrate that the structure of fungal communities residing in the wood differed significantly among symptomatic and asymptomatic Heterobasidion infected trees. The result also showed that under in vitro conditions, one of the trees associated mycobiome, a dark septate endophyte (Phialocephala sphaeroides, DSE) promoted the root growth of Norway spruce seedlings. The DSE significantly reduced H. parviporum transcripts (by 92%) during co-infection. A specific transcriptional response to P. sphaeroides inoculation was the increased transcripts of genes involved in jasmonic acid biosynthesis and plant hormone signal transduction. The P. sphaeroides experienced a shift from cell growth to anti-stress, while it repressed H. parviporum carbohydrate/polysaccharide-degrading enzyme machinery. We conclude that H. parviporum triggered reprogramming of host metabolism whereas the endophyte counteracted the negative effects of pathogen.

P3.1-010

CHARACTERIZATION OF NRPS AND PKS GENE CLUSTERS AND FAMILIES IN ALTERNARIA DAUCI AND DEMONSTRATION OF THE ALDAULACTONE BIOSYNTHESIS PATHWAY THROUGH KO MUTANTS

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Text

Quantitative disease resistance in necrotrophic pathosystems involves various mechanisms, including chemical warfare. *Alternaria dauci*, a necrotrophic fungus, synthesizes toxins conferring pathogenicity to carrots. Aldaulactone, a phytotoxic benzenediol lactone from *A. dauci*, has been shown to be central in both the pathogenicity of *A. dauci* and carrot partial resistance toward the fungus. Secondary metabolite biosynthesis is thus of prime importance in this interaction. Our study provides the first comparative examination of the SM genetic basis in *Alternaria*. Using transcriptome data, we assembled the *A. dauci* genome data set and identified 19 putative SM clusters. Comparison of these genomic data with the already published genomes of other *Alternaria* species predicted 55 putative families of SM core genes in the *Alternaria* genus. Exploitation of phylogeny allowed us to pinpoint cluster 8 as a candidate for aldaulactone biosynthesis. This cluster harbors *AdPKS7* and *AdPKS8*, homologs of genes encoding a reducing and a non-reducing polyketide synthase necessary to produce benzenediol lactones. The expression patterns of *AdPKS7* and *AdPKS8* correlated with aldaulactone production. We also produced KO-mutant *A. dauci* strains for both PKS genes. Aldaulactone production in the transformed strains was abolished as determined by HPLC analysis. Our results provide strong evidence that *A. dauci* PKS cluster 8 harboring *AdPKS7* and *AdPKS8* is responsible for aldaulactone biosynthesis.

P3.1-011

A COMPARATIVE STUDY OF SA-JA-ABA CROSS-TALK IN RESPONSE TO ALTERNARIA BRASSICAE IN SUSCEPTIBLE AND RESISTANT BRASSICA SPECIES.

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Text

An extensive yield-oriented breeding approach, climate change and a faster rate of adaptation of pathogens have rendered cultivated mustard (Brassica juncea) susceptible to many diseases including Alternaria blight caused by Alternaria brassicae. Past research has established that the disease resistance mechanism in plants is regulated by SA and JA-mediated pathways. However, recently the studies on the involvement of ABA in SA-JA cross-talk have gained momentum because of the diverse role of ABA in disease resistance. Depending on the phyto-patho system ABA can be a positive or a negative regulator. In terms of the A. brassicae-brassica system, the role of ABA as a regulator has not been established assertively. This study aims at understanding the existing variation concerning phytohormone signalling in susceptible B. juncea and its resistant wild relatives and to decipher the effect of the pathogen on variable SA-JA-ABA cross-talk. Our study revealed a significant difference in the initial trigger of phytohormone signalling with 3hrs of inoculation in susceptible and tolerant genotypes. Also, the involvement of ABA in SA-JA cross-talk differs considerably in susceptible and resistant genotypes. Our studies revealed ABA as the key modulator of resistant/susceptible response in Brassica-A. brassicae phyto-pathosystem by regulating the SA-JA biosynthesis pathways in the early hours of the inoculation.

P3.1-012

NOVEL GENES ASSOCIATED WITH SUSCEPTIBILITY OR CRYPTIC QUANTITATIVE DISEASE RESISTANCE TO PYRENOPEZIZA BRASSICAE IN BRASSICA NAPUS

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Text

Unlike, R gene-mediated resistance, quantitative disease resistance (QDR) can provide

crops with durable control against pathogens. QDR is a desirable trait for crop improvement, but little is known about causative genes, which makes incorporation into breeding programmes difficult. Light leaf spot, caused by Pyrenopeziza brassicae, is an important disease problem of oilseed rape (Brassica napus) in the U.K. and Europe. To identify new QDR gene loci, we have used a high-throughput screening pathosystem with P. brassicae on 195 lines of B. napus in combination with an association transcriptomics platform. We have demonstrated that all resistance against P. brassicae was associated with QDR and not R genes. We have used genome-wide association mapping with an improved B. napus population structure to reveal four loci significantly associated with QDR in regions showing linkage disequilibrium. In addition, eight gene expression markers (GEMs) were associated with QDR against P. brassicae. For seven of these, expression was positively correlated with resistance whereas for one, an HXXXD-type acyl-transferase negative correlation indicated a potential susceptibility gene. Pathogen-induced gene expression was detected in resistant B. napus lines for five out of seven GEMs tested. The acyl transferase was only induced in susceptible B. napus lines. A TILLING mutant with a D167N substitution was more resistant against P. brassicae than the cv. Cabriolet background.

P3.1-013

NOVEL RESOURCES FOR SOUTHERN BLIGHT DISEASE RESISTANCE BREEDING IN COMMON BEAN

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Text

Southern blight of common bean caused by *Sclerotium rolfsii* Sacc. is an economically important disease commonly occurring in the tropics, sub tropics and warm temperate regions. Yield losses of up to 64% have been reported due to the disease. Use of host plant resistance is the most suitable option for management of the disease. Southern blight is currently the most important root rot disease of common bean in Uganda. The National Legumes Research Programme of the National Crops Resources Research Institute has initiated research activities that will lead to the development and release of Southern blight resistant varieties in Uganda. Accordingly, 200 isolates of *S. rolfsii* obtained from within Uganda have been observed to be culturally, morphologically and phenotypically diverse. We are currently undertaking molecular characterization to determine genetic diversity of the isolates. In our efforts to identify sources of resistance to Southern blight disease, we have a collection of 582 diverse Common bean germplasm from disease nurseries, interspecific lines, advanced breeding lines, etc. Preliminary findings have shown significant differences in the reaction of the germplasm lines to *S. rolfsi*i infection. With further evaluations, we hope to identify reliable sources of resistance that can be used 1) to map Southern blight resistance and develop markers to accelerate breeding in Common bean and 2) as parents in Southern blight resistance breeding programmes.

P3.1-014

HETEROLOGOUS EXPRESSION IN NICOTIANA BENTHAMIANA IDENTIFIES CANDIDATE EFFECTOR PROTEINS FROM PHYLLACHORA MAYDIS THAT SUPPRESS CELL SURFACE-TRIGGERED IMMUNE RESPONSES

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Text

Fungal pathogens often secrete virulence proteins into plant cells during the infection process to modulate host immune responses. Recent genomic and transcriptomic studies of Phyllachora maydis which causes tar spot disease in maize, revealed 163 putative effector proteins, eighteen of which are abundantly expressed during disease development. Here, we used heterologous expression in N. benthamiana to elucidate whether any of these eighteen candidate effector proteins have effector-like functions. Live-cell imaging of N. benthamiana epidermal cells revealed that a majority of the putative effectors localized to the nucleus and cytosol. We also show that though all candidate effectors expressed detectable protein, none were able to suppress cell death triggered by BAX or INF1 when transiently expressed in N. benthamiana, indicating that these putative effectors likely do not function as general cell death suppressors. Importantly, some candidate effectors consistently suppressed cell surface-triggered immune responses including chitin-induced reactive oxygen species production and MAP kinase activity, revealing these putative effectors contribute to inhibition of immune responses. These results provide valuable insights into the putative functions of candidate effectors from P. maydis and will stimulate new research aimed at elucidating the molecular mechanisms potentially manipulated by this fungal pathogen.

P3.1-015

IDENTIFICATION OF A PATHOGENICITY CHROMOSOME IN FUSARIUM OXYSPORUM F. SP. CEPAE.

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Text

Fusarium oxysporum species complex is a cosmopolitan, soil-borne, plant pathogenic fungus with a wide host range spanning over 120 species. In *F. oxysporum*, a pathogenicity chromosome (necessary for pathogenicity toward its host but not for vegetative growth) has been reported. However, the presence of a pathogenicity chromosome has not been verified in *F. oxysporum* f. sp *cepae* (*Foc*), which causes Fusarium basal rot disease on onions.

Therefore, the aim of this study was to determine whether a pathogenicity chromosome is present in *Foc*. We initially screened for effector candidates in the Japanese strain *Foc*_TA using genomic sequence data. Twenty-one effector candidates were identified, of which five were expressed during infection. Interestingly, four of the expressed effector candidates were located on the 4-Mb chromosome in *Foc*_TA. To elucidate the relationship between pathogenicity and the 4-Mb chromosome in *Foc*_TA, nine putative 4-Mb chromosome loss strains were generated by benomyl (a mitosis inhibitor drug) treatment. Pathogenicity testing with these putative 4-Mb chromosome loss strains revealed impaired pathogenicity toward onion. Moreover, genome analysis of the putative 4-Mb chromosome loss strains demonstrated that the 4-Mb chromosome functioned as a pathogenicity chromosome in *Foc*_TA, and a 2.7-Mb region within the 4-Mb chromosome is necessary for full pathogenicity toward onion.

P3.1-016

A TRANSCRIPTOMIC ANALYSES OF THE ROLE OF OTHER FUNGI IN BOTRYTIS CINEREA CAUSED NOBLE ROT OF GRAPEVINE

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Text

Botrytis cinerea is one of the key fungi of grape production, which can lead the formation of noble rot under certain environmental conditions. Noble rot results unique metabolic profile, changes the physical texture and chemical composition. The functional genes during the process have been poorly characterized. We generated metatranscriptomic data from *Botrytis cinerea* infected grape berries representing the four phases of noble rot, from the healthy to the fully dried out berry. The genes were significantly enriched characterizing the carbohydrate and protein metabolism of the fungi involved in the breakdown of the berry skin structure. In addition, we identified genes expressed by the most abundant filamentus fungi and yeast during the noble rot process belonging to enriched pathways that can be crucious in grapevine cultivation. These fungi have been found to compete with *Botrytis* and to play an important role in the chemical composition of grapes

P3.1-017

METATRANSCRIPTOMIC ANALYSIS OF POSSIBLE CHANGES IN OENOLOGICALLY RELEVANT COMPONENTS OF GRAPE BERRIES DURING NOBLE ROT

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Text

Noble rot of grapevine (*Vitis Vinifera*) berries caused by Botrytis cinerea together with other filamentus fungi and yeasts. This development causes a unique aromatic profile of botrytized wines. To have more insight into this process we generated metatranscriptomic data representing the four NR stages (I-IV) from the Tokaj wine region of Hungary over three months. The most abundant filamentous fungi and yeast include Alternaria alternata, Botrytis cinerea, Epicoccum nigrum, Aureobasidium pullulans and Rhodotorula graminis, RNAseq reads were aligned to the latter species. Gene module clusters generated by WGCNA clustering, enriched pathways involved in the synthesis of aromatic compounds such as amino acid-, carbohydrate- and lipid metabolism co-jointly expressed by all filamentous fungi and yeast were identified within the turquoise module. It was found that the enzymes involved in the synthesis of aromatic compounds are expressed and up-regulated during the later stages (III-IV) of the NR process. This study has indicated that beside the *B. cinerea* other microbes are playing important role in aromatic development of grape berries during the noble rot process.

P3.1-018

COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF MAPK-MEDIATED REGULATION OF PATHOGENICITY, STRESS RESPONSES AND DEVELOPMENT IN CYTOSPORA CHRYSOSPERMA

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Text

MAPK cascades are highly conserved signal transduction pathways that mediate cellular responses to various biotic and abiotic signals in plant pathogenic fungi. Our previous studies have shown that CcPmk1 is a core regulator of fungal pathogenicity in Cytospora chrysosperma, the causal agent of canker disease in a wide range of woody plants. Here, we identified and functionally characterized the other two MAPK genes (CcHog1 and CcSlt2), and then compared the transcriptional differences among these three MAPKs in C. chrysosperma. We found that the MAPKs shared convergent and distinct roles in fungal development, stress responses and virulence. For example, CcHog1, CcSlt2 and CcPmk1 were all involved in conidiation and response to stresses, including hyperosmotic pressure, cell wall inhibition agents and H2O2, but only CcPmk1 and CcSlt2 were required for hyphal growth and fungal pathogenicity. Transcriptomic analysis showed that numerous hyperosmosis and cell wall related genes significantly reduced their expression levels in ΔCcHog1 and ΔCcSlt2, respectively. Moreover, two secondary metabolite gene clusters were significantly down-regulated in ΔCcPmk1, ΔCcSlt2 and/or ΔCcHog1. Importantly, some virulence-associated genes were significantly down-regulated in ΔCcPmk1 and/or ΔCcSlt2, such as candidate effector genes. Collectively, these results provide a better understanding of the regulation network of MAPKs in C. chrysosperma.

P3.1-019

TRANSGENIC BRASSICA NAPUS SEEDLINGS OVEREXPRESSING RICE ACYL-COA-BINDING PROTEIN OSACBP5 ARE PROTECTED AGAINST SEEDLING INFECTION BY FUNGAL PHYTOPATHOGENS

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Text

Oilseed rape (*Brassica napus* and its related species), the second largest oilseed crop in the world, is particularly susceptible to fungal pathogens. The most significant of these are blackleg caused by *Leptosphaeria maculans* and *L. biglobosa*, followed by Sclerotinia stem rot caused by *S. sclerotiorum*, and Alternaria blight caused by *Alternaria brassicae* and *A. brassicicola*. It has been reported that the Class III acyl-CoA-binding proteins (ACBPs) from dicots (Arabidopsis and grapevine) protect against biotrophic pathogens. Also, the overexpression of the monocot *Oryza sativa* (rice) OsACBP5 in transgenic Arabidopsis and rice has been demonstrated to enhance broad-spectrum disease resistance against selected phytopathogens. In this study, transgenic rapid-cycling *Brassica napus* (*B. napus*-RC) and canola cv. Westar OsACBP5-OEs were generated and tested against Alternaria blight, blackleg and Sclerotinia stem rot diseases. Alternaria blight and blackleg pathogen assays were based on infecting young cotyledons, while detached true leaf assay was used to test the tolerance of *B. napus* plants toward *S. sclerotiorum*. OsACBP5-OE plants exhibited resistance 5 days after inoculation with *Alternaria brassicae*, 12 days after inoculation with *Leptosphaeria maculans*, and 24 h after inoculation with *Sclerotinia sclerotiorum*. This study provides an insight into the usefulness of OsACBP5 in enhancing resistance to necrotrophic phytopathogens.

P3.1-020

IDENTIFICATION AND USE OF A CONSERVED LOCUS FOR STANDARDISED TARGET SITE INTEGRATION IN FUSARIUM GRAMINEARUM

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Text

Fusarium graminearum (*Fg*) is a destructive fungal pathogen of wheat, barley, maize and other crop species. Infections cause the production of different mycotoxins that contaminate grains making them unsafe for human and animal consumption. Transformation protocols have been developed for *Fg* allowing gene/protein function studies in different ways such as overexpression and/or fusions to fluorescence tags. These protocols exploit fungal recombination to insert an expression cassette randomly into the Fg genome. However, this may result in variable gene expression of off-target genes. To address this problem, we identified a 3.2 kb intergenic region contained within a conserved *Fg* locus on chromosome 1 suitable for standardised target site integration (STSI). We created an efficient cloning vector

system based on the Golden Gate method and show evidence that the expression cassette integration into this locus does not affect fungal virulence or fungal growth under different stress conditions. In addition, the activities of a metabolism-dependent promoter and an effector promoter were not altered by STSI. Finally, we established a protocol to study protein secretion in wheat coleoptiles using confocal microscopy and STSI for stable expression of different gene fusions.

P3.1-021

IDENTIFICATION AND CHARACTERIZATION OF CANDIDATE EFFECTORS FROM THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Text
Zymoseptoria tritici causes Septoria tritici blotch on wheat. The biotrophic lifestyle of *Z. tritici* makes this fungal pathogen an attractive model to investigate infection phase-specific gene expression. Differential gene expression, Gene Ontology and KEEG enrichment were determined in comparisons among *Z. tritici* during a compatible interaction with the susceptible cultivar Taichung29, two incompatible interactions with the resistant cultivars Veranopolis and Israel493, and one non-host interaction with barley, at 1, 3, 6, 10, 17 and 23 days after inoculation (DAI). We found 978 up-regulated genes at 1 DAI and 2,317 up-regulated genes at 3 DAI in the compatible compared to the non-host interaction. *Z. tritici* activates 1,300 genes at 10 DAI in the compatible compared to the incompatible interactions which correlates with the initiation of the necrotrophic lifestyle. Of the *Z. tritici* genes that are significantly up-regulated at 1 DAI in the compatible interaction, 31 are predicted to be effectors. Examination of the protein sequences of several of the candidate effectors revealed that they likely target specific subcellular organelles, including the nucleus, chloroplasts, and mitochondria. We are currently investigating the subcellular localization patterns of super yellow fluorescent protein (sYFP)-tagged *Z. tritici* effectors using a *Nicotiana benthamiana*-based heterologous expression system.

P3.1-022

RESISTANCE TO SCLEROTINIA SCLEROTIORUM BY OVER-EXPRESSING EXECUTER1, DEFENSIN, SYNTAXIN OF PLANTS (SYP121) OR LECRK A4.3 IN TRANSGENIC BRASSICA NAPUS

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Text
Brassica napus genes conferring resistance to *Sclerotinia sclerotiorum* were identified in a genome wide association study. Selected genes were cloned from resistant lines and together with promoter CaMV35S, transformed into a susceptible control. Homozygous single gene-insert lines were obtained after 3-6 generations. Stems were inoculated with mycelium at full flower to simulate yield loss. To ascertain resistance was long lasting, symptoms were rated 21 days after inoculation (dai). The following four gene products were constitutively expressed in stem tissue, upregulated 1-14 dai, and provided sclerotinia resistance. Importantly Executer1, which causes programmed cell death (PCD) when exposed to $^1O_2$ in photosynthesis, also triggered PCD during the initial oxidative burst caused by infection. Defensin accumulates in vacuoles and has antifungal activity. Syntaxin of plants (*SYP121*) regulates fusion of bilayer lipid membranes vital for secretion of vacuolar contents into the apoplast. Lectin receptor kinase A4.3 in the cell membrane is activated by pathogen-associated molecular patterns that induces defense cascades. Amino acid differences were insignificant between the inserted gene and homolog genes in the control. Resistance was therefore due to the gene promoter which ensured the protein was present before infection and expressed at higher levels than the native protein. Consequently, the above results could not have been obtained using gene modification such as CRISPR.

P3.1-023

GENETIC CHARACTERIZATION AND PATHOGENICITY SCREENING OF FUSARIUM SPP. ISOLATES CAUSING POST FLOWERING STALK ROT IN MAIZE

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Text
Post Flowering Stalk Rot (PFSR) of maize caused by the Fusarium species complex. Seventy-one isolates were collected from 40 sites in five agro-climatic zones of India to assess the diversity of Fusarium spp. associated with maize crops showing symptoms of PFSR in the field. In-vitro, Kharif, and Rabi seasons were utilized to investigate the pathogenicity of Fusarium spp.-causing PFSR. The isolates were judged to be virulent based on their ability to decrease seedling vigour in in-vitro situations and high disease severity in field experiments. Pathogenicity test in Kharif season showed 12 isolates with virulent reactions causing mean severity ranging between 50 to 67% whereas in Rabi season, only five isolates were considered virulent, and the mean severity ranged between 51.9% and 66.7%. Molecular identification of the ten most virulent isolates was from the partial sequence of the Tef-1 α. Based on the examination of pathogenicity in in-vitro, Kharif, and Rabi field trials, ten strains, namely, Fusarium acutatum (FUR11, F10), Fusarium verticillioides (Syn. Fusarium fujikuroi) (Davanagere, Raichur, FUG9, FUR15, F21, F13, and F59), Fusarium andiyazi (F18), (FUR15), recorded the highest diseases index. Morphological characterization, molecular identification, and information on the geographical distribution of virulent Fusarium isolates will be helpful for the efficient management of PFSR, including screening for resistance in maize-inbred lines.

P3.1-025

ISOLATION AND STEPS TOWARDS MOLECULAR CHARACTERISATION OF ASCOCHYTA RABIEI EXOSOMES

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Text

Across kingdoms, exosomes are produced by pathogenic organisms that are associated with signalling for host recognition and invasion, including by fungi and when in contact with their plant hosts. However, very little is known about plant pathogenic fungal exosomes, with data limited to a few early studies. Meanwhile, *Ascochyta rabiei* is an widespread necrotrophic ascomycete fungus that causes significant impact on chickpea production. Very few *A. rabiei* avirulence (avr) factors have been proposed and none have been functionally assessed for how they are delivered to the chickpea plant. Therefore, the current study focuses on determining if *A. rabiei* exosomes are the carrier mechanism for the avr sequences involved in the initial recognition and establishment on chickpea. To determine their presence, exosomes were initially isolated from *A. rabiei* broth cultures through optimised ultrafiltration methods, and morphologically characterized under TEM. This revealed typical cup-shaped structures with double membranes of 30-150 nm in size. Subsequently, ribonucleic acid was extracted from the exosome-concentrated fractions. Methods to remove non-exosome material from the exosome fraction were then optimised and the purified exosome fraction was sent for full transcriptome sequencing with and without exposure to chickpea host material. Differentially expressed transcripts were investigated for those potentially functionally associated with host recognition and early invasion.

P3.1-026

OXALIC ACID METABOLISM CONTRIBUTES TO FULL VIRULENCE AND PYCNIDIAL DEVELOPMENT IN THE POPLAR CANKER FUNGUS CYTOSPORA CHRYSOSPERMA

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Text

Poplar Cytospora canker, which is mainly caused by *Cytospora chrysosperma*, is one of the most destructive and widespread tree diseases worldwide. Although oxalic acid (OA) is demonstrated as an important virulence determinant in several necrotrophic fungi, specific functions of OA during pathogenesis remain controversial. Here, we identified three genes (*CcOah*, *CcOdc1*, and *CcOdc2*) directly involved in OA biosynthesis and catabolism in *C.*

chrysosperma. We demonstrated that *CcOah* is required for OA biogenesis. All three genes were found to be highly upregulated during early infection stages of the poplar stem. The deletion of any of the three genes led to an obvious reduction of pycnidial production but no abnormality of hyphal growth and morphology. Furthermore, the individual deletion strain exhibited significantly limited lesion sizes on poplar twigs and leaves. Exogenous application of OA or citric acid can complement the virulence defects of Δ*CcOah* and Δ*CcOdc1* strains. We further found that the Δ*CcOah* strain strongly promoted reactive oxygen species burst of poplar leaves during infection. Finally, induced secretion of OA was observed by monitoring color change of the plates after poplar stem extracts were added in the cultures; however, we failed to quantify OA concentration by high-performance liquid chromatography. Taken together, the present results provide insights into the function of OA acting as an important virulence factor of *C. chrysosperma*.

P3.1-027

VDTPS2 MODULATES PLANT SYMPTOM DEVELOPMENT AND STRESS RESPONSES IN VERTICILLIUM DAHLIAE

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Text

Trehalose is critical for protection against several environmental stresses and virulence in plant and human pathogens. Trehalose phosphate synthase/trehalose phosphate phosphatase (TPS/TPP) are widely conserved components of trehalose biosynthesis in fungi. Although the TPS complex has been studied in many fungi, seldom of them have been functionally characterized in the vascular phytopathogenic fungi *Verticillium dahliae*. In this study, we identified the TPS complex including VdTps1, VdTps2, and VdTps3 in *V. dahliae* and found that VdTps1 and VdTps3 appeared dispensable for fungal development, trehalose biosynthesis, and virulence. However, the deletion of *VdTps2* severely restrained growth which is likely caused by abnormal hyphal tip swelling. The Δ*VdTps2* strain showed promoted microsclerotia formation and melanin biosynthesis and is more resistant to cell wall perturbation, reactive oxygen species (ROS) stress, and high-temperature stress. Virulence assays showed that VdTps2 regulates disease symptom development by regulating the spread of V. dahliae in the host above the crown. However, the deletion of *VdTps2* promoted plant colonization by increasing penetration peg formation under ROS stress. Additionally, our results also revealed the role of VdTps2 as a regulator of autophagy. Together, these results indicate that VdTps2 modulates disease development, radial growth, melanized microsclerotia formation, stress response, and autophagy in *V. dahliae*.

P3.1-028

SEPTINS REGULATE VIRULENCE IN VERTICILLIUM DAHLIAE AND DIFFERENTIALLY CONTRIBUTE TO MICROSCLEROTIAL FORMATION AND STRESS RESPONSES

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Text

Verticillium dahliae is a notorious root-infecting pathogen causing vascular wilt diseases on many woody plant species worldwide. Its infection requires VdSep5 to form a specialized fungus-host interface for effector delivery. However, the roles of other septins are unclear and the different functions of VdSep5 also need to be investigated further in *V. dahliae.* Herein, we characterized and studied the functions of septin-coding genes (*VdSep3*, *VdSep4*, *VdSep5*, and *VdSep6*) in microsclerotia formation and various stress responses. Lossing, any of four septins led to weakened hyphal expansion into plant cells and attenuated virulence. All the septin gene deletion mutants showed an abnormality in chitin distribution but varied in their responses to several stresses examined. VdSep4 and VdSep6 regulated melanized microsclerotia formation positively while VdSep3 and VdSep5 are dispensable for it. Deletion of *VdSep3* or *VdSep4* increased sensitivity to reactive oxygen species (ROS) and reactive nitrogen species (RNS) stress, whereas VdSep6 played a role in RNS stress response but not in ROS stress response. Deletion of *VdSep4* or *VdSep5*, but not *VdSep3*, resulted in hypersensitivity to high-temperature stress. VdSep3 and VdSep4 played a contrary role in response to benomyl. Taken together, our results indicate that four septins play diverse roles in regulating melanized microsclerotia development and stress responses, while they are all required for full virulence in *V. dahliae*.

P3.1-029

TRANSCRIPTOME VARIATIONS IN VERTICILLIUM DAHLIAE IN RESPONSE TO TWO DIFFERENT INORGANIC NITROGEN SOURCES

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Text

Verticillium dahliae causes vascular wilt in hundreds of plant species. Research into the control of this fungus has focused on infection processes such as penetration and effector secretion, but the ability of the fungus to acquire and utilise nutrients is often overlooked and may offer additional potential for formulating new disease control approaches.The molecular mechanisms of nitrogen acquisition and uptake in *V. dahliae* are poorly understood. This study used RNA sequencing and gene expression analysis to investigate differentially expressed genes in response to nitrate and ammonium in *V. dahliae*.In response to nitrate and ammonium treatments, a total of 3244 and 2528 differentially expressed genes, respectively, were identified. The data suggest that nitrate metabolism requires additional energy expenditure, whereas ammonium metabolism reduces certain cellular processes. Furthermore, we showed that mutants of three differentially expressed transcription factors (VdMcm1, VdHapX, and VDAG_08640) exhibited abnormal phenotypes under nitrate and ammonium treatment compared to wild-type. This suggests that nitrogen assimilation requires regulation of the bZIP transcription factor family and involves the cell cycle.Overall, our results reveal convergent and distinct regulatory mechanisms between

preferred (ammonium) and alternative (nitrate) nitrogen metabolism at the transcriptome level, leading to a better understanding of inorganic nitrogen metabolism in *V. dahliae.*

P3.1-030

CHARACTERIZATION OF THE PROMOTER OF THE AVIRULENCE GENE AVRPI9 IN THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE

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Text

Rice Blast disease, caused by the fungus Magnaporthe oryzae, poses a significant threat to global food security. During the interaction between rice and the blast fungus, the fungus's effector proteins encoded by avirulence (Avr) genes are secreted into the plant tissue. This process decreases the plant's defense signals and supports the growth of fungus. In Thailand's rice blast population, AvrPi9 gene is predominantly found, but there is a lack of knowledge on the specific regulatory elements for this gene. In this study, the minimum AvrPi9 promoter was identified and the 200 bp located between -900 to -700 upstream of the promoter was investigated. A protein-DNA pull-down assay was used to identify MoHOX6, which contains a Homeobox domain, as a potential transcription factor for AvrPi9. It was observed that the expression of AvrPi9 was induced 24 and 48 hours after inoculation but was delayed in ΔMoHOX6 mutant. The results suggest that MoHOX6 plays a role in accelerating the expression of AvrPi9. Furthermore, the AvrPi9 promoter was able to direct the expression of the luciferase reporter gene in transfected rice protoplasts. Additionally, co-transfection of the AvrPi9 promoter fused with the luciferase gene and expression of MoHOX6 resulted in a significantly increased luciferase signal. This is the first glimpse into the regulation of the AvrPi9 promoter of M. oryzae and has the potential to advance our understanding of fungal infection mechanisms in the future.

P3.1-031

OCCURRENCE AND PARASITIC SPECIALIZATION OF LARGE-SPORED ALTERNARIA SPECIES AS-SOCIATED WITH EARLY BLIGHT OF POTATO AND TOMATO IN ALGERIA

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Text
Occurrence and parasitic specialization of large-spored Alternaria species associated with early blight of potato and tomato in Algeria

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Potatoes and tomatoes are two important solanaceous crops in Algeria. The two crops are often grown in succession in many coastal regions. Symptoms of early blight and morphological characteristics of the isolates do not allow a clear distinction between the different species of Alternaria large spored. Using specific primers, PCR/RFLP by double enzymatic digestion of a portion of calmodulin gene and sequence analysis with calmodulin and RPB2 genes, allowed us to identify four distinct species: Alternaria solani, A. linariae, A. grandis and A. protenta. To our knowledge, this is the first report of A. linariae on potato in the world, and the first occurrence of A. protenta as pathogen on potato in Algeria. Pathogenicity tests for the four species, confirmed that all were pathogenic to potato and tomato, with varying virulence. These results suggest that parasitic specialization of these Alternaria species should be reconsidered, and good agricultural practices for the control early blight diseases.

P3.1-032

THE RICE BLAST FUNGUS SR PROTEIN 1 REGULATES ALTERNATIVE SPLICING WITH UNIQUE MECHANISMS

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Text
Serine/arginine-rich (SR) proteins are well known as splicing factors in humans, model animals and plants. However, they are largely unknown in regulating pre-mRNA splicing of filamentous fungi. Here we report that the SR protein MoSrp1 enhances and suppresses alternative splicing in a model fungal plant pathogen *Magnaporthe oryzae*. Deletion of *MoSRP1* caused multiple defects, including reduced virulence and thousands of aberrant alternative splicing events in mycelia, most of which were suppressed or enhanced intron splicing. A GUAG consensus bound by MoSrp1 was identified in >94% of the intron or/and proximate exons having the aberrant splicing. The dual functions of regulating alternative splicing of MoSrp1 were exemplified in enhancing and suppressing the consensus-mediated efficient splicing of the introns in *MoATF1* and *MoMTP1*, respectively, which both were important for development and virulence. Interestingly, MoSrp1 had a conserved sumoylation site that was essential to nuclear localization and enhancing GUAG binding. Further, we showed that MoSrp1 interacted with a splicing factor and two components of the exon-joining complex via its N-terminal RNA recognition domain, which was required to regulate mycelial growth, development and virulence. In addition, only orthologues from Pezizomycotina

species could completely rescue defects of the deletion mutants. This study reveals that the fungal conserved SR protein Srp1 regulates alternative splicing in a unique manner.

P3.1-033

BIOLOGICAL CHARACTERISTICS CONTRIBUTING TO VIRULENCE ENHANCEMENT IN FUSARIUM OXYSPORUM F. SP. CUCUMERINUM AFTER THE SELECTIVE PRESSURE OF RESISTANT CUCUMBER

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Text
Cucumber is commonly suffering from Fusarium wilt disease, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (*Foc*). Although resistant cultivars play certain role in Fusarium wilt disease control, the enhancement of virulence of *Foc* has been found after monoculture of wilt-resistance cultivar. To investigate biological characteristics of which contribute to the virulence evolution of Foc, a wild type strain foc-3b (WT) and its virulence-enhanced variant Ra-4 (InVir) were compared in growth characteristics, stress tolerance, and infection process. The InVir strain showed similar culture characteristics on PDA media as the WT strain, but exhibited less pigment accumulation than the WT. The InVir strain produced significantly more conidia with distinctly higher germination rate than the WT strain. In addition, the InVir WT strain produced visible more macroconidia than microconodia, inversely, the WT produced more microconidia than macroconidia. Colony diameter of the InVir strain increased faster than the WT strain on PDA plates, however, mycelia dry weight of the InVir was significantly less than that of the WT harvesting from PDB. The InVir strain exhibited significantly increased tolerance to osmolality. The InVir strain infected and propagated in the cucumber vascular faster than the WT strain. These results would provide insight into the virulence evolution, and help to understand the mechanisms underlying the evolutionary biology of *F. oxysporum*.

P3.1-035

REGULATORY MECHANISMS OF IN PLANTA SPECIFIC EXPRESSION OF A FUNGAL EFFECTOR GENE, MOHTR1, IN THE RICE BLAST FUNGUS

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Text
During plant-phytopathogen interactions, fungi secrete effector proteins in the host cells to subvert the immune system or alter host metabolism for successful infection. Despite the functions of many effector genes have been elucidated so far, there are only limited information on the mechanisms of regulating *in planta* expression of effector genes. To understand the *in planta* specific expression of effector genes, we characterized the promotor of a nuclear effector gene of *Magnaporthe oryzae*, *MoHTR1*. To identify the cis-elements regulating transcription of *MoHTR1*, we performed a truncation analysis in the promoter region of *MoHTR1* with *sGFP* tagging. By evaluating the fluorescence intensity to determine the promoter activity, we found the 8 bp of cis-element (TATTTCGT). Transversion substitution mutation of these 8-bp sequences led to reduced virulence of the fungal pathogen similar to the deletion of *MoHTR1*. We further unveiled that in planta specific expression of *Slp1* is also regulated by the same *cis*-element of *MoHTR1*. Through promoter switching, we verified that *MoHTR1*'s promoter can induce expression of other genes, which are not expressed during infection, in the biotrophy stage. We are now identifying transcriptional factor(s) binding to this *cis*-element of *MoHTR1* using pull-down assay and yeast one hybrid. This study will provide comprehensive insights into the regulatory mechanisms of *in planta* specific expression of fungal effector genes.

P3.1-036

DISSECTION OF THE SDS2-MEDIATED CELL DEATH AND DEFENSE PATHWAY TO MAGNAPORTHE ORYZAE IN RICE

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Text
Programmed cell death (PCD) plays an important role in plant immunity against pathogens. SPL11 cell-death suppressor 2 (SDS2), a receptor-like protein kinase, positively regulates PCD by phosphorylating the U-box E3 ligase SPL11, OsRLCK118 and OsRLCK176 that leads to OsRbohB-mediated ROS production and defense activation. However, how SDS2 is activated and how the activation triggers downstream signaling are still unknown. In this study, we performed RNA-seq and identified many up-regulated genes in the SDS2 overexpression line. Among them, the leucine-rich repeat (LRR)-containing gene OsBDG1 was highly induced in the SDS2 overexpression line, and the protein interacted with SDS2 in different interaction assays. The subcellular localization assay showed that OsBDG1 was localized on the cell membrane and in the nucleus. The luciferase and GUS assays indicated that co-expression of SDS2 and OsBDG1 in rice protoplasts led to decreased luciferase and GUS activities compared to the expression of single genes. DAB staining after co-expression of SDS2 and OsBDG1 in N. benthamiana caused ROS accumulation and cell death. Additionally, luciferase complementation imaging assays revealed that OsBDG1 also interacted with OsRbohB, OsRLCK118 and OsRLCK176. Furthermore, we identified an E3 ligase that may regulate the protein level of OsBDG1. These results demonstrate that OsBDG1 might be an important component in the SDS2-mediated cell death and defense pathway in rice.

P3.1-037

FUNCTION AND HOST CELL LOCALIZATION OF SIX3 AND SIX5 EFFECTORS IN FUSARIUM OXYSPORUM F. SP. CEPAE

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Text

Fusarium oxysporum f. sp. *cepae* (FOC) is a causal agent of Fusarium basal rot disease of onions, contributing to substantial economic losses worldwide. The FOC genome contains the effector genes *SIX3* and *SIX5*, the functions of which have yet to be determined. In this study, we sought to establish the functions and host cell localizations of SIX3 and SIX5. A *SIX3*-disrupted mutant was found to be characterized by reduced pathogenicity on onion plants susceptible to FOC, whereas a *SIX5*-disrupted strain showed high virulence against FOC-resistant shallot plants. These findings indicate that SIX3 functions as a virulence factor that contributes to determining pathogenicity against onions, whereas SIX5 serves as an avirulence factor against shallots. Immunostaining using anti-SIX3 and anti-SIX5 antibodies was performed to clarify cell localization in the basal plate. In susceptible onion plants, SIX3 and SIX5 proteins were broadly localized in the xylem vessels and apoplastic spaces in basal plates, and FOC spores and mycelia were observed at 21 dpi. Contrastingly, in resistant shallots, we detected comparatively few SIX3 and SIX5 signals, and observed no FOC spores or mycelia. Moreover, yeast two-hybrid assays revealed that SIX3 interacts directly with SIX5. Collectively, our findings indicate that the SIX3 and SIX5 effectors jointly contribute to determining the virulence of FOC against onions by localizing in the xylem and apoplast of the basal plate.

P3.1-038

ANALYSIS OF MSP1-INDUCED POST-TRANSLATIONAL MODIFICATION DYNAMICS UNVEILED NOVEL INSIGHTS INTO RICE-MAGNAPORTHE ORYZAE INTERACTION

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Text

Magnaporthe oryzae snodprot1 homologous protein (MSP1) has been shown to act as a pathogen-associated molecular pattern (PAMPs) and trigger PAMP-triggered immunity (PTI) response including programmed cell death and expression of various defense-related genes in rice. The involvement of several post-translational modifications (PTMs) in the regulation of plant immune response, especially PTI, during pathogen infection is well established, however, the information on the regulatory roles of these PTMs in response to MSP1-induced signaling in rice is currently elusive. Here, we report the phosphoproteome,

ubiquitinome, and acetylproteome to investigate the MSP1-induced PTMs alterations in MSP1 overexpressed rice. Our analysis identified a total of 4,666 PTMs-modified sites in rice leaves including 4,292 phosphosites, 189 ubiquitin sites, and 185 acetylation sites. Among these, the PTM status of 437 phosphorylated, 53 ubiquitinated, and 68 acetylated peptides was significantly changed by MSP1. Functional annotation of MSP1 modulated peptides by MapMan analysis revealed that these were majorly associated with cellular immune responses such as signaling, transcription factors, DNA and RNA regulation, and protein metabolism, among others. Taken together, this study uncovers the MSP1-induced PTM changes in rice proteins and identified several novel components of rice-MSP1 interaction.

P3.1-039

ASSAY FOR TRANSPOSASE ACCESSIBLE-CHROMATIN WITH HIGH-THROUGHPUT SEQUENCING (ATAC-SEQ) REVEALS THE MOLECULAR RESPONSES OF POSTHARVEST PEAR DURING PENICILLIUM EXPANSUM INFECTION

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Text

In pears, blue mold decay caused by *Penicillium expansum* is a destructive postharvest disease around the world, which leads to stasis of the pear industry and creates substantial economic losses. Transcription factors play a crucial role in the defense of pears against infection, and some of their functions are witnessed. Despite this, the roles of many TFs have not been explored in the defense of pear against *P. expansum*. Therefore, this study used ATAC-seq analysis to screen the TFs of postharvest pear. According to GO enrichment and KEGG pathway to analyze the peak-related genes which were expressed differentially. In our results, the up-regulated genes involved in MAPK signaling pathway-plant, plant hormone signal transduction pathway, plant-pathogen interaction pathway, glutathione metabolism pathway, phenylpropanoid biosynthesis, flavonoid biosynthesis pathway and carotenoid biosynthesis pathway were identified. Especially the transcription factors TGA, gene *SAUR* and *CYCD3* in plant hormone signal transduction pathway and *NCED*, *CYP707A* in carotenoid biosynthesis pathway were differentially regulated. The accuracy of ATAC-Seq analysis of *P. expansum*-infected pear was confirmed by RT-qPCR analysis on twelve randomly selected differentially expressed peak-related genes. In this study, TFs of pears against *P. expansum* were screened by using ATAC-seq, thereby understanding the chromatin-mediated gene regulation in the defense of pear against infection.

P3.1-040

CCEG1, A GLYCOSIDE HYDROLASE 12 PROTEIN FROM CYTOSPORA CHRYSOSPERMA, CAN TRIGGER PLANT IMMUNITY BUT IS NOT REQUIRED FOR FUNGAL VIRULENCE

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Text

Phytopathogens secrete numerous effectors to host cells which can facilitate their infection and colonization. However, little is known about the pathogenic mechanism of effectors in the *Cytospora chrysosperma*, the causal agent of canker disease of many woody plants. In this study, we identified five glycoside hydrolase family 12 (GH12) effectors in *C. chrysosperma* genome, which were all significantly upregulated during the infection stage. Among them, *CcEG1*, containing an additional carbohydrate-binding module family 1 domain (CBM1) at the C-terminal, was selected for further analysis. Transient expression found that *CcEG1* was localized to the apoplast region of *Nicotiana benthamiana* and acted as a pathogen-associated molecular pattern (PAMP) to induce cell death, which could also activate the expression of downstream immune signaling pathway. Furthermore, the GH12 domain was sufficient for cell death-inducing activity without the CBM1 domain. Additionally, both the leucine-rich repeat (LRR) receptor-like kinases *NbBAK1* and *NbSOBIR1* were required for *CcEG1* to induce plant defense responses. Intriguingly, *CcEG1* mutant did not affect fungal growth, pathogenicity, and response to cell wall stress factor, but it is required for cellulase degradation. Collectively, our results suggest that cellulase *CcEG1* can be recognized by conserved receptors *NbBAK1* and *NbSOBIR1* to activate plant immune responses.

P3.1-041

THE LYSM PROTEIN BDLM1 OF BOTRYOSPHAERIA DOTHIDEA PLAYS IMPORTANT ROLES IN FULL VIRULENCE AND INHIBITS PLANT IMMUNITY BY BINDING CHITIN AND PROTECTING HYPHAE FROM HYDROLYSIS

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Text

Botryosphaeria dothidea infects hundreds of woody plants, causing a severe economic loss in apple production through fruit rot and warts, rough skins, and cankers on stems. We described the characterization of proteins containing the LysM domain and BdLM1 containing one LysM domain from B. dothidea. BdLM1 suppressed programmed cell death (PCD) caused by the mouse protein BAX (BT-PCD) through Agrobacterium-mediated transient expression in N. benthamiana and increased the lesion size of Phytophthora nicotianae with the accumulation of H2O2. The expression of BdLM1 was dramatically induced at 6 h in wounded apple fruit, and strongly induced at 7 dpi, peaked at 20 dpi on intact shoots. The knockout mutants of BdLM1 had a significant decrease in disease severity on intact apple shoots (20%), in lesion length on wounded apple shoots (40%), and in lesion size on wounded apple fruit (40%). Moreover, BdLM1 binds chitin and is able to protect fungal hyphae against degradation by plant hydrolytic enzymes. Results here indicated that the LysM effector BdLM1 is required for full virulence of B. dothidea and plays different roles during the colonization of fruit and branches. Furthermore, BdLM1 mediates plant immunity probably via dual functions including the perturbation of the activation of chitin-triggered host immunity and protecting hyphae against chitinases.

P3.1-042

THE GENE OF A GPI-ANCHORING PROTEIN A PROMISING NEW TARGET FOR THE CONTROL OF THE CUCURBIT POWDERY MILDEW PODOSPHAERA XANTHII

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Text

One of the main limitations of the cucurbit crops production is the powdery mildew disease, caused by the biotrophic fungus *Podosphaera xanthii*. An integrated management, using several strategies, is carried to control the disease but the application of fungicides is the most effective one. The problem is that *P. xanthii* has been classified by the Fungicide Resistance Action Committee (FRAC) as a pathogen with a high risk of resistance developing, in addition of the strong restrictions on the use of phytosanitary products at a European level. For this reason, new phytosanitary tools are necessary to allow a sustainable control of this devastating disease such as the use of the RNA interference (RNAi) technology. In this work, dsRNA targeting a *P. xanthii* gene, which encodes a protein that appears to be essential for the correct assembly of the fungal cell wall, was evaluated. Preliminary gene silencing results have shown a significant reduction of fungal development on melon plants suggesting that this gene may be a promising target for the control of powdery mildew of cucurbits.

This work has been funded by AEI (PID2019-107464RB-C21).

P3.1-043

DISTRIBUTION OF AVIRULENCE GENES OF THE RICE BLAST FUNGUS COLLECTED FROM THE NORTHERN AREAS OF KOREA IN 2021

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Text

The rice blast fungus is a devastating fungal disease in rice-cultivated areas worldwide. In 2021, rice leaf and stem blast occurred severely in the northern area (Yeoncheon, Gyeonggi province) of Korea. In order to determine the cause of the outbreak, we investigated the genetic diversity of the rice blast fungus using the genetic variation of the avirulence gene. A total of 165 isolates were collected and subjected to select representatives using repetitive element-based polymerase chain reaction (rep-PCR). Approximately all of the 58 isolates were separated among isolates in over 83% similarity, showing genetically identical of the collected isolates. Based on this result, we selected five representative isolates and then

examined the presence of avirulence genes through pathogenicity test using over 25 near-isogenic lines. Further analysis will be displayed. [This work was supported by a grant from the Rural Development Administration (PJ0152782023).]

P3.1-044

ROLE OF ENDOGENOUS EUGENOL IN THE RESISTANCE TO BOTRYTIS CINEREA OF THE HYBRID GRAPEVINE CULTIVAR “BACO BLANC”

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Text

Eugenol, widely distributed in various plants including cloves, is a well-known powerful antifungal and antibiotic molecule that is concentrated in hybrid grapevines, notably in the cultivar Baco blanc (*Vitis vinifera x Vitis riparia x Vitis labrusca*) created by F. Baco in the 19th century. Under biotest conditions, by assessing fruit rot incidence and severity, we confirmed that this variety is highly resistant to *Botrytis cinerea* by comparing with two Vitis vinifera cultivars, also of prime importance in the Armagnac region: Folle Blanche and Ugni Blanc. The marked varietal resistance was also confirmed in the vineyards and may arise from differential chemical feature of the berry skin. The Baco blanc berry skin was highly concentrated in eugenol, notably at veraison (1118 to 1478 µg/kg), contrarily to the two other cultivars (e.g. Ugni Blanc 22 to 28 µg/kg). Furthermore, significant intra-varietal differences in *B. cinerea* resistance among six Baco blanc clones were shown in terms of incidence and severity of fruit rot and/or sporulation. These differences in resistance were related to different fruit chemical composition, including the berry skin eugenol content. Finally, a significant negative correlation was highlighted between the fruit technological maturity and the skin eugenol content, allowing us to propose eugenol as a key fruit skin biomarker of the ontogenic resistance of the hybrid cultivar, Baco blanc, to such a major necrotrophic pathogen in grapevine.

P3.1-045

SYSTEMIC COLONIZATION OF POTATO PLANTS BY CO-INFECTION WITH VERTICILLIUM DAHLIAE AND FUSARIUM OXYSPORUM

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Text
Global potato production is plagued by multiple pathogens, among which the Verticillium. dahliae and Fusarium oxysporium is the casual agents of Potato verticillium wilt and potato fusarium wilt respectively. In present study, Agrobacterial mediated transformation system was used to generate transformants of both V. dahliae and F. oxysporum, which were isolated from a single potato diseased sample. A green fluorescent protein (GFP) and a red fluorescent protein (mCherry) tagged isolates of V. dahliae (V.dG9) and F. oxysporum (F.oM4) were used to study the co-infection progression in potato. The results showed that the conidia of two inoculated strains could germinate and the mycelia expanded alone the cortical cells and catheter of the root tip, suggesting that the root tip of potato was the earliest infection site. V.dG9 colonized the vascular bundles much faster than F.oM4 ; however, the mycelium proliferation rate of F.oM4 was significantly faster than that of V.dG9 inside the potato root vascular system. The expansion speed of V.dG9 was much faster than that of F.oM4 above ground tissues. Both V.dG9 and F.oM4 were able to colonize inside the vascular bundles of potato stolons after 35 days of post co-inoculation. However, only the GFP or mCherry signal could be detected separately inside the vascular bundles of the tuber. This is the first study on characterizing co-infection of both V. dahliae and F. oxysporum on potato systematically.

P3.1-046

PESTICIDE APPLICATION CAUSES CHANGES IN GENE EXPRESSION OF THOUSAND CANKERS DISEASE HOST AND PATHOGEN

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Text
Thousand cankers disease is caused by the fungus *Geosmithia morbida* and threatens *Juglans nigra* trees. Host-pathogen interactions at a molecular level have not been well characterized. Furthermore, the effects of potential disease management strategies on these interactions are unknown. To address this knowledge gap, we used transcriptomic approaches to identify gene expression changes in both host and pathogen following treatment with chemical (PHOSPHO-jet) and biological (RootShield) management strategies. These treatments were compared to water-only controls using 24 trees per treatment (N=72). One week following treatment, 36 trees (12 per treatment) were inoculated with *G. morbida*, and potato dextrose agar (PDA) was used as a negative control. At 14-, 28-, and 56-days post-treatment application (PTA), RNA was extracted and sequenced from canker-bordering phloem tissues. For *J. nigra*, the number of differentially expressed genes (DEGs) between *G. morbida* and control trees declined over time with 4,386 DEGs at day 14 to 148 DEGs at day 56 PTA. Furthermore, *G. morbida* gene expression changed with time, with the greatest number of DEGs (1560) occurring between days 14 and 56 PTA. Additionally, *G. morbida* gene expression in PHOSPHO-jet trees differed from control trees at each sampling date. This study will provide a deeper understanding of mechanisms related to *G. morbida* pathogenicity and identify how management strategies influence pathogen virulence at a molecular level.

P3.1-047

RNA SEQUENCING-BASED TRANSCRIPTOME ANALYSIS OF TWO CONTRASTING S. LYCOPERSICUM VARIETIES INFECTED BY BOTRYTIS CINEREA.

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Text

Botrytis cinerea is one of the most important pathogens in the tomato industry. The indiscriminate use of chemicals to control *Botrytis* has generated more resistant pathogens and a growing interest in safer alternatives. The study of plant transcriptomic response to infection has traditionally provided a better understanding of crop defense mechanisms. Multiple papers have compared *S. lycopersicum* to more resistant *Solanum* species. However, little research compares the transcriptomic response of contrasting commercial varieties. In this work, we compare the defense response to *Botrytis* of a highly susceptible and traditional Chilean variety (Rosado) with a resistant variety (Marmande). Results show that 4406 genes are downregulated, and 309 genes are upregulated during infection in Marmande compared to Rosado. When the main gene ontology differentially expressed was analyzed both varieties have upregulated processes related to lipid, jasmonic acid, and fatty acid response. However, processes related to hormone and ethylene stimulus were only upregulated in Marmande samples, meanwhile, Rosado samples have upregulated processes related to the salicylic acid pathway, response to oxidative burst, and senescence. Besides, most of the gene ontology categories downregulated are shared in both varieties, including photosynthesis and carbohydrate metabolism. These results are the base to develop new tools to defeat *B. cinerea* infections in commercial and traditional Chilean varieties.

P3.1-048

UNRAVELLING THE CELL WALL INTEGRITY PATHWAY OF FUSARIUM GRAMINEARUM

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Text
F. graminearum is an important fungal pathogen of cereal crops, responsible for Fusarium head blight disease and the accumulation of harmful mycotoxins in grain products. The cell wall integrity (CWI) pathway of fungi is important for normal fungal growth and reproduction, and plays a role in plant-pathogen interactions. The mitogen-activated protein kinases (MAPKs) cascade, FgBck1-FgMkk1-FgMgv1, is thought to regulate the CWI pathway in F. graminearum, and disruption of any of these 3 genes results in slow growth phenotypes and an apparent loss of virulence. To further characterize this pathway, a series of knockout and overexpression (OX) strains targeting the CWI MAPK cascade are being investigated. OX of FgMgv1 had no effect on growth or virulence. Meanwhile, OX of FgMkk1, either as the wild-type gene or as a presumed 'hyperactive' variant, resulted in slow growth on potato dextrose agar compared with the wildtype strain, similar to observations made of hyperactive mutants of the yeast orthologue. Surprisingly, neither set of FgMKK1 OX strains seem to activate FgMgv1, which is thought to be turned on by FgMkk1. Furthermore, it appears that FgMkk1 is a negative regulator of chemotropic growth towards wheat exudate or horse radish peroxidase, whereas FgMgv1 was previously shown to be necessary for chemotropism. Additional experiments are underway to understand the implications of these results.

P3.1-049

IDENTIFICATION OF SOLANUM LYCOPERSICUM SMALL RNA TRANSFERRED TO BOTRYTIS CINEREA DURING THE INFECTION PROCESS.

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Text
Botrytis cinerea is an important pathogen to the tomato industry. The current strategy for *B. cinerea* control is the use of chemical fungicides. However, the indiscriminate use of these chemicals has generated more resistant pathogens and a growing interest in safer alternatives. The recent discovery of bidirectional small RNA transfer between *Solanum lycopersicum* and *B. cinerea* represents a new level of transcriptional regulation with great biotechnological potential. Therefore, in this work we sought to study and identify small RNAs transferred from tomato to the fungus in response to infection and explore new ways to combat the disease. For this purpose, a susceptibility study of twelve commercial tomato varieties to *B. cinerea* was conducted. Marmande and Rosado varieties were the most resistant and susceptible cultivars, respectively. Money Maker showed an intermediate susceptibility. Subsequently, sRNAseq libraries were generated from set of tomato leaves control and inoculated with *B. cinerea* B05.10 and a list of small RNAs differentially expressed in response to the fungus was obtained and their putative mRNAs target in *B. cinerea* were identified. Those sRNA targeting fungi genes involved in virulence and growth were selected. These results will allow us to identify sRNA candidates that are potentially transferred by the plant to the pathogen and that may affect the virulence of the fungus, thus allowing us to explore new alternatives for *B. cinerea* control.

P3.1-050

TRANSCRIPTOME ANALYSIS OF DIFFERENT POTATO CULTIVARS REVEALS THE CULTIVAR-SPECIFIC MOLECULAR EVENTS IN EARLY BLIGHT DISEASE

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Text

Early blight, caused by Alternaria solani is an economically important disease affecting potato tuber yield worldwide. Chemical plant protection agents mainly control the disease and over-using these chemicals can lead to the evolution of resistant strains. For sustainable early blight management, identifying genetic disease resistance factors is crucial. Therefore, we performed transcriptome sequencing of Magnum Bonum, Désirée, and Kuras potato cultivars with varying levels of early blight resistance at 18 and 36 hours post-infection (hpi) to identify key host genes and pathways in a cultivar-specific manner. The number of differentially expressed genes (DEGs) increased with susceptibility and infection time. Interestingly, the up-regulated DEGs were twice in number as compared to down-regulated ones in all cultivars and time points, except Kuras at 36 hpi. Many transcription factor families were enriched, of which a significant number were up-regulated. Key transcripts involved in the jasmonic acid, ethylene biosynthesis, mevalonate pathway, and terpene biosynthesis were up-regulated across the potato cultivars and time points. Compared to Magnum Bonum and Désirée, multiple components of the photosynthesis machinery, starch biosynthesis and degradation pathways were down-regulated in the most susceptible potato cultivar, Kuras. The results provide important insights into the molecular events at the early stages of disease development and help to shorten the knowledge gap.

P3.1-051

TRANSCRIPTOMIC ANALYSES REVEALED PATHOGENICITY-RELATED GENES IN THE FUNGAL PATHOGEN VERTICILLIUM LONGISPORUM

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Text

Verticillium longisporum is a soil-born fungal species causing sever disease on mainly Brassicaceae family across the world. The fungus secretes diverse small proteins, known as effectors, to evade plant immunity. However, little information is available about the mechanisms of pathogenicity of this species. Genome analysis of the V. longisporum stain VL1, showed that it contains more than 60 candidate effector genes. Transcriptome analyses of 14 candidate genes in this study revealed that the effector genes VIsPLA2 phospholipase, and an endolysin-like gene were highly expressed during the infection on its host. The functional characterization of candidate effectors indicated that overexpression of the VlPLA2

increased virulence of the fungus. Protein expression in E. coli cells, showed that it is an active A2 phospholipase. Transient expression in *Nicotiana benthamiana* plants also confirmed that PLA2 associated with vesicle associated membrane proteins (VAMPs), to be transferred to the nuclear envelope, facilitating the entry to the nucleoplasm, and altering the expression of genes involved in plant defense. Overall, our data indicated that VlPLA2 is a fungal virulence factor targeting host nuclei and suppressing basal plant immunity responses. The results from the current study give us deep knowledge towards understanding the precise role of these lytic enzymes in fungal infection biology.

P3.1-052

TRANSCRIPTOMIC RESPONSES CONTROLLING AGGRESSIVENESS OF ASCOCHYTA RABIEI DURING ASCOCHYTA BLIGHT INFECTION IN CHICKPEA

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Text

Ascochyta blight of chickpea, caused by *Ascochyta rabiei*, is one of the most important foliar diseases in chickpea, with substantial worldwide economic impact on production quality and yield. Foundational research on plant-pathogen molecular interactions have provided clues to the molecular mechanisms underlying *A. rabiei* infection in early stage of chickpea seedlings. These studies identified several signaling molecules (effectors) and transcription factors that have suggested to play key roles in the infection and establishment of the disease. To reveal the underlying molecular mechanisms that contribute to the aggressiveness of *A. rabiei*, a dual RNA-Sequencing approach was chosen to compare the transcriptomic responses of both the plant and the pathogen between highly aggressive and mildly aggressive isolates while infecting vegetative and reproductive parts of chickpea plant. The identified differentially and co-expressed genes have been functionally annotated using homology and domain searches and cross-referenced to a database of *A. rabiei* predicted effector proteins. The results of this study will help to identify the key genes contributing to *A. rabiei* aggressiveness and unravel the pathogen's strategy to overcome the chickpea defense responses. This knowledge will be useful for developing targeted disease management strategies and breeding for resistance chickpea varieties.

P3.1-053

UNCOVERING GLOBAL DIVERSITY AND EVOLUTION OF VIRULENCE GENES TOXA AND TOXB IN TAN SPOT PATHOGEN PYRENOPHORA TRITICI-REPENTIS

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Text

In this study, we investigated the global diversity and evolution of two major virulence genes, ToxA and ToxB, in the tan spot pathogen Pyrenophora tritici-repentis. A total of 427 isolates collected from regions across the Americas, Europe, North Africa, the Fertile Crescent, and Asia were analysed. Over the past 30 years, only one haplotype of the necrosis-inducing effector gene ToxA had been reported in tan spot. However, in our study, we identified additional five haplotypes from Argentina, Japan, Canada, and North Africa, all of which induced necrosis on susceptible wheat. We also identified twenty haplotypes in the chlorosis-inducing effector gene ToxB, but only three of these were associated with the ability to induce chlorosis. ToxB upstream sequences revealed the presence of short sequence repeats of 25 nucleotides, present in tandem repeats, that may have an impact on gene expression. We identified ToxB-like proteins in species associated with plant hosts, either as pathogens or endophytes, in the Dothidiomycetes and Sordariomycetes, as well as for the first time in the Leotiomycetes class. All of these ToxB-like proteins exhibited a conserved structure. Most of the mutations in the ToxA and ToxB genes were non-synonymous, indicating that they likely evolved under positive selection pressure. A direct correlation between selection pressure and effector isoform may highlight the importance of exploring the variation in effector-encoding genes at a global scale.

P3.1-054

MAIZE ANTIFUNGAL PROTEIN AFP1 ELEVATES FUNGAL CHITIN LEVELS BY TARGETING CHITIN DEACETYLASES AND OTHER GLYCOPROTEINS

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Text

Pathogenic fungi convert chitin to chitosan to evade plant perception and disarm chitin-triggered immune responses. Whether plants have evolved factors to counteract this evasion mechanism remains obscure. Here, we decipher the mechanism underlying the antifungal activity of maize secretory mannose-binding cysteine-rich receptor-like secreted protein (CRRSP), Anti-Fungal Protein 1 (AFP1). AFP1 binds to multiple sites on the surface of sporidial cells, filaments, and germinated spores of the biotrophic fungus *Ustilago maydis*. It inhibits cell growth and budding, as well as spore germination. AFP1 promiscuously interacts with most chitin deacetylases (CDAs) by recognizing the conserved NodB domain to interfere with the enzyme activity. Deletion of O-mannosyltransferase 4 reduces the protein mannosylation, which correlates with reduced AFP1 binding and antifungal activity, suggesting that AFP1 interacts with mannosylated proteins to exhibit an inhibitory effect. AFP1 also has extended inhibitory activity against *Saccharomyces cerevisiae*; however, AFP1 did not reduce binding to the double *Sccda*1,2 mutant, indicating that the targets of AFP1 have expanded to other cell-surface glycoproteins, probably facilitated by its mannose-binding property. Increasing chitin levels by modulating the activity of cell-surface glycoproteins is a universal feature of AFP1 interacting with a broad spectrum of fungi to inhibit their growth.

P3.1-055

COLLAPSE OF AERIAL HYPHAE AS A POTENTIAL SIGNAL TO INDUCE PERITHECIUM FORMATION IN THE CEREAL PATHOGEN FUSARIUM GRAMINEARUM

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Text

Fusarium graminearum, the causal agent of Fusarium Head Blight in cereal crops, produces a sexual fruiting body (perithecium) on plant debris as an overwintering and dissemination strategy. In an artificial condition (e.g. carrot agar medium), a *F. graminearum* Z3643 strain was able to produce perithecia mostly at the central region of fungal culture where aerial hyphae were naturally collapsed. To explore the molecular mechanism underlying hyphae collapse-mediated sexual development in this fungus, we focused on a total of 699 genes differentially expressed at the collapsed region, among which only 13.9% were overlapped with those controlled at the transcriptional level by the *MAT* loci, master regulators of sexual development in *F. graminearum*. For further functional analysis, we generated transgenic strains of Z3643, which carried single deletions of the selected 26 genes. Most strains exhibited no dramatic changes in hyphal growth and sexual development, but those deleted for 5 genes significantly differed from Z3643 in pattern of hyphae collapse, and/or perithecium formation. Particularly, the deletion strain of FGSG_09210, which was highly induced during hyphal collapse but not regulated by *MAT*, showed no clear hyphal collapse, and produced no perithecia on carrot agar. Taken all together, it is possible that aerial hyphae collapse, if occurred on plant debris, would act as a physical signal leading to sexual development in *F. graminearum* in a natural condition.

P3.1-056

USTILAGO MAYDIS N-GLYCOSYLATED APOPLASTIC EFFECTOR ALE1 TARGETS MAIZE ZMTAXI TO COUNTERACT ITS ATTACK ON UMXYLANASE 11A

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Text

Plant cell wall is a dynamic barrier to pathogen infection. Successful pathogens secrete cell wall degrading enzymes (CWDEs) to support their virulence. However, fungal CWDEs could be inhibited by plant inhibitors, such as TAXI-I which inhibits the activity of xylanases from Botrytis cinerea and Aspergillus niger. Whether fungal pathogens have overcome this inhibitory action is less explored. Here, we report that Ustilago maydis effector ALE1 is

upregulated and secreted to the maize apoplast during biotrophic stages to promote fungal virulence. ALE1 is N-glycosylated and has a conserved virulence function among smut fungi in a glycosylation-dependent manner. ALE1 confers its virulence function by interacting with maize TAXI-I, presumably negating its inhibition on U. maydis xylanase Xyn11A. However, glycosylation of ALE1 is not required for the interaction of ALE1 with TAXI-I, suggesting that glycosylation of ALE1 may involve an as-yet-undetermined function. Indeed, a maize hypothetical protein preferentially interacts with un-glycosylated ALE1. This indicates a possibility that glycosylation of ALE1 help in escaping the interference by the hypothetical protein. Our study attempts to reveal the strategy of U. maydis in counteracting maize's sophisticated defense mechanisms at the apoplastic interface between fungus and plant.
Keywords: Ustilago maydis, TAXI, N-Glycosylated ALE1

P3.1-057

PATHOGENIC EFFECTOR VMUSP1 CONTRIBUTES TO THE FULL VIRULENCE OF VALSA MALI AND INTERACTS WITH APPLE HEAT SHOCK PROTEIN 70 AS A POTENTIAL TARGET

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Text
Apple Valsa canker caused by *Valsa mali* results in a huge economic loss each year. Pathogens manipulate host immunity to achieve colonization and infection by secreting effector proteins, which are typically identified based on the presence of signal peptides. Similar to other pathogens, effector proteins are important pathogenic weapons for *V. mali*. Here, we show that an effector protein VmUSP1, not only inhibits BAX-induced cell necrosis but also contributes to the complete virulence of *V. mali*. The pathogenicity of the *VmUSP1* gene knockout mutant was significantly reduced to that of the wild type, and overexpression of *VmUSP1* in apple promoted host susceptibility to V*. mali*. Interestingly, VmUSP1 lacks a typical signal peptide but exhibits characteristics of unconventional secretion. In assays of yeast two-hybrid system, bimolecular fluorescence and, coimmunoprecipitation, it's confirmed that VmUSP1 targets apple (*Malus domestica*) heat shock protein 70 (MdHSP70). MdHSP70 participated in the immune response of apple to *V. mali* and induced the accumulation of callose and reactive oxygen species. In addition, overexpression of *MdHSP70* enhanced the resistance of apple to *V. mali*. However, VmUSP1 greatly compromised the MdHSP70-mediated resistance of apple to *V. mali*. Taken together, our results revealed a mechanism by which a *V. mali* effector protein VmUSP1 promotes the infection of *V. mali* by interfering with MdHSP70-mediated plant immunity.

P3.1-058

STRATEGIES TO DISCOVER DISEASE RESPONSE GENES AGAINST FUSARIUM HEAD BLIGHT IN WHEAT

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Text

Development of cereal crop varieties with resistance to Fusarium head blight (FHB) is a main breeding goal worldwide. Currently, AAC Tenacious is the only spring wheat cultivar in Canada to receive a resistant (R) rating to FHB and its resistance is unparalleled. We are integrating genetics, genomics, and host-pathogen responses to elucidate the genetic and physiological mechanisms of resistance in AAC Tenacious. We have performed transcriptomic and histological analyses of AAC Tenacious after inoculation with Fusarium graminearum. In addition, from a cross between AAC Tenacious and Roblin, we evaluated FHB response in a doubled haploid (DH) population grown under inoculated field conditions. A linkage map of 8951 SNP markers (Infinium iSelect 90k SNP wheat array) was generated and QTLs associated with resistance to FHB were identified. In a current project, we have used gamma irradiation mutagenesis of seeds to develop a TILLING population of AAC Tenacious. Through preliminary field evaluations, we have observed that this treatment has induced susceptibility, suggesting some of these mutations have “knocked-out” resistance genes such that they no longer function, resulting in TILLING lines that have become susceptible. By sequencing the genome of AAC Tenacious and comparing that to the DNA sequence of the susceptible TILLING mutants, we can point the exact mutations resulted in the susceptible phenotype.

P3.1-059

A VALSA MALI VIRULENCE EFFECTOR VMPR1C REPRESSES APPLE RESISTANCE TO VALSA CANKER BY COMPROMISING MDVQ29-MEDIATED IMMUNITY

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Text

The pathogenic fungus *Valsa mali* (*Vm*) is the major causal agent of apple Valsa canker (AVC) that causes great economic loss. Effector proteins secreted by the phytopathogens play important roles in virulence to manipulate host immunity. qRT-PCR analysis showed that the effector protein VmPR1c of *Vm* was significantly up-regulated during the early stages of infection, and the virulence of the Δ*VmPR1c* was significantly reduced compared with that of the wild-type strain, indicating that VmPR1c was an important virulence effector of *Vm*. However, the molecular mechanism of its virulence function remains largely unknown. Here, we reported that VmPR1c interacted with a VQ-motif containing protein MdVQ29 and overexpression of *MdVQ29* enhanced apple resistance to AVC. In addition, MdVQ29 interacted with MdWRKY23, which was further shown to bind the promoter of *MdCOI1* and activated its expression. Overexpression of *MdWRKY23* and *MdCOI1* enhanced apple resistance, whereas silencing them attenuated resistance to AVC. Importantly, *MdVQ29*

promoted the transcriptional activity of MdWRKY23, however, *VmPR1c* compromised *MdVQ29*-mediated resistance. Taken together, our findings revealed that the *Vm* effector protein VmPR1c subverts host immunity by targeting disease-resistant related factor MdVQ29 to facilitate infection.

P3.1-060

USTILAGO MAYDIS PR-1-LIKE PROTEIN HAS EVOLVED TWO DISTINCT DOMAINS FOR DUAL VIRULENCE ACTIVITIES

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Text
Diversification of effector function, driven by a co-evolutionary arms race, enables pathogens to establish compatible interactions with their hosts. Structurally conserved plant pathogenesis-related PR-1 and PR-1-like (PR-1L) proteins are involved in plant defense and fungal virulence, respectively. However, how fungal PR-1L counteracts plant defense to promote fungal virulence has not been elucidated. Here, we demonstrate that *Ustilago maydis Um*PR-1La and *Saccharomyces cerevisiae Sc*PRY1 localize to the fungal cell surface mediated by their serine/threonine-rich domains to protect against plant phenolics. Despite it, *Um*PR-1La has gained additional specialized activity in eliciting hyphal-like formation upon phenolic perception, suggesting that *U. maydis* deploys *Um*PR-1La to sense environmental signals and direct their growth within the plant host. *U. maydis* also hijacks maize Cathepsin B-like 3 (CatB3) to release a functional CAPE-like peptide upon cleavage of the conserved CNYD motif of *Um*PR-1La to subvert plant immunity and promote fungal virulence. However, it is unclear how CatB3 selectively avoids cleaving plant PR-1s to release CAPE peptides, despite the presence of the same conserved CNYD motif. Our work highlights that *Um*PR-1La has acquired additional functional roles to suppress plant defense and sustain the infection process of fungal pathogens.

P3.1-061

DISSECTION OF THE UBIQUITIN E3 LIGASES-MEDIATED DISEASE RESISTANCE MECHANISM IN RICE AGAINST MAGNAPORTHE ORYZAE

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Text
Rice blast, caused by the fungal pathogen Magnaporthe oryzae, is one of the most serious rice diseases. The ubiquitin-proteasome system (UPS) is one of the most important protein

turnover mechanisms. Among the three main UPS enzymes, E3 ubiquitin ligases (E3s) are highly flexible and diverse. The regulation mechanism of E3s in the rice-M. oryzae interaction is still unclear. We found the RING-type E3 APIP10 promotes the degradation of two rice transcription factors OsVOZ1 and OsVOZ2, while silencing of OsVOZ1/2 decreases the NLR protein Piz-t accumulation and mediated resistance, indicating that the UPS is essential for the NLR-mediated immunity. Moreover, we found that the U-box-type E3 OsPUB73 positively regulates rice resistance against M. oryzae by promoting the degradation of OsVQ25. Knockout mutants of OsVQ25 exhibit enhanced resistance to the pathogen without a growth penalty, revealed that the E3 fines tune plant immunity and growth. To rapidly identify the cognate E3s of ubiquitinated proteins, we generated a complete ubiquitin E3 library containing 98.94% of the 1515 E3 genes in the rice genome. Using this library, we identified the hub F-box-type E3 OsFBK16 that promoted the degradation of phenylalanine ammonia lyase family (OsPALs). Loss-of-function of OsFBK16 displayed enhanced blast resistance, indicating that OsFBK16 negatively regulates rice immunity. Taken together, these results indicate that E3s play important roles in rice immunity against M. oryzae.

P3.1-062

IMPACTS OF MYRTLE RUST DISEASE ON THE SOIL MICROBIAL COMMUNITY ASSOCIATED WITH THE NAÏVE HOST LOPHOMYRTUS BULLATA

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Text

Lophomyrtus bullata (ramarama; Myrtaceae) is endemic to New Zealand and although widespread, is now considered threatened due to the ongoing spread of *Austropuccinia psidii,* the causal agent of myrtle rust. *Lophomyrtus bullata* is a shrub or mid-storey tree, which is widespread but uncommon in coastal and lowland forests especially on riparian margins. This species is often present in lowland podocarp riparian forest and is extremely susceptible to myrtle rust. Since it's relatively uncommon, *L. bullata* had not been the focus of many studies until the 2017 myrtle rust incursion into New Zealand. We investigated the associated soil microbial diversity, focussing on fungi and bacteria, in the vicinity of myrtle rust symptomatic and asymptomatic *L. bullata* to assess the impact of the disease on the surrounding soil community. The study site included three 60 metre long transects containing twenty-four 5 × 5 metre subplots; three soil samples were taken from subplots containing at least one tagged *L. bullata* plant. A comprehensive disease assessment of 146 *L. bullata* plants within the transects was conducted simultaneously; over 37500 leaves were assessed for presence of *A. psidii* urediniospores. Five loci were targeted for metabarcoding analyses to cover the microbial diversity present along the disease gradient. We specifically wanted to investigate what, if any, impact this devastating disease has on soil microbial networks.

Molecular aspects of plant-fungal interactions Part 2: Mechanisms of infection

C7.1-1

FUNCTIONAL ANALYSIS OF THE MEP EFFECTOR GENE REPERTOIRE OF THE BLAST FUNGUS MAGNAPORTHE ORYZAE

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Text

Magnaporthe oryzae is the causal agent of rice blast disease, one of the most serious diseases affecting rice cultivation around the world. During plant infection, M. oryzae forms a specialised infection structure called an appressorium, which uses mechanical force to breach the rice cuticle and utilises a second infection structure, the transpressorium, to move between rice cells. During invasive growth, the blast fungus deploys a large repertoire of at least 546 Mep (Magnaporthe effector protein) effectors, which are expressed in a co-ordinated manner during plant infection. These include structurally conserved ADP-ribosyl transferase and MAX effectors, which are temporally co-regulated. We are investigating the function of the Mep effectors by identifying their host targets based on the generation of transgenic rice and barley lines expressing them and co-immuno-precipitation experiments. In parallel we are analyzing targeted effector mutants and the localisation and delivery of Mep effectors. Using these approaches, we have identified a sub-set of effector proteins that can target host chloroplasts and interact with photosystem II components to suppress chloroplast immunity responses, and a second group associated with transpressorium function. We have also characterized the contribution of effectors to pathogen fitness based on competition assays and identified regulatory proteins necessary for transcriptional regulation of MEP effectors. Progress in the functional characterization of the effector repertoire of M. oryzae will be presented.

C7.1-2

REGULATION OF EFFECTOR GENE EXPRESSION AS CONCERTED WAVES IN LEPTOSPHAERIA MACULANS: A TWO-PLAYERS GAME INVOLVING A CHROMATIN REMODELER AND A SPECIFIC TRANSCRIPTION FACTOR

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Text
Leptosphaeria maculans is a phytopathogenic fungus responsible for oilseed rape stem canker that displays a particularly complex lifecycle. Different sets of effector-genes are expressed at each stage of oilseed rape infection. Repeat-rich regions of L. maculans genome are enriched in effector-genes specifically expressed during biotrophic stages of infection. These regions show a repressed chromatin state during mycelial growth in vitro. We showed the importance of chromatin remodeling in the control of effector-genes expression. As such, the repressive histone modification H3K9me3 deposited by the methyltransferase KMT1, is involved in the regulation of these genes not expressed in vitro but highly expressed during infection. However, inactivation of KMT1 did not de-repress the expression of effector-genes in vitro at the same level as observed during infection, suggesting additional actors involved, such as transcription factor(s) (TF). We investigated the involvement of Pf2, a fungal specific Zn2Cys6 TF, in the control of effector-gene expression. Deletion of LmPf2 lead to a non-pathogenic mutant. Its over-expression was not sufficient to express effector-genes in vitro. In contrast, its over-expression in a Kmt1 mutant background induced the expression of effector-genes in vitro to the same level as during plant infection. These results demonstrated for the first time a dual control of effector-gene expression involving a chromatin remodeler and an infection specific TF.

C7.1-3

TIGHT REGULATION OF CELL WALL DEGRADING ENZYMES IS CRITICAL FOR VIRULENCE OF THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Text
The plant cell wall is a major defence barrier against pathogens and a major source of nutrients for colonizing microorganisms. Pathogenic fungi hydrolyse cell wall polymers with a diverse set of secreted cell wall degrading enzymes (CWDEs). Here, we demonstrated that the cellulase ZtCel45A from the wheat pathogen *Zymoseptoria tritici* hydrolyses wheat cell wall cellulose and mixed-linked glucans (MLGs). We showed that *ZtCel45A* is tightly regulated and only expressed at the necrotrophic infection stage. Early misexpression of *ZtCel45A* impairs fungal virulence and enhances the production of cello-oligomers and MLG-oligomers. Exogenous treatment of wheat seedlings with MLG43 and cello-triose enhances resistance to Z. tritici. These results demonstrate that the balance between cell wall degradation and release of resistance-inducers, such as wall damage-associated molecular patterns (DAMPs), by fungal CWDEs governs the outcome of host invasion. We suggest that this balance drives the evolution of fungal CWDE expression regulation to favour plant colonization.

C7.1-4

ADRESSING REDUNDANT ROLES OF PHYTOTOXIC PROTEINS FOR NECROTROPHIC INFECTION OF B. CINEREA BY MULTI-K.O. MUTAGENESIS

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Text

Botrytis cinerea is a wide host range necrotroph. During invasion, it quickly kills host cells and colonizes dead tissue, supported by secretion of CWDE, cell death inducing proteins (CDIPs) and metabolites, and tissue acidification. However, it is still unclear how the fungus induces host cell death. Based on a highly efficient CRISPR/Cas9 protocol, we have constructed a series of up to 22-fold *B. cinerea* mutants, lacking all currently known CDIPs. The mutants showed normal growth, but decreased virulence with increasing numbers of deleted CDIPs. The 22x mutant caused strongly reduced lesion formation on leaves and almost no infection of fruits of different species. High resolution secretome analysis of the mutants confirmed the loss of the deleted CDIPs. The search for remaining CDIPs is ongoing, to generate finally a non-necrotrophic *B. cinerea* mutant. This is one of the first systematic approaches to address functional redundancy of fungal virulence factors. B. cinerea triggers the plant hypersensitive response (HR) as an infection strategy. We assume that CDIPs contribute to HR by activating plant pattern-triggered immunity (PTI). Infection of mutants or silenced tissues of Arabidopsis or tobacco lacking the coreceptors of pattern recognition receptors (PRRs), BAK1 and SOBIR1, did not reveal differences to WT plants in susceptibility against *B. cinerea*. More mutants are tested to identify cell death pathways that are activated by isolated CDIPs and the invading fungus.

C7.1-5

SUPPRESSION AND COUNTER-SUPPRESSION OF PLANT IMMUNITY BY AN EXO-BETA-1,3-GLUCANASE OF GH17 FAMILY AND AN ELONGATION FACTOR 1ALPHA OF THE RICE BLAST FUNGUS

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Text

Fungal cell walls undergo continual remodeling during hyphal growth, development, infection and adaptation to environmental stress. The cell wall remodeling generates β-1,3-glucan fragments by diverse endo-glycosyl hydrolases (GH), which are well-known pathogen-associated molecular patterns (PAMPs). How fungal pathogens evade the plant immunity triggered by β-1,3-glucan fragments and associated GH proteins is not known. Here, we

report a novel mechanism of immune evasion underlying the suppression of ?-1,3-glucan-triggered plant immunity by the blast fungus Magnaporthe oryzae. An exo-β-1,3-glucanase of the GH17 family, named Ebg1, is found important for fungal cell wall integrity and virulence of M. oryzae. Ebg1 can hydrolyze β-1,3-glucan and laminarin into glucose to prevent β-1,3-glucan-triggered plant immunity, but also acts as a PAMP, independent of its hydrolase activity. Surprisingly, M. oryzae engages an elongation factor 1alpha protein (EF1α) to interact and co-localize with Ebg1 in the apoplast to suppress Ebg1-triggered immunity. Further, both Ebg1 and EF1α are widely distributed in fungi, and their orthologues from Fusarium graminearum were revealed to interact with each other and rescued the phenotype defects in EBG1 and EF1α deletion mutants in M. oryzae. Together, our results suggest that the interaction between Ebg1 and EF1α may be a conserved mechanism whereby fungal pathogens evade plant immunity and safeguard cell wall remodeling during infection.

C7.1-6

THE FUNGAL PATHOGEN USTILAGO MAYDIS MODULATES HOST GENE EXPRESSION TO TRIGGER TUMOR FORMATION IN MAIZE

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Text

Ustilago maydis is a biotrophic fungus causing common smut in maize, which is characterized by plant tumors localized to the infected organs. In leaves, U. maydis infection results in two types of tumor cells: hypertrophic cells that develop from mesophyll, and hyperplasic cells which result from de novo cell division of bundle sheath1. Multiple studies have shown that U. maydis virulence depends on the activity of secreted effector proteins2. However, little is known about the mechanistic basis of fungal effectors to orchestrate tumorigenesis. A cross-species transcriptome analysis between U. maydis and the closely related maize pathogenSporisorium reilianum, which does not induce tumors, identified effectors being differentially regulated in both pathogens and likely evolved diversified virulence functions3.

Here, we show that such effectors trigger the formation of plant tumors by modulating host gene transcription. One example is Sts2, which induces the formation of hyperplasic tumor cells. Sts2 acts as a transcriptional activator in the host nucleus, where it activates maize key regulators of leaf meristem development. Besides Sts2, we found at least one additional U. maydis effector that actively modulates maize gene expression to trigger tumor growth and executes a distinct molecular mechanism. Together, our findings show that a fungal pathogen evolved a highly sophisticated molecular toolbox to directly reprogram plant developmental processes to trigger disease.

F7.1-2

SPATIOTEMPORAL ANALYSIS OF TAN SPOT IN WHEAT USING TRANSCRIPTOME AND HIGH-RESOLUTION ELEMENTAL IMAGING

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Text

Pathogen attacks elicit dynamic and widespread molecular responses in plants. While our understanding of plant responses has advanced considerably, little is known of the responses in the asymptomatic “green” regions (AGR) adjoining lesions. Here, we explore spatiotemporal transcriptome data and elemental maps to report the changes in the AGR of two wheat cultivars infected with a necrotrophic fungal pathogen, Pyrenophora tritici-repentis. We show, for the first time, that calcium oscillations are modified in the susceptible cultivar, resulting in possible “frozen” host defence signals at the mature disease stage, and silencing of the host’s recognition and defence mechanisms which would otherwise protect it from further attacks. In contrast, Ca accumulation and heightened defence response was observed in the moderately resistant cultivar. Other findings include the inability of the AGR of the susceptible wheat to recover post disease disruption and expression of eight predicted pathogen proteinaceous effectors. The targeted tissue sampling for gene expression analysis resulted in a unique dataset which creates capability to study plant-pathogen interactions at a higher resolution compared to classic bulk leaf tissue analysis. Collectively, our results highlight the benefits of spatially resolved molecular analysis in providing high-resolution spatiotemporal snapshots of host-pathogen interactions, paving the way for detangling complex disease interactions in crop plants.

P7.1-001

ACTIVATION OF WHEAT TANDEM KINASE 1 INDUCES EXPRESSION OF GENES INVOLVED IN PATHOGEN RECOGNITION, SIGNAL TRANSDUCTION, AND HYPERSENSITIVE CELL DEATH

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Text

Plant diseases risk global food security and significantly limit wheat production. Stripe rust is one of the most wheat-devastating diseases caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*). The most cost-effective and sustainable solution is the development of disease-resistant food crops carrying resistance genes. *Yr15* encodes a protein with a tandem kinase-pseudokinase architecture designated wheat tandem kinase 1 (WTK1) that confers broad-spectrum resistance to *Pst*. To investigate the transcriptional changes activated by WTK1 in response to infection with *Pst*, we conducted transcriptional time-course analyses of a resistant durum wheat genotype transformed with WTK1 under its native promoter and compared with its susceptible sister line (near-isogenic lines, NILs) after inoculation with *Pst*. Data sets from 80 samples were processed and analyzed using the R package Moanin for

time-course gene expression analysis. Using spline clustering, we identified 10 differentially expressed gene clusters that exhibit various patterns of regulation. Six clusters were upregulated in the resistant line and were enriched in genes involved in pathogen recognition, signal transduction, phytohormone production, and hypersensitive cell death. To conclude, whole transcriptome analysis of wheat NILs revealed that WTK1 activates defense-associated transcriptional reprogramming upon pathogen infection leading to disease resistance.

P7.1-002

IDENTIFICATION OF EFFECTOR GENES IN PHELLINUS NOXIUS, THE CAUSE OF BROWN ROOT ROT DISEASE

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Text

Brown root rot is a common tree disease affecting a wide variety of fruit trees and woody horticultural trees in tropical and subtropical regions. The pathogen *Phellinus noxius* often causes root rot, making the trees susceptible to falling and resulting in economic losses or safety hazards. Little is known about the pathogenesis of *P. noxius* and how it can infect a wide range of hosts. We found that following inoculation with *P. noxius*, rapid cortical collapse can be observed prior to the appearance of symptoms. To determine whether effectors are involved in the pathogenesis, we analyzed the genome and transcriptome data. We used the ab initio gene prediction method to predict 9,684 coding sequences of *P. noxius*. 472 potential secreted proteins without transmembrane regions were predicted using SignalP6.0 and TMHMM2.0. The small secreted proteins were predicted by EffectorP3.0, and a total of 129 candidate effectors were selected. As a preliminary screening, a set of candidate effector genes from the cDNA of a highly virulent *P. noxius* strain FBS71 infecting *Populus trichocarpa* will be expressed in the *Nicotiana benthamiana* leaves by agroinfiltration method to analyze their function in inducing plant cell death. For the cell death-inducing candidate effector genes, *Agrobacterium*-mediated transformation of the *P. trichocarpa* roots will subsequently be conducted to observe their effects on root tissues and analyze the activation or suppression of plant defense-related genes.

P7.1-003

DUAL TRANSCRIPTOMIC ANALYSES UNVEIL THE INTERACTION BETWEEN PHELLINUS NOXIUS AND POPULUS TRICHOCARPA

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Text

Brown root rot disease, caused by the white rot fungus *Phellinus noxius*, can infect more than 200 hardwood and softwood tree species. However, the pathogenic mechanisms of *P. noxius* remain to be elucidated. In this study, we compared the transcriptomes of high- and low-virulence isolates inoculated on *Populus trichocarpa* and the grain inoculum at 2-day post-incubation. A total of 1305 genes were differentially expressed. Of these, 123 carbohydrate-active enzymes were up-regulated, and 61 of them were *glycoside hydrolase* (*GH*) family proteins. These enzymes might be involved in the degradation of plant tissues, and some of them were known to be virulence factors in other pathogens. In addition, a phytotoxin gene *cerato-platanin* was induced during the infection of *P. noxius*, and a significantly higher level of expression was observed in the high-virulence isolate. On the other hand, the transcriptome data indicated that the pathogenesis-related (PR) genes *PR1*, *glucan endo-1,3-ß-glucosidases* (*PR2*), *chitinases* (*PR4*), *germin-like proteins* (*PR16*), and the cell wall reinforcement genes *extensins*, *pectinesterases*, and *lignin-forming anionic peroxidases* might contribute to the early defence in poplar. These results provide new insights into the early interaction between *P. noxius* and the host, and candidate virulence genes of *P. noxius* will be verified by double-stranded RNA-induced gene silencing.

P7.1-004

STRIPE RUST FUNGAL CONSERVED EFFECTORS PSTGSRE1 AND PSTGSRE4 DISRUPT THE WHEAT ROS-INDUCED IMMUNITY TO FACILITATE PST INFECTION

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Text

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), gives great threats to wheat production. Therefore, it is very important to uncover molecular mechanisms of important and conversed *Pst* effectors regulating wheat immunity for sustainable control of stripe rust. We have identified an important glycine-serine-rich effector family PstGSREs and found that PsGSRE1 and PstGSER4 are important pathogenicity factors of Pst. PstGSRE1 and its glycine-serine-rich motif PstGSRE1-m9 could disrupt nuclear localization of TaLOL2 and suppressed ROS-mediated cell death induced by TaLOL2, thus compromising wheat immunity. Interestingly, unlike the PstGSRE1, the PstGSRE4 does not contain m9 motif and does not interact with TaLOL2. Furthermore, we found that PstGSRE4 can interact with TaCZSOD2 and reduce the enzyme activity of TaCZSOD2, indicating that PstGSRE4 can reduce H2O2 accumulation by inhibiting the enzyme activity of TaCZSOD2, thereby promoting Pst infection. Further study revealed that TaGAPDH2 interacts with PstGSRE4 and hijacked by the PstGSRE4 as a plant immunity negative regulator to promote *Pst* infection. Taken together, we systematically analyzed the function of PstGSREs during wheat-*Pst* interactions. Our studies expanded the understanding of the biological functions of rust fungal effectors, and laid a foundation for the development of a lasting and effective

strategy for the prevention and control of wheat stripe rust.

P7.1-005

IN SEARCH OF CANDIDATE GENES FOR RESISTANT TO VENTURIA OLEAGINEA IN OLIVE (OLEA EUROPAEA SUBSP. EUROPAEA)

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Text
The phytopathogenic genus *Venturia* is composed different pseudo-biotrophic species highly specialized. Among them are *V. oleaginea*, causal agent of olive leaf scab, and *V. inaequalis*, which cause the apple scab. One of the most important control methods for apple scab is the use of resistant varieties, but genetic resistant has been poorly studied in the case of olive scab. In apple, which may be considered here a model pathosysthem for olive, 20 major *V. inaequalis* resistance genes (Rvi) have been identified. In this study, we performed a search for homologs to Rvi in olive by studying both their structural and functional organization. Using the reference sequence of the apple genome (ASM211411v1), we mapped the QTL (Rvi1, Rvi3, Rvi5, and Rvi12) to the corresponding genomic regions in the olive genome assembly (CNAG: Oe9) through BLAST searches. Further analysis based on functional annotation using gene ontology terms (GO) narrowed eventually the list to 28 annotated genes. Specific primers for quantitative real-time PCR have been designed and qPCR assays and transcriptome profiles analysis on contrasting plant material are ongoing.

P7.1-006

MTA1-MEDIATED RNA M6A MODIFICATION REGULATES AUTOPHAGY AND IS REQUIRED FOR INFECTION OF THE RICE BLAST FUNGUS

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Text
In eukaryotes, N6-methyladenosine (m6A) is abundant on mRNA, which plays key roles in the regulation of RNA function. However, the roles and regulatory mechanisms of m6A in phytopathogenic fungi are still largely unknown. Combing with biochemical analysis, MeRIP-seq and RNAseq methods, as well as biological analysis, we showed Magnaporthe oryzae *MTA1* gene is an ortholog of human *METTL4*, which is involved in m6A modification and plays a critical role in autophagy for fungal infection. The Δ*mta1* mutant showed reduced virulence due to blockage of appressorial penetration and invasive growth. Moreover, the autophagy process was severely disordered in the mutant. MeRIP-seq identified 659 hypomethylated m6A peaks covering 595 mRNAs in Δ*mta1* appressorium, 114 m6A peaks was negatively related to mRNA abundance, including several ATG gene's transcripts.

Typically, the mRNA abundance of *MoATG8* was also increased in the single m6A site mutant ?*atg8/MoATG8*^{A982C}, leading to an autophagy disorder. Our findings reveal the functional importance of the m6A methylation in infection of M. oryzae and provide novel insight into regulatory mechanism of plant pathogenic fungi.

P7.1-007

IDENTIFICATION OF FUNGAL EFFECTOR TARGETS INVOLVED IN SUSCEPTIBILITY OF BREAD WHEAT TO FUSARIUM HEAD BLIGHT

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Text
Pathogen development in plant tissues can be facilitated by the expression of specific host genes known as susceptibility genes. By targeting these genes, small secreted proteins called effectors act as a key determinants of disease development. Recent works on Fusarium graminearum have described the nature and dynamics of effectors upon early stages of the infection progress, especially by innovative dual-omics approaches of infected and non-infected wheat varieties.
Starting from this relevant effector set, we selected 31 core-effectors shared by different strains and propose their functional validation. A structural characterization of their sequence demonstrated canonical sequences of either chloroplast or nuclear localization signals but failed to define common protein motifs/domains. In order to define the localization of the effectors in the host cell, we designed GFP-fused effector proteins and screened their expression patterns in transient expression in tobacco. Most effectors localized to the plant nucleus with some enrichment in the nucleolus or at the envelope.
Merging all information allowed the selection of a reduced numbers of candidate effectors to determine whether they can physically interact with wheat proteins by using Yeast two-hybrid system along with a specific wheat spike cDNA library. This work will allow connecting effectors to their supposed targets in wheat and will contribute to the identification of new and unpredictable susceptibility genes.

P7.1-008

CONSTRUCTION OF SEXUAL HYBRID POPULATION OF PUCCINIA STRIIFORMIS F. SP. TRITICI AND CANDIDATE GENETIC INTERVAL OF AVRYR10 AND AVRYR26

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Text
Puccinia striiformis f. sp. *tritici* (Pst), the causal agent of wheat stripe rust, is of massive destructiveness to wheat production worldwide. New variants overcome the resistance of

wheat cultivars due to pathogenic variation. Identification and cloning of an avirulence gene provide an insight into understanding mechanism of virulence variation of Pst in molecular level. We developed a sexual population by crossing CYR32, avirulent to *Yr10* and *Yr26*, and CYR34, virulent to *Yr10* and *Yr26* on Berberis aggregata seedlings. All of F_1 progeny showed resistance at both *Yr10* and *Yr26* loci. A F_2 population consisting of 221 progeny was developed by selfing a F_1 progeny on B. aggregata seedlings, showing identical phenotypes with a avirulence and virulence segregation ratio of 167:54 (≈3:1, $\chi 2 = 0.01$, $P = 0.92$) at either of both loci. These results indicated that avirulence of Pst against *Yr10* and *Yr26* was controlled by an dominant gene with independent effect, respectively. Two DNA bulks of both parental races, together with genome avirulent and virulent DNA bulks were constructed for bulked segregation analysis (BSA). Totally, 210,260 SNPs were obtained, 22,322 of which caused non-synonymous mutations. While InDel detection obtained 34,931 Small InDel. Using the ED and SNP index for association analysis, one candidate region with a genetic interval of 0.02 Mb on Chr14 with 3 candidate genes were obtained, which will facilitate the fine mapping and cloning of both avirulence genes.

P7.1-010

INVESTIGATION ON HIGH POLYPHENOLIC WHEAT GENOTYPES RESISTANCE TO FUSARIUM HEAD BLIGHT (FHB)

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Text

Fusarium head blight (FHB) is one of the most harmful diseases affecting cereal crops. Polyphenols provide a protective effect against biotic and abiotic stresses. It is therefore interesting to focus on genotypes with a high concentration of these compounds in the aleurone and pericarp. This research examines the interaction between five different pigmented wheat cultivars (Purendo, Skorpion, Rosso, Vanilnoir and Indigo) and the fungal pathogen Fusarium graminearum. Data obtained from phytopathological experiments and AUDPC calculation, demonstrate that blue aleurone genotypes are highly sensitive to FHB while genotypes with purple pericarp are less sensitive. Notably, Vanilnoir demonstrated a disease incidence identical to the resistant control Sumai3 (less than 10% after 21 days of infection). FDK evaluation and the total phenolic content showed no differences between genotypes. Furthermore, the quantification of the fungal genome by RT-qPCR showed that the pathogen had a lower diffusion in the Vanilnoir cultivar (compared to the more vulnerable varieties such as Skorpion), suggesting the involvement of a type II resistance mechanism. As a further level of investigation, a transcriptomic (mRNAseq) experiment was performed on the Vanilnoir and Rosso cultivars with the aim of identifying genes involved in the mechanisms of SAR induction at 2 days after infection.

This work was supported by the project MULTIFRU funded by POR FESR Lazio 2014-2020 action 1.2.1

P7.1-011

LECANOSTICTA ACICOLA MODULATES ITS PROTEOMIC PROFILE DEPENDING ON PINUS INNATE RESISTANCE TO BROWN-SPOT NEEDLE BLIGHT DISEASE

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Text

Pine needle blights are serious fungal diseases affecting European forests. Brown-spot needle blight disease (BSNB), caused by the phytopathogenic ascomycete *Lecanosticta acicola* (von Thümen) Sydow, is responsible for crown transparency and severe productivity losses. Genetic variation for disease resistance exists among pine species, where *P. radiata* is considered susceptible and *P. pinea* is relative resistant.

The main objective of our work was to explore how *L. acicola* modulates proteome and secretome in the presence of these pines with different degrees of susceptibility to BSNB. *Lecanosticta acicola* was grown in liquid medium (control) and media amendment with *P. radiata* or *P. pinea* needles. The secretome and the cellular proteome were analysed by LC-MS/MS.

The total number of proteins was higher when *L. acicola* faced pine needles. As expected, proteome presented higher number of proteins when compared to secretome.

A differential modulation of *L. acicola* protein profile was observed depending on the pine species. Secretome analysis - which is the host-fungi interface, showed that *L. acicola* expresses proteins able to overcome the first physical plant barrier, the cell wall. Cell wall degrading proteins were particularly overexpressed when *L. acicola* faced *P. pinea*.

This work demonstratesthat the fungus is able to distinguish between hosts and modulate its protein profile set to succeed during the infection process.

P7.1-012

OSMBR, A PUTATIVE RECEPTOR OF MAGNAPORTHE ORYZAE SNODPROT 1 (MSP1), OVEREXPRESSION CONFERS RESISTANCE TO RICE BLAST DISEASE IN RICE

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Text

Pathogen-associated molecular patterns (PAMPs) play a key role in triggering PAMPs triggered immunity (PTI) in rice. Recently, *Magnaporthe oryzae* snodprot1 homolog (MSP1) has been identified that functions as pathogen-associated molecular pattern (PAMP) and triggering PTI in rice and tobacco. However, potential receptor responsible for recognition of

MSP1 during PTI was elusive until recently. In this study, we successfully identified a putative MSP1 binding receptor (MBR) protein using transcriptomic analysis and showed up-regulation during infection of *M. oryzae*, Cochliobolus miyabeanus, and Xanthomonas oryzae in rice leaves. Moreover, to determine the interaction between MSP1 and MBR proteins, co-immunoprecipitation (Co-IP) assay was performed and it revealed that MSP1 binds with high affinity to the extracellular domain of MBR. Besides, we further confirmed that overexpression of MBR in rice showed enhanced resistance to rice blast disease. However, it still requires further investigation of MBR-MSP1 interaction-mediated downstream signaling during rice-M. oryzae interaction.

P7.1-013

PERCEPTION AND SIGNALLING OF MYCORRHIZA INDUCED RESISTANCE IN TOMATO PLANTS AGAINST BOTRYTIS CINEREA

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Text

Upon perception of biotic stress, some endogenous peptides known as phytocytokines, such as the peptide hormone systemin, are released to the apoplast functioning as endogenous regulators of plant immune responses. When a plant perceives mechanic damage or is attacked by a necrotrophic pathogen or a herbivore, systemin is released from its precursor prosystemin and perceived by specific membrane receptors (SYR1/2) triggering a signalling cascade that induces jasmonic acid-dependent responses. It is possible to enhance plant resistance by using beneficial microorganisms such as Arbuscular Mycorrhiza Fungi. This process is known as Mycorrhiza Induced Resistance (MIR). Previous studies showed that mycorrhizal tomato plants perceive faster *Botrytis cinerea* infection through JA-dependent responses. In this study, to understand why mycorrhizal tomato plants are more sensitive to *B. cinerea* infection, we studied the perception of systemin in tomato mycorrhizal plants, since both, systemin and MIR, involve JA responses. Mycorrhizal plants displayed higher basal levels of systemin compared to control plants. We also observed a priming profile in SYR1/2 gene expression. Finally, SYR1/2-silenced mycorrhizal plants displayed higher disease symptoms, indicating that MIR is mediated by systemin.

P7.1-014

DETECTION OF GENES FOR RESISTANCE TO THE WHEAT BLAST FUNGUS IN OATS AND THEIR CORRESPONDING AVIRULENCE GENES.

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Text

Wheat blast caused by the *Triticum* pathotype of *Pyricularia oryzae* is now spreading from South America to Asia and Africa. To control this devastating disease, it is crucial to identify resistance genes, their corresponding avirulence (AVR) genes, and monitor their dynamics. We focused on oat (*Avena sativa*) as a potential source of wheat blast resistance genes. The avirulence of *Triticum* isolate Br48 on oat is controlled by a single gene designated as *PAT1*. Br48ΔPAT1, a *PAT1* disruptant, showed virulence on most of oat cultivars. However, two oat cultivars, Red Algeria (Ot-5) and Winter Culberson (Ot-22), were still resistant to Br48ΔPAT1. These resistant cultivars were crossed with susceptible cultivars, PI173579 (Ot-25) and Byzantina 11 (Ot-28), respectively. Infection assays of these F_2 seedlings with Br48ΔPAT1 suggested that each resistant cultivar carries a single resistance (R) gene. To identify AVR genes corresponding to these R genes, we screened F_1 progeny derived from Br48ΔPAT1 x Br58 (*Avena* isolate) on Ot-5 and Ot-22. Although both Br48ΔPAT1 and Br58 were avirulent on Ot-5, one fourth of the F_1 hybrids showed virulence, suggesting that each parent carry an AVR gene against Ot-5 at different loci. Similar results were obtained on Ot-22. In addition, F_1 hybrids avirulent on both, avirulent on Ot-5 alone, avirulent on Ot-22 alone, and virulent on both segregated in a 9:3:3:1 ratio, suggesting that the AVR genes against Ot-5 and Ot-22 are inherited independently.

P7.1-015

BASIDIOMYCETES IN ESCA COMPLEX OF DISEASES: PHENOTYPICAL CHARACTERISTICS AND DEGRADATION CAPABILITIES WITH A FOCUS ON THE NON-ENZYMATIC PATHWAY

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Text

The Esca complex of diseases (ECD) is a widespread threat to viticultural panorama worldwide, with varying degrees of severity depending on location. Despite its economic importance, there is still a lack of understanding of the connection between the presence of fungal pathogens in the trunk and the development of typical leaf symptoms. The associated ascomycetes *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* are found worldwide, while the wood rot basidiomycete species vary according to geographical location. The degradation ability of the European white rot agent *Fomitiporia mediterranea* (Fmed) has been well studied, and recent research has shown that Fmed can trigger both enzymatic and non-enzymatic mechanisms to degrade lignocellulose. However, the phenotypical characteristics and degradation abilities of other grapevines white-rot agents besides Fmed are largely unknown. To fill this gap of knowledge, this study aimed to: i) a phenotypical characterization of the different species, and ii) screen all the major steps of the non-enzymatic radical-generating pathway among the *Vitis*-white rot agents (namely, the ability to acidify the microenvironment, produce Fe^{3+}-reducing compounds, ultimately leading to hydroxy radicals production through redox cycling). The preliminary results indicate

differences both in growth and wood degradation abilities among ECD basidiomycetes, encouraging further research in this direction.

P7.1-016

ATTENUATED ISOLATE GIBELLULOPSIS NIGRESCENS VN-1 ENHANCES RESISTANCE AGAINST VERTICILLIUM DAHLIAE IN POTATO

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Text

Abstract: *Verticillium* wilt in potatoes caused by *Verticillium dahliae* is a devastating disease that is difficult to control. To identify potential avenues for disease control, the pathogenicity of 72 *V. dahliae* isolates was tested here. We also tested the resistance to the most virulent isolate (Vd-36) induced by the attenuated isolate *Gibellulopsis nigrescens* Vn-1. Induction of *Verticillium* wilt resistance was strongest when using attenuated isolate Vn-1 to inoculate potatoes with a spore suspension concentration of 1×10^6 conidia mL^{-1}, followed by infection with isolate Vd-36 at 5 d intervals. And reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) were produced and accumulated in potato leaves 12 h post-inoculation. The changes in respective defense enzymes, except phenylalanine ammonia-lyase, were consistent with the changes in ROS and H_2O_2 levels. Furthermore, the content of salicylic acid (SA) in inoculated plants was higher than the control, and biosynthesis-related genes StNPR1, StPR1b, StPR2, StPR5 were activated. However, there was no significant difference in the jasmonic acid and ethylene (JA/ET) content between the treatment and control groups. These results demonstrated that the attenuated isolate Vn-1 enhanced resistance to *Verticillium* wilt by inducing the SA signalling pathway and weakly activating the JA/ET signalling pathways in potatoes.

Keywords: *Gibellulopsis nigrescens* Vn-1; *Verticillium dahliae* Vd-36; *Verticillium* wilt; induced resistance;

P7.1-017

DETECTING GENETIC SOURCES OF RESISTANCE TO CHOCOLATE SPOT IN FABA BEAN: DETACHED-LEAF SCREENING OF A DIVERSE POPULATION.

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Text

Protein crops, like faba beans, are an inexpensive, readily available source of protein, complex carbohydrates, vitamins and minerals, making them a valuable food and feed

commodity. Furthermore, faba bean is an excellent break crop in rotations, and like other legumes, has the potential to reduce N-requirements in the following cereal crop. However, yield instability, associated with abiotic and biotic stresses, has reduced the crops' acceptance by farmers and the production area in Europe remains small.
Chocolate spot (CS) is one of the major diseases compromising yield stability. This work aimed to develop rapid screening methods to identify genetic sources of CS resistance within a diversity panel of 220 faba bean lines (ProFaba), which have been yield-tested in European field conditions.
We successfully established a rapid screening assay for CS using detached leaf assays. Plant material grown in controlled conditions (4-6 weeks) was infected with an Irish isolate of *Botrytis fabae*, using a multiple droplet technique. Lesion development was recorded every 24h, for a period of five days post inoculation, with a digital camera. Lesion area was estimated using image analysis software APS Assess, generating a quantitative phenotype dataset, which was then used in GWAS analysis. This methodology allowed early stage selection of highly susceptible lines as well as tolerant lines. Scoring of intermediate resistance levels required whole plant screening in glasshouse conditions.

P7.1-020

TRANSCRIPTOMIC AND FUNCTIONAL APPROACHES TO STUDY HOST-PATHOGEN INTERACTIONS UNDERLYING DUTCH ELM DISEASE

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Text
Populations of American elm (*Ulmus americana*) were greatly impacted by successive pandemics of Dutch elm disease (DED) caused by *Ophiostoma ulmi* and *O. novo-ulmi*. In order to unravel *Ulmus*-*Ophiostoma* interactions, we inoculated *U. americana* saplings with strains representing the moderately virulent *O. ulmi*, three genetic lineages of the highly virulent *O. novo-ulmi*, the related pathogen *O. himal-ulmi*, and the saprobe *O. quercus*, and analyzed plant and fungal transcriptomes at days 3 and 10 post infection. Differential expression analyses of 8640 *Ophiostoma* genes and over 23000 *U. americana* genes showed that gene expression in both organisms differed depending on the strain inoculated and the length of infection. Genes overexpressed in the more virulent strains of *Ophiostoma* included genes that encode hydrolases that may act synergistically, as well as genes for putative cytoplasmic effectors. Elms genes overexpressed in response to the most virulent strains of *Ophiostoma* were linked to the synthesis of secondary metabolites and to the degradation of xenobiotics. Based on the results of fungal transcripts analysis *in planta*, we produced a first set of CRISPR-*Cas9* targeted deletion mutants for 23 *Ophiostoma* candidate pathogenicity genes and are currently phenotyping *O. ulmi* and *O. novo-ulmi* mutants *in vitro* and *in planta*. Mutants analyzed so far include weakly virulent, virulent, and hypervirulent individuals which will help improve our understanding of DED.

P7.1-021

CHROMOSOME-LEVEL GENOME RESOURCE FOR CACAO IDENTIFIES THE GENETIC BASIS FOR RESISTANCE TO VASCULAR STREAK DIEBACK

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Text

Vascular Streak Dieback (VSD) of cacao is caused by the fungal pathogen Ceratobasidium theobromae. The pathogen is an obligate parasite that causes significant crop losses in Southeast Asia and the Pacific and presents a serious biosecurity threat to cacao farming in West Africa and Latin America. VSD resistance has been shown to be highly heritable and durable, with evidence for both qualitative and quantitative resistance. QTLs associated with resistance have been mapped and confirmed on chromosomes 8 and 9 using a population derived from a cross between the cacao genotypes S1 (VSD resistant) and CCN51 (VSD susceptible). Interestingly, one of these QTLs co-localized with a chromosomal region identified for Phytophthora Pod Rot (PPR) and for Frosty Pod Rot (FP) resistance. We built new genome resources for a susceptible progeny and conducted fine scale investigation of these genomic regions to determine the resistance gene complements inherited from both parents. Our investigations provide insights into resistance to VSD and potentially other serious diseases of cacao.

P7.1-022

MOLECULAR INSIGHTS INTO HIGH-TEMPERATURE SEEDLING PLANT RESISTANCE AGAINST PUCCINIA STRIIFORMIS F. SP. TRITICI IN XIAOYAN 6 WHEAT CULTIVAR

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Text

Wheat stripe rust, caused by the Puccinia striiformis f. sp. tritici (*Pst*), is a widespread disease that poses a significant threat to wheat production. Xiaoyan 6, a wheat cultivar conferring high-temperature seedling plant (HTSP) resistance, which is non-race-specific and durable, has maintained consistently resistant to *Pst* for more than 40 years in China, making it the promising candidate for elucidating the genetic basis of the resistance. In our study, we conducted transcriptome sequencing on the *Pst*-infected Xiaoyan 6 seedlings under different temperature conditions and identified 1395 differentially expressed genes (DEGs). Among these DEGs, receptor-like kinase (RLK) genes, *TaXa21*, *TaCRK10*, *TaSERK1* and *TaRIPK*, were identified to serve as the sensors for *Pst* infection and high temperature, which activate a series of defense responses through phosphorylation. In additional to the RLKs,

transcriptional factors *TaWRKY70*, *TaWRKY62* and *TaWRKY45* were involved in defense responses, which might receive the signal from phosphorylated RLKs to regulate the expression of related resistance genes. The resistance genes *TaRPS2* and *TaRPM1* were also found to positively associated with HTSP resistance, as evidenced by accumulation of reactive oxygen species and number of necrotic cells when exposing to *Pst* under high temperature. The insights gained from these results could advance our understanding of the HTSP mechanism, and potentially assist in enhancing and utilizing the resistance.

P7.1-023

EVOLUTION OF THE WHEAT BLAST FUNGUS THROUGH STEPWISE LOSSES OF FUNCTION OF AVIRULENCE GENES PARTIALLY ACCOMPANIED BY INTER-CHROMOSOMAL TRANSLOCATIONS

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Text

Pyricularia oryzae is composed of host genus-specific subgroups such as *Oryza* pathotype (PoO), *Setaria* pathotype (PoS), *Eleusine* pathotype (PoE), *Lolium* pathotype (PoL), *Avena* pathotype (PoA) and *Triticum* pathotype (PoT), which are exclusively pathogenic to rice, foxtail millet, finger millet, perennial ryegrass, oat, and wheat, respectively. This host specificity at the genus level is conditioned by gene-for-gene interactions. We previously cloned *PWT3* and *PWT4* conditioning avirulence of Br58 (a PoA isolate) on common wheat and *PWT6* conditioning avirulence of MZ5-1-6 (a PoE isolate) on common wheat. In the present study, we cloned an avirulence gene from Br58 through the map-based method and designated it as *PWT7*. *PWT7* homologs were widely distributed in PoE and PoL isolates and infrequently in PoS, but completely absent in PoT isolates. The *PWT7* homolog found in PoE was one of the five genes involved in its avirulence on wheat. Phylogenetic relationships and distribution of *PWT7*, *PWT6*, and *PWT3* in *P. oryzae* suggested that, in the course of parasitic specialization toward PoT, a common ancestor of PoE, PoA, PoL, and PoT first lost *PWT6*, secondly *PWT7*, and finally the function of *PWT3* through recombination events. *PWT7* or its homologs were located on core chromosomes in PoS and PoE isolates but on supernumerary chromosomes in PoA and PoL isolates. This is an example of inter-chromosomal translocations of effector genes between core and supernumerary chromosomes.

P7.1-024

DETECTION OF RMG8, A GENE FOR RESISTANCE TO THE WHEAT BLAST FUNGUS, IN AEGILOPS UMBELLULATA

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Text
Wheat blast, caused by *Pyricularia oryzae* pathotype *Triticum* (PoT), first emerged in Brazil in 1985 and has recently spread to Asia and Africa. Previously, we identified a resistance gene in common wheat cultivar S-615, and designated it as *Rmg8*. We also identified and cloned its corresponding avirulence gene, *AVR-Rmg8*. Subsequently, we screened 520 worldwide local landraces of common wheat with Br48 (a PoT isolate), and found 18 resistant lines carrying *Rmg8*. In the present study, we screened *Aegilops* spp., ancestral wild species of wheat, and found that 27 out of 201 accessions of *Ae. umbellulata* were resistant to Br48. Interestingly, all resistant accessions were susceptible to Br48ΔA8, an *AVR-Rmg8* disruptant of Br48, but resistant to Br48ΔA8+eI, a transformant of Br48ΔA8 carrying the eI type of *AVR-Rmg8*. This indicated that all resistant accessions of *Ae. umbellulata* recognize *AVR-Rmg8*. In F_2 populations derived from their crosses with susceptible accessions, resistant and susceptible individuals segregated in 3:1 ratios, suggesting that a single major gene is involved in the resistance. On the other hand, crosses among resistant accessions yielded no susceptible individuals, suggesting that all resistant accessions tested share the same resistance gene. This gene should be *Rmg8* (or its homolog) because it recognizes *AVR-Rmg8*. These results suggest that *Rmg8* originated in Triticeae tribe before the differentiation of *Triticum* and *Aegilops*.

P7.1-025

TERPENOIDS ARE INVOLVED IN EXPRESSION OF SYSTEMIC INDUCED RESISTANCE IN AUSTRIAN PINE

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Text
Phenolics are known to play a critical role in systemic induced resistance (SIR) of Austrian pine to the tip blight and canker pathogen Diplodia pinea. Here, we explored the role of terpenoids in this phenomenon at a very early stage in the interaction. We induced Austrian pine saplings by either wounding or inoculating the lower stems with D. pinea. The seedlings were then challenged after 12 h, 72 h, or 10 d with D. pinea 15 cm above the induction. Lesion lengths and terpenoids were quantified at both induction and challenge locations. Key terpenoids were assayed for antifungal activity in in vitro bioassays. SIR increased over time and was correlated with inducibility of several compounds. α-Pinene and a cluster of b-pinene, limonene, benzaldehyde, dodecanol, and n-dodecyl acrylate were positively correlated with SIR and were fungistatic in vitro, while other compounds were negatively correlated SIR and served as a carbon source for D. pinea. This study shows that, overall, terpenoids are involved in SIR in this system, but their role is nuanced, depending on the type of induction and time of incubation. We hypothesize that some, such as a-pinene, could serve in SIR signaling.

P7.1-026

UNCOVERING FUNGAL MICROBIOME ASSOCIATED WITH WINTER PEA NODULES

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Text

Rhizobia are not the only inhabitants in legume nodules. Other nodule-occupying bacteria are known to originate from the soil and interact with the nodule microbiome to affect N fixation and fitness of the host plant. However, little is known about the composition of the nodule-occupying fungal community and its role in establishment and maintenance of effective rhizobium-legume symbiosis. In this study we assessed the composition of the fungal root and nodule microbiome associated with 4 winter pea (Pisum sativum L.) cultivars across several fields in Washington state. Our data indicated that field location was the strongest factor affecting community structure (R2=0.15). Additionally, tissue type and cultivar were also significant factors affecting fungal microbiome. When the community from individual fields were analyzed separately, ~16% and ˜15% of fungal community variation was explained by tissue type and cultivar, respectively. Several fungal genera, including Didymella andFunneliformisI, were differentially represented between the root and nodule microbiomes or between cultivars. The nodule microbiome exhibited less Shannon diversity than the root microbiome. This data indicate that a fungal community is part of the complex interaction between a N-fixing legume-host and the soil microbiome and its role in establishment of effective symbiosis should be further investigated. The cultivar genotype should be also considered as a factor affecting legume-fungi interaction.

P7.1-027

RE-EMERGENCE OF A PATHOGEN: SEQUENTIAL BREAKDOWN OF CF-9, THE MOST COMMONLY DEPLOYED LEAF MOULD RESISTANCE LOCUS IN COMMERCIALLY CULTIVATED TOMATO BY FULVIA FULVA (SYN. CLADOSPORIUM FULVUM)

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Text

Leaf mould is a serious disease of tomato plants and is caused by the fungus *F. fulva*. Resistant tomato varieties can recognize specific *F. fulva* effectors by means of receptors called Cf proteins, resulting in a hypersensitive response. *Cf-9*, which recognizes F. fulva effector *Avr9*, is currently the most widely deployed resistance gene against *F. fulva* and is part of a locus harbouring five homologous genes, of which two are known to contribute to resistance. *Cf-9* itself confers resistance in all stages of growth, whilst its close homolog *Cf-9B* confers resistance to adult plants only. In the last decade, *F. fulva* strains have emerged that break the resistance conferred by both *Cf-9* and *Cf-9B*. It was previously determined that *Avr9* is deleted in these strains, however the identity of *Avr9B* had remained elusive so far. In this study, we identified two major candidates for *Avr9B* by means of a comparative genomics approach, and by means of transient expression and gene complementation assays we identified one of these to be the true *Avr9B* gene. Next, we assessed the allelic variation of *Avr9B* in a large selection of *F. fulva* isolates and found a striking correlation between deleterious mutations in this gene, and the ability to overcome *Cf-9B*-mediated resistance. This work is exemplary for the way in which plant pathogens can evolve to sequentially break down the resistance conferred by an introgressed resistance locus carrying multiple functional resistance genes.

P7.1-028

PATHOGENICITY OF FUSARIUM GRAMINEARUM AND F. POAE CAUSING FUSARIUM HEAD BLIGHT IN BARLEY UNDER CONTROLLED CONDITIONS

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Text

Fusarium head blight (FHB) is one of the most devastating diseases of barley. FHB is caused by a species complex of Fusaria, of which Fusarium graminearum Schwabe is the species responsible for most FHB epidemics. Field surveys show that two or more Fusarium species often co-exist within the same field and F. poae is as another dominant species in barley. This study investigated the effect of the interactions between F. graminearum and F. poae on FHB and mycotoxin accumulation. Two susceptible barley genotypes were spray-inoculated with Fusarium conidiospore suspensions and the disease severity and fungal accumulation was evaluated based on symptom and genomic DNA. There was a significant difference in FHB severity between F. graminearum and F. poae infections, where F. graminearum produced severe FHB disease symptoms while F. poae did not cause FHB. When heads were co-inoculated with both Fusarium species, the resulting FHB severity was unchanged relative to heads inoculated with F. graminearum which was reflected in the DNA quantification of the species. The mycotoxin profile of the co-inoculated treatment appeared to be most influenced by F. graminearum-related metabolites with a minor influence by F. poae-related metabolites. Forty-six features were annotated with metabolite study and which shows F. graminearum appears to outcompete F. poae in its ability to establish infection in barley and as a result contributes the majority of mycotoxin contamination within this crop.

P7.1-029

IDENTIFICATION AND CHARACTERIZATION OF A LARGE MULTIGENE FAMILY OF COOPERATING EFFECTOR GENES FACILITATING CELL-TO-CELL MOBILITY CONSERVED IN DOTHIDEOMYCETES AND SORDARIOMYCETES

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Text

With the increasing availability of high-quality fungal genomes, effectors conserved among species and genera have been uncovered. Two avirulence effectors, AvrLm10A and AvrLm10B, of Leptosphaeria maculans, responsible for stem canker of oilseed rape, are members of such a large family of conserved effectors. AvrLm10A and AvrLm10B are neighboring genes in divergent transcriptional orientation. Sequence searches within the L. maculans genome showed that AvrLm10A/AvrLm10B belong to a multigene family comprising five pairs of genes with similar tail-to-tail organization, specifically expressed during biotrophic stages of infection. Two of the corresponding protein pairs, including AvrLm10A and AvrLm10B, have the ability to physically interact. AvrLm10A homologues were identified in more than 30 Dothideomycete and Sordariomycete plant-pathogenic fungi. One of them, SIX5, is an effector from Fusarium oxysporum f.sp. lycopersici (Fol) physically interacting with the avirulence effector Avr2 and required for the movement of Avr2 from cell-to-cell through plasmodesmata. We demonstrated that members of the AvrLm10A family in L. maculans can complement SIX5 function in plant cell-to-cell mobility assays and Fol virulence. We found that AvrLm10A/SIX5 homologues were associated with at least eight distinct effector families, suggesting an ability to cooperate with different effectors. These results point to a general role of the AvrLm10A/SIX5 proteins as “cooperator proteins”.

P7.1-030

VOLATILE ORGANIC COMPOUNDS AS BIOMARKER TO ASSESS THE PHENOTYPE OF SUSCEPTIBLE AND RESISTANT APPLE CULTIVARS

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Text

Plants of varying genotypes, species, and taxa exhibit unique patterns of volatile organic compound (VOC) emissions, either constitutive or induced. However, the functional correlation between genotypes and phenotypes is not well understood, especially in plant-environment interaction studies. This research aimed to investigate the correlation between genotypes and VOC emissions in apple cultivars to identify potential pheno(chemo)typic

markers for differentiation between origins and provenances. The study analyzed ten different apple cultivars for their VOC emissions using thermal desorption-GC-MS and assessed their potential role in disease resistance. Results showed that resistant cultivars (Florina, Firdous, liberty) emitted more sesquiterpenes than susceptible cultivars and contained distinct markers such as 3-hexanoic acid and benzoic acid-methyl ester. Further investigation revealed that 3-Hexenoic acid exhibited potent antifungal activity against *Venturia inaequalis*, a common apple pathogen, and could serve as a promising alternative to traditional fungicides. The use of 3-Hexenoic acid as a biomarker may help distinguish between susceptible and resistant cultivars, allowing for more targeted and efficient breeding programs. These findings highlight the potential of VOC analysis as a valuable tool for identifying plant genotypes that exhibit desirable traits in terms of pathogen resistance and other key factors.

P7.1-032

SIGNIFICANCE OF WHEAT RESISTANCE GENE(S) LOCATED ON CHROMOSOME 1DS AS A HOST BARRIER TO NON-ADAPTED PATHOTYPES OF PYRICULARIA ORYZAE

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Text

Pyricularia oryzae, a causal agent of blast disease on various gramineous plants, is composed of pathotypes with host specificity at the plant genus level. The avirulence of an *Eleusine* pathotype (specific to finger millet) on common wheat is controlled by at least five genes. Here we report map-based cloning of the fourth avirulence gene, *PWT8*, and identification of its corresponding resistance gene, *Rwt8*. An F_1 culture derived from MZ5-1-6 (*Eleusine* isolate) x Br48 (*Triticum* isolate) was backcrossed with Br48 twice. When resulting BC_2F_1 cultures were sprayed onto wheat cultivar Norin 4 (N4), avirulent and virulent cultures segregated in a 1:1 ratio, suggesting that a single gene condition the avirulence on it. Fosmid clones and their subclones were introduced into Br48. Complementation tests revealed that one predicted ORF on chromosome 4 of MZ5-1-6 conditions avirulence on N4. We designated this gene as *PWT8*. Segregation analyses of F_2 seedlings with Br48+PWT8 (a transformant of Br48 carrying *PWT8*) indicated that N4 carries a single resistance gene corresponding to *PWT8*. This gene was designated as *Rwt8*. Interestingly, a result of molecular mapping with 93 F_3 lines derived from N4×Hope showed that *Rwt8* was closely linked to *Rwt3* and *Rwt6*, previously reported genes involved in the incompatibility between common wheat and MZ5-1-6. There is a possibility that one resistance gene involved in the pathotype- genus specificity corresponds to three different avirulence genes.

P7.1-033

INDUCTION OF GINSENOSIDES USING FUNGAL ENDOPHYTES ISOLATED FROM MOUNTAIN-SIMULATED GINSENG

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Text

As a well-known traditional herbal medicine, ginseng has various pharmacological effects. Ginsenoside, which belongs to the group of triterpenoid saponin, is one of the bioactive compounds of ginseng. Endophytes exist inside of plant tissues and they have harmonious symbiotic relationships with their host plants. Various Trichoderma species have been discovered as one of many endophytic fungi that could increase host plants resistance, and promote plant growth and secondary metabolites of their host plants through various modes of action. Our preliminary study shows that formulated Trichoderma isolates are successfully used as biological control agents, bio-fertilizers, and bio-stimulants (elicitors). We also observe various changes in molecular and metabolic processes after treatment of the fungal endophyte, Trichoderma, isolated from mountain-simulated ginseng. One of our goals is to investigate the role of total metabolite produced by Trichoderma as an elicitor for ginsenoside production. To achieve the objective, it is required to assess the optimal growth condition of the fungal endophyte (carbon / nitrogen sources, temperature, and shaking condition). The extracted total metabolite will be formulated and the effect of ginsenoside induction will be analyzed after treatment into the mountain-simulated ginseng.

P7.1-034

DEVELOPMENT OF A NOVEL ASSAY FOR THE STUDY OF HYPHAL CONSTRICTION AND ITS GENETIC BASIS IN THE RICE BLAST FUNGUS, MAGNAPORTHE ORYZAE

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Text

Ability to constrict hyphae is essential for go through complex structures in the soil, fungal development, and pathogenesis. The hemi-biotrophic rice pathogen, Magnaporthe oryzae is one of the most socio-economically important crop diseases in the world. During host infection, successful colonization is dependent on the ability of the fungus to move to the adjacent cells through pit field by undergoing severe hyphal constriction up to ~ 0.5 μm. We developed a method to evaluate hyphal constriction ability and investigated its genetic basis in the rice blast fungus. We demonstrated nitrocellulose membranes can be used to test hyphal constriction by monitoring whether the fungus pass through the membrane and form a colony on the underlying medium. Electron microscopy confirmed the passage of M. oryzae is not due to degradation of the membrane but the constriction of hyphae. Assays using mutant lines showed MAP kinase pmk1 mutant, which was shown in previous study to be required for cell-to-cell movement, cannot pass through the membrane. Furthermore, we found M. oryzae sirtuin, MoSir2 is required for the efficient passage of hyphae through the

membrane. Sheath assay for Mosir2 mutant confirmed that cell-to-cell movement of the mutant was not as efficient as that of the wild type. RNA-seq experiments using our assay gives insight into the genomic basis of hyphal constriction. We anticipate that our work would provide a model for studying genetic mechanisms and the evolution of hyphal constriction.

P7.1-035

STUDY OF NUCLEOLAR DYNAMICS USING A NUCLEOLAR MARKER MONOP1 IN THE RICE BLAST FUNGUS, MAGNAPORTHE ORYZAE

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Text

The nucleolus has emerged as a central hub for nuclear functions in eukaryotic cells. Recent studies in mammals and the model yeast Saccharomyces cerevisiae showed that the nucleolus is essential for nuclear functions including but not limited to ribosome biogenesis. It has been well established that nucleolar functions such as rDNA transcription and subsequent ribosome assembly positively correlate with the size of the nucleolus. However, little is known about the nucleolus of filamentous fungi. Here, we investigated in the plant pathogenic filamentous fungus, Magnaporthe oryzae, changes of nucleoli in response to nutrient availability and infection-related development by monitoring localization of the nucleolar marker protein, MoNOP1 tagged with red fluorescent protein (RFP). This showed that nucleoli of M. oryzae remain constant in size, while nuclei shrank under low nutrient availability. Our observations were further supported by 1) no significant difference in steady-state level of rRNA between nutrient-rich and -poor media and 2) down-regulation of key genes involved in nuclear membrane synthesis. Nucleoli in conidia monitored by the signal of RFP were not very active but became active during appressorium differentiation and maturation. We propose that such distinctive nucleolar dynamics may reflect the strategy of filamentous fungi under low nutrient availability to forage for food resources as well as roles of the nucleolus during fungal development.

P7.1-036

JMJD2 GENE ENCODING A HISTONE DEMETHYLASE IS REQUIRED FOR FUNGAL DEVELOPMENT AND PATHOGENICITY THROUGH TRANSCRIPTIONAL REGULATION OF RIBOSOMAL DNA AND NUCLEAR GENES IN THE RICE BLAST FUNGUS

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Text

Histone demethylases play crucial roles in transcription regulation and genome integrity by regulating histone methylation status via interplay with histone methyltransferase. Histone lysine demethylases have been classified into two families, which are JmjC (Jumonji C) and LSD (Lysine Specific Demethylase). Here we report that a JmjC domain-containing histone demethylase, MoJMJD2 regulates transcription of both rDNA loci in nucleolus and a set of nuclear genes, which are development and pathogenesis related genes, via H3K9 and H3K36 demethylation in *Magnaporthe oryzae*. We showed that alternative splicing in *MoJMJD2*, which appears to be generated in an environment-dependent manner generates a protein isoform lacking a putative nucleolar localization sequence. Deletion of *MoJMJD2* led to defects in vegetative growth, asexual reproduction, pigmentation, penetration, invasive growth and cell wall synthesis, and re-introduction of wild-type copy of gene into the mutant was able to complement all these defects. Moreover, rDNA and some of the key conidiogenesis, melanin synthesis and cell wall synthesis genes were directly regulated by MoJMJD2-mediated histone demethylation. Taken together, we propose that MoJMJD2 coordinates transcriptional regulation of rDNA in nucleolus and nuclear genes via histone demethylase activity and alternative splicing, linking ribosome biogenesis to transcription of nuclear genes in response to environmental cues.

P7.1-037

INVESTIGATING THE CELL BIOLOGY OF PLANT INFECTION BY THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE

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Text

Rice blast disease, caused by the filamentous, ascomycete fungus, *Magnaporthe oryzae*. is one of the most important limiting factors in rice production. Infection starts when a conidium lands on the leaf surface, carried by wind or splash dispersal. To enter the rice leaf, the fungus forms a dome-shaped infection structure called an appressorium. The melanized appressorium develops a penetration peg, which differentiates into a narrow primary invasive hypha (IH) and subsequently into bulbous invasive hyphae that colonize the first host cell. Previous live-cell imaging studies have demonstrated that IH are surrounded by the extra-invasive hyphal membrane (EIHM), an invagination of the host plasma membrane, which bounds IH as they proliferate in epidermal rice cells. We have generated a series of rice (cv Kitaake) transgenic lines expressing green fluorescent protein (GFP)-labelled subcellular components, including the plasma membrane, endoplasmic reticulum (ER), Golgi, nuclei, F-actin, microtubules, early and late endosomes. Using these transgenic lines, we aim to characterize the temporal development of blast disease and create a spatial atlas of the major cellular changes associated with blast infection, in both compatible and incompatible interactions. We have classified major changes in plasma membrane and F-actin organization and integrity during invasive growth. We will report the major plant cellular changes that accompany host tissue colonization by *M. oryzae*.

P7.1-039

A NUCLEUS-TARGETING EFFECTOR OF STRIPE RUST DISTURBS THE PLANT PHASE SEPARATION TO MANIPULATE HOST IMMUNITY

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Text

Phytopathogens produce a large number of host intracellular effectors to suppress host immune responses by targeting diverse cell organelles, such as nucleus. And nucleus are vital during the growth and immunity of plants. Here, we identified that Hasp170, an early-induced nucleus-targeting effector secreted from the wheat stripe rust pathogen, Puccinia striiformis f. sp. tritici (Pst), could suppress plant immunity. As a virulence factor of Pst, silencing of Hasp170 markedly reduced Pst growth and development. Hasp170 interacted with a novel wheat liquid-liquid phase-separated protein TaMad1. The intrinsically disordered region 1 (IDR1), which was required for driving phase separation of TaMad1, played a crucial role in TaMad1-mediated wheat resistance to Pst. Hasp170 could bind IDR1 domain to disturb the phase-separated bodies formation of TaMad1 in the nucleus and interference the host immunity. Therefore, our data demonstrate that the nucleus-targeting effector Hasp170 suppresses host immunity by disturbing the liquid-liquid phase separation of TaMad1, thereby enhancing the pathogenicity of Pst to wheat.

P7.1-040

THREE AVRPM60D CANDIDATE GENES HAVE BEEN ACHIEVED FROM THE HIGH-DENSITY LINKAGE MAPPING METHOD

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Text

Wheat powdery mildew, caused by Blumeria graminis f.sp. tritici (Bgt), poses a serious threat to wheat production worldwide. Monitoring the virulence and diversity of Bgt population can guide the spatio-temporal deployment of R genes in the field. The genetic mapping and cloning of avirulence gene (Avr gene) is the prerequisite and foundation of monitoring Bgt population, and is critical for understanding pathogenesis of Bgt as well as interaction between Bgt and wheat. In this study, Isolates E21 and HB-24 was virulent and avirulent respectively on differentials carrying Pm60d. A cross was performed between the two strains, and a suitable mapping population of 128 progenies was produced. Using denovo sequenceing Bgt isolate 96224 from Switzerland as the reference genome, We performed whole genome re-sequencing of these 128 progenies and generated 20766 SNPs markers to construct the high-density linkage map in 11 chromosomes. Combined with the gene annotation results from reference genome 96224?we identified three candidate genes for AvrPm60d. The construction of high-density linkage map provides a useful platform for genetic mapping of all other Avr genes of Bgt if the parent isolates E21 and HB-24 have

significance difference in the differentials carrying corresponding powdery mildew resistance genes, and it will help us to clone these Avr genes.

P7.1-041

NOVEL TRICHODERMA ASPERELLOIDES-NT33 ALLEVIATE WATER DEFICIT STRESS IN SUSCEPTIBLE TOMATO GENOTYPES

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Text

Water deficit stress (WDS) is the most destructive abiotic stress limiting global crop productivity. The application of growth-promoting fungi Trichoderma has been established as a sustainable tool to mitigate the negative impact of WDS. However, the mechanism by which Trichoderma inoculation impacts WDS in tomatoes remains unknown. In addition, plant genotype also influences fungal colonization and stress-mitigating potential. This work evaluates the ability of 43 novel isolates from several species of Trichoderma, primarily native to Nepal, to improve growth in different tomato genotypes. The results demonstrate differences in the ability of Trichoderma isolates to confer tolerance to WDS. Trichoderma asperelloides-NT33, isolated from a dry region, delivered consistent performance advantages in tomato partners. NT33 inoculation improves the physiological, and biochemical parameters in drought susceptible tomato genotype ‘Jaune Flamme’ and ameliorates the negative impact of WDS. Further, transcriptomic analysis was conducted to elucidate the key molecular mechanisms involved in WDS response. The study showed that the metabolic pathways involved in secondary metabolite production and antioxidative defense are the key mechanisms adopted by NT33 inoculated plants for the maintenance of homeostasis balance under WDS. This research delivers insights into mechanisms adopted by novel Trichoderma isolates to increase plant growth and induce defense responses in inoculated plants.

P7.1-042

ANCIENT VARIATION IN AVIRULENCE EFFECTORS UNDERLIES THE RAPID RESISTANCE BREAKDOWN OF TWO INTROGRESSED RYE RESISTANCE GENES IN WHEAT

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Text
Crop wild and domesticated relatives are valuable sources of new resistance gene specificities against fungal plant pathogens. The durability of such resistance gene introgressions is highly variable, a phenomenon that remains poorly understood mainly because the corresponding avirulence effectors are unknown. Until their breakdown, the resistance genes *Pm8* and *Pm17*, located on independent rye to wheat translocations, conferred resistance against the wheat powdery mildew disease caused by *Blumeria graminis* f.sp. *tritici*. We used genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping in mildew to identify the corresponding *AvrPm8* and *AvrPm17* effectors both encoding small, secreted proteins that are highly expressed during the early stages of infection. Diversity analysis in powdery mildew collections from major wheat growing areas as well as related powdery mildew lineages revealed that several gain-of-virulence variants of *AvrPm17* and one variant of *AvrPm8* are likely ancient and predate the introgressions of *Pm17* and *Pm8* from rye to wheat. We concluded that standing genetic variation can underlie rapid resistance breakdown of introgressed resistance genes. Our studies demonstrate the relevance of pathogen-based genetic approaches to understand resistance gene durability. We, therefore, argue that the effort to identify durable resistance genes cannot be dissociated from studies of pathogen avirulence genes.

P7.1-043

THE ECTOMYCORRHIZA FUNGUS PROMOTES GROWTH OF SCOTS PINE SEEDLINGS AND MITIGATES NEGATIVE EFFECTS OF CONIFER ROOT PATHOGEN

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Text
Mycorrhizal fungi always associate with the roots of plants including forest trees, which can improve the nutrient status of their host plants, influencing disease resistance and growth. In this study, it was hypothesized that mycorrhizal fungal inoculation could improve the growth and disease resistance of Scots pine (Pinus sylvestris L.) against Heterobasidion. annosum. Scots pine seedlings were inoculated with ectomycorrhizal fungus (Suillus luteus) prior to H. annosum infection. The result showed that Scots pines with ectomycorrhizal inoculation grew much better than the seedlings without ectomycorrhizal inoculation. Ectomycorrhizal inoculation was able to promote the growth of the primary roots. Transcriptomic changes due to mycorrhiza or pathogen challenge were monitored. The results will be presented and discussed.

P7.1-044

A SECRETED LEUCINE-RICH REPEAT EFFECTOR SUPPRESSES PLANT IMMUNITY

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Text
Z. tritici is a hemibiotrophic pathogen, which has an extended latent growth phase through the wheat leaf (approximately two weeks) before inducing necrotic lesion symptoms. The molecular interactions that occur during Z. tritici infection are poorly understood, and so we are examining effectors with the ability to suppress host-immune responses. Among these, we have identified a secreted leucine-rich repeat (Zt-sLRR) that is highly expressed during the biotrophic phase of infection. We have found that the Zt-sLRR is able to suppress pathogen triggered immunity (PTI) when transiently expressed in Nicotiana benthamiana. Structural prediction shows that the Zt-sLRR is predicted to be a structural homologue of extracellular plant immune receptors. We have also identified various plant plasmid membrane-associated proteins that are known to interact with these plant receptors as also being interaction partners of the Zt-sLRR effector. We believe that the effector is functioning as a mimic for plant leucine-rich proteins and inhibits functionality of the BAK1/FERONIA signaling complex and interacting protein partners.

P7.1-047

PROFILING DEGS POST INOCULATION BY STRIPE RUST (PUCCINIA STRIIFORMIS F. SP. TRITICI) IN WHEAT USING AN RNA-SEQ APPROACH

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Text
With the drastic change in climate, wheat is affected by multiple biotic stresses that decreases its production. Among others, diseases caused by *Puccinia striiformis f. sp. tritici* pathogen affect wheat production annually. Hence, the development of varieties that confer durable resistance/tolerance to rust infections is a sustainable strategy to control the diseases in wheat. Herein, an RNA-seq approach was utilized to profile differentially expressed genes (DEGs) in response to rust infection in a putative stripe rust resistant Senqu variety that is well grown in South Africa. A total of 713 and 417 significant genes were differentially expressed at 14 and 21 days post infection. Gene annotations revealed upregulation of genes associated with defence response, including the disease resistance proteins, LRR domain proteins and transcription factors, among others. Furthermore, gene ontology analysis showed enrichment of upregulated genes under the molecular function

categories. The expression patterns of these genes have also been associated with both environmental stress responses in various crops including Arabidopsis and rice. Based on our results, the variety used in this study may be considered tolerant to stripe rust. However, more analysis should be done to understand the mechanisms underlying the DEGs profiled in response to stripe rust infection in this variety. Overall, our findings will also assist in paving a way for wheat breeding regimes in SA.

P7.1-048

DISTRIBUTION OF WHEAT BLAST RESISTANCE GENES IN TETRAPLOID WHEAT

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Text

Distribution of wheat blast resistance genes in tetraploid wheat
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Wheat blast, caused by *Pyricularia oryzae* pathotype *Triticum* (PoT), is a new threat to the global wheat production. Wheat blast was first reported in Brazil and has recently spread to Bangladesh and Zambia. Previously, we identified two resistance genes in tetraploid wheat cultivar St24 and common wheat cultivar S-615 and designated them as *Rmg7* and *Rmg8*. However, *Rmg8* and *Rmg7* recognize the same avirulence gene, *AVR-Rmg8*, suggesting that these two genes are equivalent to a single gene from the viewpoint of resistance breeding. In the present study, we screened 133 tetraploid wheat accessions (*Triticum turgidum* ssp. *durum* and *T*. *turgidum* ssp. *dicoccoides*) and found that 47 out of 133 accessions were resistant to Br48 (a PoT isolate) at the seedling stage. In the resistant accessions, 38 were susceptible to Br48Δ*A8* (an *AVR-Rmg8* disruptant of Br48), but resistant to Br48Δ*A8*+eI (a transformant of Br48Δ*A8* carrying the eI type of *AVR-Rmg8*), indicating that the majority of the resistant accessions recognized *AVR-Rmg8*. They were inferred to be *Rmg7* carriers. These results suggest that *Rmg7* is widely distributed in tetraploid populations. Alternatively, 9 accessions were resistant to all of the three strains, suggesting that these accessions may carry novel resistance genes to wheat blast.

P7.1-049

USING TETRACYCLINE CONDITIONAL PROMOTORS TO CONTROL GENE EXPRESSION IN BOTRYTIS CINEREA

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Text
In reverse genetic, one issue when studying essential or lethal genes is the difficulty to characterize them unless their expression is finely regulated. The Tetracycline-regulated expression system (widely used in eukaryotes) has the potential to circumvent this difficulty. By using tetracycline (or an analog), it is possible to down-regulate (with the Tet-off system) or to up-regulate (with the Tet-on system) the expression of a gene of interest in a concentration-dependent manner. In this study, we focus on the development of the Tet-on and Tet-off systems for inducible gene expression in the phytopathogenic fungus *Botrytis cinerea*. Using classical molecular approaches, coupled with fluorescence microscopy, we are assessing the Tetracycline system in various biological contexts such as axenic cultures and during plant infection. Altogether, this study will bring a molecular tool that will facilitate the functional characterization of essential and lethal genes involved in the development and pathogenicity of the phytopathogenic fungus.

P7.1-050

METABOLOME REPROGRAMING AND PHENOME CHARACTERIZATION ON BASIL AFTER THE INFECTION BY FUSARIUM AND ITS INTERACTION WITH TRICHODERMA ATROVIRIDE ANTAGONIST UNDER SALINE AND NON-SALINE CONDITIONS

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Text
Sweet basil (Ocimum basilicum L.) is a medicinal and aromatic plant with low tolerance to salinity. This conditions its performance to face biotic stresses. Fusarium wilt is a common disease and the causal agent of the production constraint, because of without any symptoms during the growth. However, no effective biological treatment is available to control the disease. In this study, basil plants were submitted to the infection by Fusarium in the presence or absence of treatment of Trichoderma atroviride AT10, under saline stress or mock condition. The phenotypic changes were recorded using non-destructive methods based on digital imaging. The physiological changes were complemented by metabolomic analysis of the leaf using UHPLC-IM-qTOF. Results highlighted that under saline conditions carboxylic acids, and especially amino acids were depleted in basil. These plants reacted upregulating salicylic acid pathway. Moreover, defense compounds like eugenol were detected in non-saline conditions but diminished after salt treatment. Infected plants in contact with Trichoderma AT10 strain increased the synthesis of tetrapyrroles preventing the loss of photosynthetic pigments. Surprisingly, the addition of Trichoderma AT10 strain to the infected plants showed the increase of salicylic acid and ethyl salicylate in a greater way. Overall, these studies provide technical insight of phenotyping into metabolic level and promise to develop microbial solution to control fungal disease.

P7.1-051

PWL2 MODULATES PAMP-TRIGGERED IMMUNITY THROUGH INTERACTION WITH A HOST ISOPRENYLATED HMA

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Text

Magnaporthe oryzae, causes the most serious disease of cultivated rice . The ability of Magnaporthe isolates to infect weeping lovegrass (Eragrostis curvula) was reported to be controlled by a single gene, PWL2. Our data shows that PWL2 belongs to a gene family and unlike other members in the family, PWL2 is present in all limited host forms and outside M. oryzae species. PWL2 occurs as multiple copies in the rice blast genomes and we use CRISPR/Cas9 to generate Dpwl2 mutants in a M. oryzae strain, which has three copies, resulting in gain of virulence towards weeping lovegrass. Additionally, constitutively expressing PWL2 in transgenic rice and barley lead to suppressed reactive oxygen species (ROS) burst suggesting it is involved in modulating PAMP-triggered immunity. We have also performed co-IP/Mass spectrometry from stable transgenic barley plants expressing Pwl2-YFP to reveal a total of 46 proteins in the Hordeum vulgare (barley) proteome database that associated with Pwl2. One of the putative interactors was identified as an isoprenylated heavy metal binding domain containing protein called Hv-HIPP2. We have generated transgenic barley lines expressing Hv-HIPP2 and shown that they are attenuated in response to PTI like transgenic lines expressing Pwl2. Our study provides evidence that Pwl2 belongs to the MAX-fold group of conserved M. oryzae effectors and plays an important role during the biotrophic phase of infection by suppressing ROS burst.

P7.1-052

GIANT TRANSPOSONS FACILITATE HORIZONTAL GENE TRANSFER OF THE NECROTROPHIC EFFECTOR TOXA IN FUNGAL WHEAT PATHOGENS

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Text

Horizontal gene transfer (HGT) is a tool that many organisms use to rapidly adapt to novel hosts or environments. One well-known example of HGT is the movement of the necrotrophic effector ToxA between three fungal wheat pathogens, *Parastagonospora nodorum, Pyrenophora tritici-repentis* and *Bipolaris sorokiniana*. Defining the extent of horizontally transferred DNA is important because it can define the mechanisms that facilitate

HGT. Our previous analysis of *ToxA* and its surrounding 14 kb showed that this region was a class II DNA transposon we named ToxhAT due to the hAT-like transposase gene near to ToxA. Importantly, there was some evidence that this transposon may remain active and mobile in *B. sorokiniana*. Long-read genome sequencing of eight ToxhAT carrying *B. sorokiniana i*solates confirmed that ToxhAT is an active transposon with a two base-pair "TA" target site duplication. This feature suggests that it should be re-classified as a member of the Tc1/Mariner transposon superfamily. In addition to confirming ToxhAT is an active transposon, these assemblies revealed that ToxhAT was a passenger within a giant transposon (~200kb). This transposon, *Sanctuary I*, has been classified as a giant *Starship* transposon a new transposon family found in fungi. In parallel, the region carrying ToxhAT in *Pyrenophora tritici-repentis* has also been shown to be a mobile Starship, named "Horizon". This indicates two independent captures of the smaller ToxhAT by these large transposons.

P7.1-053

COMPARATIVE TRANSCRIPTOMICS REVEALS TISSUE AND GENOTYPE GENE EXPRESSION PATTERN DIFFERENCES IN NEAR-ISOGENIC TOMATO LINES DIFFERING IN VERTICILLIUM WILT RESISTANCE.

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Text

Verticillium dahliae is a soil-borne pathogen and the causal agent of Verticillium wilt on a broad range of plants, including tomato. To dissect host resistance, we created bi-grafted plants with scions from near-isogenic lines (NILs) of tomato exhibiting (R, Ve1) or lacking (S, ve1) resistance to race 1 of V. dahliae grafted onto rootstocks of both genotypes. Ten days after inoculation (DAI), scion and rootstock tissues were subjected to differential gene expression and co-expression network analyses. Symptoms only developed in susceptible scions regardless of the rootstock. Although relatively few transcripts mapped to V. dahliae, susceptible rootstocks contained more V. dahliae transcripts than resistant tissues. Signal peptides were common in highly expressed genes. The infection resulted in a dramatic alteration of tomato gene expression in resistant and to a greater extent in susceptible tissues, including pathogen receptor, signaling pathway, PR protein, and cell wall modification genes. Differences in gene expression patterns were observed between scions and rootstocks, primarily related to differences in physiological processes in these tissues. A few genes were associated with the Ve1 genotype, which was independent of infection or tissue type. This research provides a fundamental comprehensive insight into tomato responses to V. dahliae infection and helps further elucidate the tissues and mechanisms associated with resistance to this destructive pathogen.

P7.1-054

IDENTIFICATION AND CHARACTERIZATION OF CANDIDATE EFFECTORS IN THE RESISTANCE-BREAKING PATHOTYPE 3A OF PLASMODIOPHORA BRASSICAE

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Text

Clubroot of crucifers, caused by *Plasmodiophora brassicae*, has been detected in more than 80 countries and results in severe yield losses. In Canada, the widespread cultivation of clubroot-resistant canola (*Brassica napus*) cultivars has exerted significant selection pressure on *P. brassicae* populations, leading to the emergence of novel resistance-breaking pathotypes. Transcriptome analysis of the effector repertoire of the resistance-breaking pathotype 3A was conducted at 7, 14, and 21 days after inoculation of resistant and susceptible *B. napus* cultivars. Two highly expressed putative effectors, SPR01261.1 and SPQ99289.1, were chosen for characterization. Each effector's signal peptide was confirmed using a yeast signal sequence trap assay. Both effectors localized to the nucleus and cytoplasm in *Nicotiana benthamiana* when monitored via fluorescent protein tagging. The function of SPR01261.1 and SPQ99289.1 will be assessed using protease and kinase assays, respectively. Experiments are underway to evaluate various heterologous protein expression systems, as *P. brassicae* cannot be cultured axenically since it is an obligate biotroph. The expression, solubility, and activity of each protein will indicate which system is most suitable for expressing *P. brassicae* proteins. Characterization of effectors deployed by the resistance-breaking pathotypes of *P. brassicae* will provide an improved understanding of the molecular interactions between this pathogen and its *B. napus* host.

P7.1-055

CELL WALL ASSOCIATED IMMUNITY AGAINST FUSARIUM OXYSPORUM IN ARABIDOPSIS BAM3 MUTANT

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Text

The plant cell wall (CW), a rigid yet dynamic polysaccharide-protein matrix, is an essential player in plant responses to external stimuli acting as the first physical barrier to outside invaders or stresses and as a source of signals to trigger downstream responses upon perception of incoming threats. CW alteration directly influences stress response pathways, especially during plant reaction to microbes that mainly live in the apoplast, like the vascular pathogenic strains of *Fusarium oxysporum* species complex (*FOSC*) that cause severe disease in more than 100 plant hosts. However, these essential plant CW modification

processes remain largely unknown. The results of the present study showed that the disruption of the gene encoding the *β*-amylase 3 protein (BAM3), the major BAM isoform that degrades starch to maltose, led to increased resistance to *F. oxysporum* f. sp. *raphani* (*For*), since the *bam3* mutant showed the lowest disease incidence and severity and contained smaller amount of fungal DNA in its vascular tissues compared to the wild type (wt) plants. Additionally, our study provides significant insights into the role of the remodeling of cell wall pectin in the regulation of Arabidopsis *bam3* defense against *For*.

P7.1-056

NEW QUANTITATIVE TRAIT LOCI (QTL) FOR RESISTANCE TO TAR SPOT IN MAIZE

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Text

Tar spot is a destructive disease of maize that was first discovered in Central and South America but has spread to the USA and Canada since 2015. During the past several years it has become the most important disease in the Corn Belt of the USA. A major quantitative trait locus (QTL) for resistance to tar spot was identified previously in tropical maize germplasm on chromosome 8 with minor QTL on other chromosomes, but a great need for additional resistance remains. To address this deficiency, progeny from the Nested Association Mapping (NAM) population of maize were selected for phenotypic analysis after a difference was identified between the resistant parent CML52 and the susceptible B73. Phenotyping of 197 recombinant-inbred lines of the CML52 by B73 plus the parents during the 2020 and 2021 growing seasons and analysis with existing molecular markers identified a major QTL on chromosome 9, a moderately strong QTL on chromosome 2 and minor QTL on chromosome 1, 4, 5, 7 and 8. The major QTL on chromosome 9 and many of the minor QTL appear to be novel and potentially provide a new source of resistance against the disease. Additional molecular markers in the region containing the QTL on chromosome 9 are being developed and the region is being screened to identify potential candidate genes. Several other NAM parental lines showed strong resistance to tar spot during field trials in North America and could provide a rich source of materials for future breeding programs.

P7.1-057

TRANSCRIPTOMIC AND METABOLOMIC RESPONSES IN ARABIDOPSIS BAM3 MUTANT TO FUSARIUM OXYSPORUM INFECTION

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Text

The *Fusarium oxysporum* species complex (*FOSC*) includes soilborne plant pathogens that cause destructive vascular wilt diseases in a wide range of economically important crops leading to high economic losses. Different strategies and means are currently being employed in agriculture to combat Fusarium wilts, with resistant host plant varieties being a key, effective, sustainable, economic and environmentally friendly component of an integrated management program. In the present study, an *Arabidopsis thaliana* mutant with disruption of the gene encoding the *β*-amylase 3 protein (BAM3) showed significantly lower susceptibility to *F. oxysporum* f. sp. *raphani* (*For*) compared with the wild type (wt) plants. To understand the molecular networks of interactions in the above pathosystem, transcriptomic and metabolomic analyses were conducted using DNA microarrays and gas chromatography-mass spectrometry (GC-MS). Alterations in starch degradation, carbohydrate and auxin metabolism were found to be components of Arabidopsis *bam*3 defense against *For*. These findings provide an essential source for the development of Fusarium-tolerant germplasm and cultivars.

P7.1-058

IDENTIFICATION AND CHARACTERIZATION OF NOVEL EFFECTOR PROTEINS REGULATED BY THE MAP KINASE PMK1 DURING CELL-TO-CELL MOVEMENT OF THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE

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Text

Rice blast pathogen *Magnaporthe oryzae* secretes a large battery of effectors to inhibit host defence mechanisms. Although effectors are associated to pathogen invasive growth, little is known about their role during cell-to-cell movement. Interestingly, the MAP kinase Pmk1 has been recently shown to regulate effectors when the fungus is moving to the neighbouring cells. In this study, using a comparative proteomics approach, we identified three putative Pmk1-regulated effectors (PREs). PREs are specifically expressed and phosphorylated during transpressorium-mediated cell-to-cell movement. Subcellular localisation of GFP-tagged PRE strains inoculated on rice leaf sheath showed effector expression *in planta* at 26-48 hpi. PRE1 localized at the Biotrophic Interfacial Complex (BIC), while PRE2 and PRE3 were found at the hyphal tip. Preliminary infection assay showed reduced virulence in *pre2* mutant strain compared to the wild-type strain. To identify the host protein targets, barley transgenic lines expressing PREs were generated and subjected to immunoprecipitation-mass spectrometry (IP-MS). Preliminary results of PREs interactomes identified potential host targets related to disease resistance and susceptibility. Furthermore, AlphaFold prediction revealed distinct structures for PREs, with no structural similarities to any known effectors. Overall, this work paves the way for further studies dissecting the functions of cell-to-cell movement effectors during rice blast disease.

P7.1-061

PHOSPHORYLATION LANDSCAPE OF EARLY APPRESSORIUM MORPHOGENESIS REVEALS NOVEL VIRULENCE DETERMINANTS DURING RICE BLAST DISEASE

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Text

Rice blast, caused by Magnaporthe oryzae, is among the most devastating fungal diseases affecting agriculture worldwide. The blast fungus enters plant cells using a specialised dome-shaped structure called an appressorium, which requires the Pmk1 MAP kinase for its morphogenesis. Although the Pmk1 pathway controls phosphorylation changes in the appressorium, little is known about the phosphorylation events on the fungal proteins. Here, we report the phosphorylation landscape of early appressorium morphogenesis in the blast fungus. Using MS1 analysis from a time series study of appressorium samples, we mapped 5,737 phosphosites corresponding to 2,101 proteins. This approach reveals a dramatic rewiring of phosphorylation during early appressorium development. Furthermore, using quantitative phosphoproteomic analysis, we identified 33 putative direct downstream targets of the Pmk1 MAPK during plant infection. One of the targets, named Vts1, is phosphorylated by Pmk1 at two proline-directed sites. Targeted mutation showed that Vts1 is necessary for mycelium growth, sporulation, appressorium development and pathogenicity. Additionally, Vts1 phosphomimetic and phosphodead mutants demonstrated the importance of its phosphorylation. Taken together, our results show how quantitative phosphoproteomics can identify novel regulators, such as Vts1 which are essential for rice blast disease.

MOLECULAR ASPECTS: plant-nematode interactions

C6.3-1

CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN GPA2-MEDIATED EFFECTOR-TRIGGERED IMMUNITY AGAINST POTATO CYST NEMATODES

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Text
Cyst nematodes are considered a dominant threat to yield for a wide range of major food crops. Current control strategies of cyst nematodes mainly dependent on crop rotation and the use of resistant crop cultivars. Various crops exhibit single dominant resistance (R) genes able to activate effective host-specific resistance to certain cyst nematode species and/or populations. An example is the potato R gene Gpa2, which confers resistance against the potato cyst nematode Globodera pallida. Gpa2 encodes an intracellular nucleotide binding-leucine-rich repeat (NB-LRR) immune receptor, which is able to specifically recognize the effector GpRBP-1. However, knowledge about the cellular and molecular mechanisms underlying effector detection and the activation of plant immune responses is still largely unknown. Here, we show that effector detection by Gpa2 occurs in the cytoplasm and that both the nuclear and cytoplasmic pools of Gpa2 contribute to effector-triggered immunity in cell death assays and nematode infection tests on transgenic potato roots. Moreover, a comparative transcriptome study was conducted to uncover differential gene expression patterns upon infection of Gpa2 resistant potato roots using virulent and avirulent nematode populations. This revealed specific DEGs involved in the activation of plant defence pathways linked to calcium binding and metabolic activity, systemic acquired resistance, salicylic acid biosynthesis and immune responses.

C6.3-2

GENOMIC VARIATIONS ASSOCIATED TO MI-1 RESISTANCE BREAKING DOWN BY MELOIDOGYNE INCOGNITA

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Text
Plants possess resistance genes to a variety of pathogens, allowing them to avoid deleterious effects of infection. Some pathogens were able to overcome these barriers during the course of evolution. One known example is the *Mi-1.2* gene from the tomato *Solanum lycopersicum* conferring resistance to the root-knot nematode *Meloidogyne incognita*. Previous studies in our lab have tested the durability of *Mi-1.2* resistance against *M. incognita*. Tomato plants carrying the resistance gene *Mi-1.2* were inoculated with a load of avirulent nematodes. Collecting the initial few offspring and reinoculating on tomato plants bearing Mi-1.2, allowed the establishment of a resistance-breaking subpopulation after 5 to 10 generations. This same experience was carried out with two initially avirulent populations, from Mexico and Russia, both yielding virulent subpopulations in the lab. We sequenced both populations from Mexico and Russia, and both avirulent and virulent phenotypes, to study whether genomic variations accompany resistance breaking down. Using long-read sequencing, we were able to identify not only single-nucleotide polymorphisms, but also structural variants (> 50 bp) exclusively present in the two virulent subpopulations (Mexico and Russia). We also studied the genome expression profiles of these populations, and whether the different genomic variations could influence the expression of candidate virulence / avirulence genes.

C6.3-3

BEECH LEAF DISEASE: AN EMERGENT THREAT TO BEECH FOREST ECOSYSTEMS

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Text

The beech leaf disease nematode, *Litylenchus crenatae mccannii*, is recognized as a new emergent species that causes beech leaf disease (BLD) in beech trees (*Fagus* spp.) in North America. Since the first report of BLD on *F. grandifolia* in Ohio in 2012, the disease has rapidly spread to other states, such as PA, NY, CT, MA, ME, MI, RI, NJ, WV, and VA, as well as Ontario. Leaf symptoms include swelling and darkening of interveinal tissues as well as chlorosis, while tissue necrosis and leaf curling occur at later stages of the disease. As a result, mortality of nematode infected understory beech trees has been reported after several years of infection in the US. To advance our understanding into this new host-nematode system, we investigated the cellular aspects of this interaction using bright-field and scanning electron microscopy. Our data reveal that these nematodes can induce dramatic morphological changes in both leaf and bud tissues. These cellular changes provide the necessary nutrients for completion of the nematode life cycle, while dramatically affecting bud and leaf morphology. In addition, we used Illumina mRNAseq to obtain insight into the transcriptome of this nematode. Spatial expression of transcripts within the esophageal glands of *L. crenatae mccannii* validated a list of novel effector genes to the Nematoda phylum. These analyses provide additional data for the understanding the mode of parasitism of this new emergent plant-parasitic nematode.

C6.3-4

SUGR: THE SUBVENTRAL GLAND “MASTER” REGULATOR OF PLANT-PARASITIC CYST NEMATODES

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Text

Plant-parasitic cyst nematodes have evolved unique and intimate relationships with their host plants. At the core of these interactions are effectors, proteins that enable nematodes to successfully parasitise their host plant. Effector production is restricted to specialised tissues, primarily two sets of gland cells, in the nematode. Investigation of this spatial confinement allowed us to discover the first regulatory mechanism that unifies effector production in the nematode, and in so doing exposes a potentially attractive target for nematode control.

We used RNA interference to identify a transcriptional regulator of effector production in cyst nematodes: the Subventral Gland Regulator (SUGR). SUGR controls the expression of 261 genes, with 45% of these genes encoding for secreted proteins, including 16 of the known 39 cell wall degrading enzymes. Furthermore, SUGR, in conjunction with the transcription factors that it also activates, directly binds the promoters of effector genes in a partially overlapping manner. Together, this reveals a regulatory network underpinning effector production.

Interestingly, at the top of this network, transcription of *SUGR* itself is activated by a plant-derived small molecule found inside roots of hosts. These data allow us to build a working model for effector regulation that ultimately describes a careful balancing of resource-sparing when you must, and promoting parasitism when it counts.

C6.3-5

DETECTION OF ROOT-KNOT NEMATODES: TRADITIONAL VS. NOVEL APPROACH WITH HYPERSPECTRAL IMAGING

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Text

Traditional detection of root-knot nematodes (RKN, Meloidogyne spp.) is based on the visual observation of non-specific symptoms such as stunning and wilting and specific symptoms on the roots, i.e. root knots or galls. The next step is sampling, usually followed by extraction of nematodes. Identification can be based on morphological characteristics, analysis of isoenzymes or molecular methods such as PCR. There is increasing evidence of the usefulness of remote sensing for detecting nematode infestations. Remote sensing with analysis of hyperspectral images enables the detection of RKN-infected plants. We have shown that analysis of hyperspectral images of the above-ground parts of potato and tomato plants, as well as images of potato tubers, can enable accurate detection of RKN-infected plants. Detection of nematode infestation by remote sensing has several advantages: Detection without having to uproot the plants (non-invasive approach); Monitoring of large production areas with cameras on tractors, drones or aircraft; Detection in the early stages of infestation in the field (finding small patches of infected plants in the field); Detection in the early stages of plant infection before the plant develops visible symptoms (pre-symptomatic detection); Distinguishing non-specific symptoms on the above-ground plant parts from very similar symptoms of drought. The detection and differentiation of closely related RKN species using hyperspectral analysis will be also discussed.

C6.3-6

SHOOT! WHERE'S MY FEEDING SITE? DEFINING A TISSUE-INDEPENDENT RESPONSE TO NEMATODE PARASITISM.

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Text

Root-parasitic cyst nematodes establish a unique feeding organ via the re-regulation of endogenous plant genes. A specific combination of gene expression defines the feeding site, some of which will be the ‘core’ to parasitism (i.e. susceptibility genes). Understanding the biology of susceptibility genes has intuitive routes to application in agriculture. In Arabidopsis

thaliana, ~ 5,500 genes are differentially regulated over the course of infection by the beet cyst nematode Heterodera schachtii. To screen mutants in all these genes for susceptibility, using the current state-of-the-art nematode infection assay, would take at least 7 years. In this work, we exploit for utility the underappreciated fact that these nematodes can also induce feeding site formation in aerial parts of the plant. By cross-referencing RNA-seq data from infected roots and infected shoots, we define a tissue-independent parasitism response, substantially reducing the number of putative susceptibility genes. To accelerate screening further, we developed a novel phenotyping platform combining low-cost and open-source 3D printed hardware with computer vision and deep-learning software. We use this novel capacity to conduct an exhaustive screen of mutants in genes that are differentially regulated by the parasite, independent of tissue. Susceptibility genes identified in this way represent attractive targets to engineer resistance using genome editing.

F6.3-1

RNAI-MEDIATED PARASITISM GENE SILENCING AS SOURCE OF CROP PROTECTION RESISTANCE TO MELOIDOGYNE INCOGNITA

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Text

Root-knot nematodes (*Meloidogyne* spp.) have evolved infection mechanisms that involve the secretion of effector proteins into host plants to suppress immune responses and facilitate parasitism in a wide range of land plants. Effector genes are, therefore, attractive targets for the genetic improvement of plant resistance to *M. incognita*. In this study, RNAi-mediated gene silencing of the *Minc03328* and *Minc16803* parasitism genes was used to generate transgenic Arabidopsis plants. We designed a T-DNA construct with the full-length regulatory region of the soybean E2 ubiquitin-conjugation promoter that modulates hairpin-type dsRNA expression in the nuclear genome of *Arabidopsis thaliana*. Data showed that transgenic Arabidopsis expressing the dsRNA-targeting *Minc03328* and *Minc16803* exhibited significantly increased resistance to nematode infection. Gall numbers and egg masses were reduced by up to 81% and 93%, respectively, in the dsRNA-*Minc03328* transgenic lines, whereas the dsRNA-targeting *Minc16803* showed 76% and 87% reduction in the same parameters. Interestingly, histopathological analyses of *M. incognita*-induced galls strongly suggest that both genes may play an important role during the early parasitism stages, encompassing amorphous giant cells with lower cytoplasmic content in the transgenic lines, besides a hallmarked effect on the nematode cuticle, reinforcing their potential as a promising specific target for application in modern crop protection development.

P6.3-002

THE LNCRNA6155-MIR169F-HAP2C MODULE REGULATES RICE IMMUNITY AGAINST THE ROOT-KNOT NEMATODE (RKN), MELOIDOGYNE GRAMINICOLA (MG)

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Text

Non-coding RNAs (ncRNAs) are involved in the regulation of diverse plant biological stresses, but it is unclear if they play a role in rice immunity against *Meloidogyne graminicola* (Mg). In previous transcriptome analyses, rice miR169f and its predicted target genes NF-YAs were differentially expressed after inoculation with Mg. Based on sequence comparisons, we found a lncRNA (lncRNA6155) that contains miR169 endogenous target mimics. It suggests that lncRNA6155 may act as the competitive endogenous RNA of miR169. In this study, we confirmed that the expression level of miR169f was significantly downregulated in rice upon Mg infection. In contrast with miR169f, the levels of lncRNA6155 and of predicted target gene *OsHAP2C* were significantly increased. Transient expression in *N.benthamiana* showed that the expression of *OSHAP2C* and lncRNA6155 was silenced by the overexpression of miR169f. However, the expression of *OSHAP2C* was strongly increased when lncRNA6155 was transiently overexpressed like that of overexpression of a target mimicry (STTM169f) that acts as a sponge to trap miR169f. Exogenous miR169f treatment reduced the expression of lncRNA6155 and *OSHAP2C* and this was associated with decreased root gall formation. Currently, transgenic over-expression lines of lncRNA1655, miR169f, and *OsHAP2C* are being generated. Our results indicate that the lncRNA6155-miR169f-HAP2C module regulates rice resistance to Mg, but more detailed mechanisms should be further investigated.

P6.3-004

UNDERSTANDING THE HYPERVARIABILITY OF HYP EFFECTORS IN POTATO CYST NEMATODES

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Text

Plant-parasitic nematodes have evolved a large repertoire of effectors. Like many effectors, HYPs are secreted into the host and are necessary for infection. HYP genes consist of a ‘hyper-variable’ domain characterized by variable number, organization, and subfamily-specific repeats. The hyper-variable domain is flanked by 410 and 94 nucleotides that have remained 95% identical for ~30 million years of evolution. The objective of this research is to understand how it is possible for the genome of an animal to permit such variability in a single domain of a gene family, while maintaining the stability of the genome in general, and HYPs in particular. In order to capture the entire HYP gene in a single read we sequenced the genomes of *Globodera pallida* and *Globodera rostochiensis* using long-read sequencing technologies.

Strikingly, we found that the dominant majority of HYP variation is allelic. To unravel the extent of such unprecedented diversity in an allelic series of a single gene, we have performed Cas9-based targeted Nanopore sequencing to enrich for HYP gene containing locus. Additionally, we have performed amplicon sequencing of multiple individuals across the lifecycle using Pacbio HiFi sequencing to understand when and how HYP variation is introduced. Latest results from these efforts at understanding the extent and nature of HYP diversity, and potentially yet unknown biology underlying HYP variation will be presented.

P6.3-005

A SMALL CYSTEINE-RICH MELOIDOGYNE JAVANICA EFFECTOR (MJCRSP) MODULATES PLANT IMMUNITY TO PROMOTE NEMATODE PARASITISM

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Text
Root-knot nematodes (RKNs), *Meloidogyne* spp., are among the most important nematodes that evolved towards plant parasitism. The host-nematode interface is mediated by a plethora of effectors, which facilitate intricate parasitic mechanisms. A vast array of potential effector proteins was previously identified from the *M. javanica* genome sequence. However, it is unknown how these effectors contribute to successful nematode infection processes. Here, we characterize a *M. javanica* core effector conserved within important RKN species. This effector encodes a small cysteine-rich secretory protein denoted as MjCRSP. Fluorescence *in situ* hybridization showed that MjCRSP is localized within amphidial glands of pre-parasitic second stage juvenile (J2s) nematodes. *Agrobacterium* transient expression analysis showed that MjCRSP does not induce a hypersensitive response (HR) but rather, suppresses infestin 1 (INF1) triggered cell death (ICD). MjCRSP also subdues defense responses triggered by Flg22. MjCRSP is localized in the nucleus and cytoplasm of *Nicotiana benthamiana* leaves when expressed transiently. Our results provide functional evidence that MjCRSP is a bona fide effector secreted from this nematode's secretory glands into host tissues to interfere with plant immunity and enhance nematode parasitism.

P6.3-006

DEVELOPMENT OF REAL-TIME DIAGNOSIS TECHNOLOGY USING PNA PROBE OF BURROWING NEMATODES

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Text
The burrowing nematode (*Radophoulus similis*) is a major pest that causes significant

economic damage in banana cultivation field in Australia, Central and South America, Africa, the Pacific, and the Caribbean, and is therefore managed as a key nematode. It has been designated as a prohibited disease and insect pest in Korea since 1996 and import restrictions on host plants from around the world are being implemented. Recently, there have been concerns about its domestic occurrence, as it has been detected in host plants from Laos and Hungary, leading to urgent restriction measures. Accordingly, we attempted to develop a root-rot nematode classification and diagnostic technology that can quickly identify domestic occurrences using artificially synthesized nucleic acid samples based on the genome information of the burrowing nematode. In this study, real-time PCR was performed using PNA probes, and the results showed that *R.similis* could be distinguished from two closely related species, *R.arabocoffease* and *R.daklakensis*, at a copy level of 10^2. If suspected cases of burrowing nematode occurrence are found in domestic foliage plants, we expect that the results of this study could be helpful for infection monitoring and diffusion information analysis through rapid species diagnosis.

P6.3-007

GENETIC DIVERSITY ANALYSIS OF FOUR CYST NEMATODES ISOLATED IN KOREA USING MIG-SEQ ANALYSIS

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Text

The cyst nematode is agriculturally important pest that cause a lot of damage to domestic cabbage, soybeans, and other crops every year. Since the first outbreak of sugar beet cyst nematode (HS) in 2011 in Taebaek, Gangwon-do, it has continued to spread to nearby cabbage fields, and three species of nematodes, sugar beet cyst nematode (HS), clover cyst nematode (HT), and soybean cyst nematode (HG), are mainly found in domestic cabbage fields. In this study, we performed a relationship analysis of four closely related cyst nematodes collected in Korea through MIG-seq analysis and investigated the genetic differences among the four nematodes. MIG-seq analysis can select thousands of genome-wide SNP markers using the multiplex ISSR genotyping method, and it is very effective for genome-based relationship analysis targeting small samples of nematodes because it can construct NGS libraries using low-quality DNA. The four species of cyst nematodes were subjected to MIG-seq analysis, and the genome-wide SNP markers was selected through various filtering steps and then subjected to phylogenetic tree, PCA, and genetic structure analysis. We were able to identify genetic differences in each of the four species of nematodes and the genetic relationships were also confirmed. The analysis of the genetic diversity of the nematode population using MIG-seq method will provide very important information for the study of genetic differences and relationships among domestic strains.

P6.3-008

CHANGES IN THE RHIZOSPHERE MICROBIOME OF WINTER WHEAT LINES WITH VARYING RESPONSE TO ROOT LESION NEMATODE INFECTIONS

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Text

Exploiting the rhizosphere microbiome to suppress root diseases is important for advancing sustainable agricultural production. This may be achieved through the deployment of plant phenotypes that favor pathogen suppressive microbial communities. The root lesion nematode, Pratylenchus neglectus (RLN, Pn) represents a major constraint of wheat production worldwide. Toward Pn control, we have bred winter wheat phenotypes displaying significant resistance to Pn in greenhouse trials. Currently, these lines, their susceptible sister lines, and commercial winter wheat varieties are being evaluated in Pn infested fields across Montana. Our aim is to release our first Pn resistant winter wheat cultivar in Montana and to examine the interactions among plant phenotype, nematode induced disease, and the surrounding microbiome. We hypothesize that nematode resistant phenotypes significantly affect the composition and services rendered by the rhizosphere microbiome. The work presented examines differences in nematode and microbial communities as affected by wheat lines with common genetic backgrounds but differing in susceptibilities to Pn. In future work, comparative rhizosphere metagenomic analysis of Pn resistant and susceptible winter wheat lines will be performed to better understand the role of these microbial communities in suppressing RLN.

P6.3-009

AI-POWERED HOLISTIC AND DYNAMIC PLANT-PATHOLOGY TO DELIVER NEW SOURCES OF RESISTANCE

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Text

Plant-parasitic nematodes are a major, and in some cases dominant, threat to food security. The major barrier to identifying new sources of resistance is phenotyping. To address this constraint, we designed and built a bespoke high-throughput, low-cost, and semi-automated phenotyping system that combines custom 3D-printed hardware and deep-learning-powered trait recognition.

Using this unprecedented capability, we screened the Arabidopsis Multiparent Advanced Generation Inter-Cross (MAGIC) population for susceptibility to the beet cyst nematode Heterodera schachtii. All 527 recombinant inbred lines, each with 20 biological replicates (approx. 10,000 plants), were phenotyped 45 times over the following 90-days (some ~400,000 phenotyping events, including tens of millions of nematodes).

This exemplifies a truly holistic (i.e. whole plants) and dynamic (i.e. the whole life cycle) phenotyping approach that sets a precedent in pathology in general. Using these extensive data, we explored novel aspects of strategic and academic merit. Firstly, we used genome

wide association studies to map the plant-loci that contribute to nematode phenotypes (i.e. novel susceptibility and resistance loci). Secondly, we define, with high confidence, new fundamental features of the nature of parasitism.
Taken together, we demonstrate the power of AI to deliver a step change in our understanding of, and ability to control, plant-parasitic nematodes.

P6.3-010

ASSESSMENT OF SOME SOYBEAN CULTIVARS AND LINES TO SOYBEAN CYST NEMATODE, HETERODERA GLYCINES

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Text
The soybean cyst nematode, (SCN; *Heterodera glycines*), is one of the most important of plant parasitic nematodes in soybean fields, which has distributed widely in the world. Most important management procedure of SCN is integrated management by applying the resistant cultivars, cultivation of non-host crops and proper rotation. In this study, 21 soybean cultivars and lines were evaluated to SCN in infested field. Katool resistance cultivar and Williams susceptible cultivar are used as controls. In field experiment, a plot with infestation history were selected in a soybean field in Mazandaran province and after preparation the plot, each line was planted in 3 meters lines. A randomized complete block design was followed for implementing the experiment containing three replications for each line. After the end of growing season, the population density of female and cyst were determined in roots and soil samples. The female index was calculated based on the average of number of female (cyst) found on each cultivar relative to the susceptible control. Soy-98-6 had resistance response (FI<10%), Saland had moderate resistance response (FI=10-30%), Saba, Sahar, SOY-98-17 and SOY-98-18 had moderate susceptible (FI=30-60%), and other cultivars had susceptible response (FI>60%). All data were subjected to analysis by GLM (Generalized Linear Model) using statistical software SPSS that indicated significant differences at among the soybean cultivars and lines for resistance to HG 0.

P6.3-011

FIRST REPORT OF THE CEREAL CYST NEMATODE (HETERODERA FILIPJEVI) ON WHEAT IN IDAHO, USA

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Text

Idaho is a key state for wheat and barley production in the United States. The continuous planting of susceptible cultivars in Southeast Idaho has allowed populations of the cereal cyst nematode pathogen to increase to economically damaging levels. During the 2018 project on identification and pathotyping of *Heterodera avenae*, cysts from an infested spring wheat field were extracted for morphological and molecular identification. Initial amplification of ribosomal RNA sequence for ITS1, 5.8S, and ITS2 regions, and partial mitochondrial cytochrome oxidase I gene (*CTC*-1) produced significant alignment with *H. filipjevi*. Further analysis on the extracted soil sample revealed mixed populations of *H. filipjevi* and *H. avenae*. Sequence analysis using a species-specific primer confirmed the presence of *H. filipjevi* in the soil samples. A PCR-RFLP protocol using seven endonucleases *Taq*I, *Hinf*I, *Pst*I, *Hae*III, *Rsa*I, *Alu*I and *Cfo*I (Yan and Smiley 2010) is in progress to distinguish individuals of any possible CCN species co-occurring in soils of Southeast Idaho. The complete results including the morphological characteristics of local populations will be included in our presentation at ICPP2023.

P6.3-012

NEMATOTOXIC POTENTIAL OBTAINED FROM DIFFERENT ACCESSIONS OF THE SAME SOLANACEAE SPECIE EFFECTIVE IN CONTROL OF MELOIDOGYNE INCOGNITA

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Text

Root-knot nematodes (RKNs) stand out negatively on agriculture worldwide, with emphasis to Meloidogyne spp. Infection by RKNs alters the root system morphology, reducing plant vigor, and product quality. The use of botanical extracts is a sustainable method for the control of phytonematodes. However, synthetic nematicidal remains very explored, even presenting an imminent risk to human and environmental health. The Solanaceae botanical family represents an abundant source of metabolites for the control of plant pathogens. The present study aimed to evaluate the nematotoxic potential of the aqueous crude extracts (ACEs) of four different Solanaceae plant accessions. The seed ACEs from the accessions 48, 52 and 78 are nematicidal, killing more than 90% of J2 M. incognita, and that activity is thermostable. The accessions 48 and 52 had the resistance reaction confirmed, and 48, 52 and 78 presented low cytotoxicity to blood cells and insects cells. The cellular viability of human keratocytes treated with these ACEs was superior to 50%, and these extracts were not phytotoxic to soybean (Glycine max) seed germination. In addition, these ACEs did not inhibit the growth and development of the beneficial soil organisms (fungi, bacteria and yeast). The accession 48 presented 100% nematicidal activity. This ACE was fractionated via HPLC-ME, and the fraction 6 that demonstrated the highest nematicidal activity, was further purified by HPLC-RP, and the molecular mass measured by MALDI-TOF

P6.3-013

CHARACTERIZATION OF PARASITIC NEMATODES OF PAPAYA (CARICA PAPAYA L.) IN BURKINA FASO AND HISTOLOGICAL ANALYSIS OF MELOIDOGYNE JAVANICA LIFE CYCLE IN ROOTS.

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Text

Papaya (*Carica papaya* L.) may be subject to attacks by plant-parasitic nematodes causing production losses. This study aims to identify the nematode species associated with papaya in the main producing regions in Burkina Faso and to determine their life cycle in papaya. Nematodes extracted from 138 soil and root samples were identified based on morphology and molecular characterization using specific PCR primers. Nine genera of nematodes were identified, with *Rotylenchulus*, *Helicotylenchus* and *Meloidogyne* genera frequently associated with papaya. Total DNA from 120 complex soil or root samples could be obtained, 52 tested positive for *Meloidogyne javanica*, 23 tested positive for MIG primers, and *Rotylenchulus reniformis* was detected in 60 samples. *M. javanica* larval development in Solo 8 papaya roots was recorded by microscopy during 5 weeks after inoculation and staining with acid fuchin. In parallel, histological sections of roots stained with toluidine blue, FASGA and Schiff-Naphthol Blue Black were prepared. Feeding sites formed in the root central cylinder and 6 to 8 multinucleated giant cells (GCs) on average rounded the nematode body. The GC cytoplasm appeared highly enriched in proteins, becoming denser from 26 to 35 days, when adult females developed. Females laying eggs in the cortex were observed at 35 days. Characterization of nematodes species and their life cycle are important data for selecting nematode-resistant papaya to control populations in Burkina Faso.

P6.3-014

DEVELOPMENT AND STANDARDIZATION OF A RAPID, HIGH THROUGHPUT PCN RESISTANCE SCREENING PROTOCOL

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Text

In Kenya, Potatoes are an important staple food as well as cash crops. In this case, they play an important role when it comes to national food security, income generation and poverty alleviation in the country. The Potato Cyst Nematode (PCN) was initially detected in Kenya in 2015. Since then, the pest has been identified as one of the major threats to the production of potatoes in the country. Resistance is a key strategy for managing pests and diseases, including PCN. In this research, we conducted both a phenotypic and genotypic screening on

64 germplasm acquired from The International Potato Centre (CIP) to assess for resistance. Five known sources of resistance were assessed using molecular means and compared with phenotypic resistance, following inoculation PCN.19 lines showed the presence of one or more Rgenes: H1, Gro 1, Gro 6, and Gpa 2. 0 lines were selected for a repeat experiment following a comparative analysis of both phenotypic and genotypic results. Separately, a high throughput method of phenotypic resistance screening was developed using genotypes identified as resistant and susceptible from the prior screening. An assessment period of 14-days post inoculation was determined as a suitable, short duration screening time for establishing resistance, based on the non-development of PCN juveniles once entering the root tissue, compared with susceptible lines in which juveniles developed into adults.

P6.3-015

THE LAY OF THE LAND: TISSUE-SPECIFIC TRANSCRIPTOMICS REVEALS A COMPREHENSIVE "EFFECTOME" OF A PLANT-PARASITIC NEMATODE.

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Text

Plant-parasitic nematode (PPN) species infect every crop. Of these, root knot and cyst nematodes pose the biggest threat. These species are sedentary endoparasites which form long-term, biotrophic relationships with the host by secreting hundred of effectors into plant cells. The vast majority of PPN effectors are produced by pharyngeal gland cells, but the PPN "effectome" remains undefined due to the technical intractability of these glands.

To address this, we take advantage of recent techniques for isolating gland cells. Here we define a stringent annotation of effectors using gland-cell-specific transcriptomics of the beet cyst nematode Heterodera schachtii at three life stages. We identify 682 effectors (317 known, 365 high-confidence candidates), in 321 families, with extremely skewed membership. The five largest families contain a quarter of effectors, and yet two fifths of effectors are the only member of their family. To understand the evolutionary origins of effectors, these data were cross-references with proteomes of 60 nematodes. This revealed the effector repertoire to be shaped by: neofunctionalization of highly-conserved genes (20 %); expansion of families present in the last common biotrophic ancestor of H. schachtii (64 %); and recent addition of orphans (7 %). These data represent the most comprehensive view of cyst nematode effectors to-date and further our understanding of an important pathogen. Ultimately, they provide the basis for crop improvement.

P6.3-017

GETTING TO THE ROOT OF PHYTONEMATODE DISEASE: TOWARD THE UNDERSTANDING OF PARASITISM REGULATION IN MELOIDOGYNE SPP

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Text

The success of plant infection in phytonematodes relies on a precise control of gene expression dedicated to parasitism. The animal produces in its secretory glands an arsenal of parasitism proteins "effectors" ultimately delivered into host cells. These effectors module and counteract important host cellular processes necessary to invade the plant. The effector gene expression is timely orchestrated in response to nematode lifecycle and plant physiology. If effectors are crucial weapons, a critical next step is to shift the focus away from effectors to “high-level” processes: how parasitism genes are regulated?

Several research groups actively work on effector biology in phytonematodes, leading to the discovery of promoter motifs unifying effector gene expression levels and consequently helping the identification of potential proteins related to parasitism regulation.

In the Mel-DOG project, I focus on the non-coding signatures in effector promoters of the root-knot Meloidogyne incognita involved at pre- and late parasitic stages. M. incognita is not transformable so far. Here, I combine two technologies to plant-nematode research - an engineered chromatin immunoprecipitation (ChIP)-CRISPR and Yeast One-Hybrid (Y1H) library assay – allowing the identification of regulatory candidates and then functionally validate their parasitism role by RNA interference.
I will discuss about their potential role in molecular mechanisms governing effector gene expression in M. incognita.

P6.3-018

NOVEL COTTON (GOSSYPIUM HIRSUTUM) PROMOTERS WITH BIOTECHNOLOGICAL POTENTIAL FOR PHYTONEMATODE AND INSECT PEST CONTROL

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Text

Gossypium hirsutum L. is the leading cotton species of economic importance for agribusiness. The main challenge to cotton productivity is the constant attack of several pests. Therefore, the aim of this study was to identify and characterize new promoters that can be applied as a biotechnological asset to drive the transcription of genes encoding molecules more efficiently than the viral-constitutive Cauliflower Mosaic Virus (pCaMV35S) promoter in vegetative and reproductive cotton tissues attacked by insects and phytonematodes. In this study, two cotton promoters were characterized: pGhERF105 and pGhNc-HARBI1. Interestingly, a comparative

analysis of these cotton promoters and pCaMV35S by quantitative real-time PCR showed that the two cotton promoters encompassed a significantly higher GUS transcription activity in plant vegetative and reproductive tissues than pCaMV35S. Additionally, the transgenic plants were subjected to bioassays with the major pests that attack cotton plants. The data indicated that the promoters were induced in at least one of the tested time-points when in the presence of RKN infestation. Finally, leaves of pGhERF105, pGhNc-HARBI1 and pCaMV35S transgenic plants were infested with 3rd instar larvae of insects. The analysis for GUS expression confirmed the induction of these promoters by pest. Overall, the present study provides new insights into a set of cotton promoters suitable for biotechnological applications in GM plants for pest resistance.

MOLECULAR ASPECTS: plant-oomycetes interactions

C5.2-1

MECHANISM OF PLANT MEMBRANE DAMAGE BY NEP1-LIKE PROTEINS

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Text

Necrosis- and ethylene-inducing 1-like proteins (NLPs) are important virulence factors of plant-associated microorganisms. NLPs exhibit cytotoxic activity towards plant cells and therefore promote infections and toxic effects in a variety of crops. They damage lipid membranes through a multi-step mechanism involving binding to lipid membranes by the plant-related sphingolipids glycosyl inositol phosphoceramides (GIPC). They then oligomerise at the plant membrane level and eventually disrupt the membrane by forming small, transient pores. We are investigating this process using structural biology, biophysical and biochemical approaches to gain unique insights. Pore formation by NLPs is distinct from other families of pore-forming toxins and is adapted to plant target membranes. Understanding pore formation at the molecular level will also enable the development of strategies to inhibit NLP activity.

C5.2-2

DISCOVERY OF PROTEIN MARKERS OF OOMYCETE EV'S

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Text
The movement of effector proteins and RNAs from pathogen into host cells during infection is known to occur. However, the exact mechanisms facilitating this movement are still being widely studied. One possible delivery route involves the secretion and uptake of extracellular vesicles (EVs) between organisms.
In this study we isolated EVs from the oomycete Phytophthora infestans, cause of potato late blight, with the aim of identifying oomycete-associated EV markers and investigating the cargo of these bodies. This is being achieved by a proteomics approach to identify both secreted and vesicular proteins during in vitro growth. We have identified some known EV proteins found widely in EV proteomes, supporting our methodology and approach. Additionally, we have identified some oomycete-specific proteins that have as yet unknown functions but appear to be transmembrane proteins, including TMP1 (Trans-Membrane Protein 1). TMP1 accumulates in the same density fraction in sucrose gradients as the RXLR effector protein, PITG_04314, during EV isolation implying they could be associated with the same EV. The overall aim of this work is to find markers of EVs that we can use to determine how these EVs are secreted and taken up into the plant cell and whether this is a mode of transport for pathogenicity factors such as RXLR effectors.

C5.2-3

KINETICS OF ZOOSPORES APPROACHING A ROOT USING A MICROFLUIDIC DEVICE

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Text
Zoospores are flagellated spores, asexual and motile cells, produced by different organisms including oomycetes. Oomycetes of the genus Phytophthora are plant pathogens (e.g. potato blight). The zoospores of oomycetes are biflagellates and are able to swim in aqueous environments by using chemotaxis.
We focus on the plant-pathogen interactions, in the first events of infection: attraction of the pathogen toward the host, adhesion, aggregation. We use a simple microfluidic device including a living root to study the movement of zoospores swimming towards a root. We investigate the telluric species P. parasitica, a polyphagous pathogen attacking a wide range of hosts, swimming towards a root of Arabidopsis thaliana.
Processing of image acquisitions around and away from the root allows to reconstruct the trajectories of zoospores and to precisely analyze their kinetics as a function of their distance from the root.

C5.2-4

EFFECT OF SMALL MOLECULE MODULATORS OF CALCIUM SIGNALLING ON PHYTOPHTHORA INFESTANS

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Text

The pathogenic oomycete Phytophthora infestans poses a significant threat to global food production and agriculture. It is the causative agent of late blight in many important crop plants including potato and tomato plants, and is well known as the trigger of the Irish potato famine during the 19th century. To this day, control of the pathogen globally is estimated to cost up to $10 billion per annum due to crop loss and fungicide use. The long-term use of fungicides to combat the spread of this pathogen has resulted in the emergence of resistant strains. Ca2+ plays a key role in regulating oomycete biology. Understanding Ca2+ signalling mechanisms in oomycetes may play a key role in developing new, more targeted, ways of controlling infection. In the current study we used commercially available small molecules that interfere with the Ca2+ signalling proteins in P. infestans. The chemicals were incorporated into Rye A agar plates at different concentrations (10μM, 20μM, and 50μM), to monitor hyphal growth of P. infestans. Complete inhibition of hyphal growth was observed using 4-chloro-3-methylphenol (4-C3-MP) at the 50μM concentration. Different structural and halogenated variants of 4-C3-MP were also tested, but only the 4-C3-MP gave a total inhibition at 50μM. 4-C3-MP has been reported to be produced by some endophytic Bacillus species of tomato, exhibiting antifungal properties, thus could be a promising anti-blight fungicide in tackling P. infestans outbreaks.

C5.2-5

USING LAB-ON-A-CHIP DEVICES TO STUDY OOMYCETE PLANT INTERACTIONS

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Text

Lab-on-a-Chip (LOC) is a term that is used to describe microfluidic devices that have the capability of integrating one or more laboratory functions. The devices allow high-through-put analyses, are typically small and compact, quick and easy to produce, and can be replicated accurately and cheaply. While their initial design and fabrication can be technically demanding, their many advantages mean that they have found widespread use in biological and medical research, and in the clinic, where they offer cheap and rapid point-of-care testing. In an attempt to better understand the interactions between pathogenic oomycetes and their hosts, at various stages of the pathogen life cycle, we have designed a number of LOC devices, which I will describe. The first device replicates the chemical and electrical environment around plant roots and allows the observation of the swimming patterns and encystment of motile *Phytophthora* zoospores. The second device incorporates pneumatic microvalves that allow the trapping and compartmentalisation of individual zoospores, their germination and the measurement of μN forces exerted by emerging germ tube tips. The third allows the measurement of forces exerted by invasive hyphae with concurrent cytoskeletal imaging. The fourth allows the generation of O_2 gradients facilitating the study of

aerotropic growth of hyphae. I will also describe devices that replicate the leaf topography of rust fungi hosts that allow study of plant/rust interactions.

C5.2-6

UNLOCKING THE SECRET TO SUCCESSFUL AVOCADO DEFENSES: THE IMPORTANCE OF NLR PROTEINS DURING PHYTOPHTHORA CINNAMOMI INFECTION

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Text

Avocado is an important crop plant in many countries worldwide. Phytophthora root rot caused by the hemibiotrophic oomycete, *Phytophthora cinnamomi*, remains one of the most destructive pathogens within the avocado industry since there are a limited number of effective control methods available to control disease. One such method includes the use of partially resistant avocado rootstocks, which demonstrates stronger immune responses during infection. Plant Nucleotide binding-Leucine rich repeat (NLR) proteins play a significant role during the activation of Effector triggered immune responses, however, a genome-wide set of avocado *NLR* genes have only recently been identified. During this study, RNA-sequencing data showed significant differences in *NLR* expression levels when compared between a partially resistant and susceptible rootstock, following *P. cinnamomi* inoculation. The partially resistant rootstock showed increased and prolonged *NLR* expression when compared to the susceptible rootstock. Genome comparisons revealed little differences between the partially resistant and susceptible rootstock *NLR* promoter sequences, however, differing transcription factor expression patterns may explain *NLR* expression differences between the two rootstocks. Understanding the role of NLR proteins during *P. cinnamomi* infection, and how the expression of *NLRs* are regulated, is critical to unravelling the molecular mechanisms underpinning rootstock resistance towards *P. cinnamomi* infection.

F5.2-1

PLATFORM DEVELOPMENT USING A MODEL HOST PLANT FOR HIGH-THROUGHPUT OMICS AND CRISPR/CAS GENE EDITING IN PHYTOPHTHORA CINNAMOMI

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Text

Phytophthora cinnamomi poses a serious threat to biodiversity, especially in Australia, with a host range of almost 5000 species. Elicitins, a group of highly conserved sterol-binding proteins are produced by *Phytophthora* species, one function of which is to support their auxotrophic lifestyle, among other putative, yet elusive, functions. Multi-omics approaches along with gene editing have expanded opportunities for functional genomics. However, success with CRISPR/Cas gene editing of these coenocytic and multinucleated oomycetes has been limited. To address these challenges, we have optimized the critical steps for transformation of *P. cinnamomi* via the use of PEG/$CaCl_2$, optimally viable starting material, nucleated protoplasts, proper guide RNA construction and suitable selection markers. The polyploid genome of *Phytophthora* presents a challenge in the selection of homokaryotic transformants. To overcome this constraint, we have compared approaches for detection of non-homologous end joining-mediated gene editing which includes T7 endonuclease assays, restriction enzyme digestion and high-resolution melt curve analysis. To test the virulence of transformants and utilise omics technologies to understand their interaction with plants, we established a soil-free growth system for the model host *Nicotiana benthamiana*. These combined approaches are enabling our studies of elicitins and will assist in expanding the strategies to reduce *Phytophthora* diseases.

P5.2-001

THE INFECTION BIOLOGY OF PLASMODIOPHORA BRASSICAE-CAUSE OF CRUCIFEROUS CLUBROOT DISEASE

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Text

Clubroot disease, caused by the soilborne protist pathogen-*Plasmodiophora brassicae*, is a devastating disease to cruciferous crops worldwide and leads to a huge economic loss annually. As an intracellular obligate biotrophic phytopathogen, the life cycle of *P. brassicae* was complicated and not fully understood. Combined the fluorescent probe-based confocal microscopy and the transmission electron microscopy, we systematically investigated the infection process of *P. brassicae* and proposed a refined model of *P. brassicae* life cycle. In this model, we have made four major improvements: 1) *P. brassicae* also initiates the primary infection in root epidermal cells; 2) documented and characterized almost all the life forms; 3) firstly captured the sexual behaviours of secondary zoospores; 4) indicated the diploid nature of the secondary plasmodia. Based on above results, we compared the infection process of *P. brassicae* in susceptible hosts, immune hosts and nonhosts. We found that *P. brassicae* could initiate the primary infection in both immune hosts and nonhosts. However, host resistance blocked the secondary infection phase at the stage of uninucleate secondary plasmodium and nonhost resistance blocked the primary infection phase at the stage of uninucleate primary plasmodium. Our study provided new insights into understanding of the complex life cycle of P. brassicae and the cellular and molecular mechanisms underlying clubroot host and nonhost resistance.

P5.2-002

PHYTOPHTHORA CINNAMOMI CRN EFFECTORS: FOOT SOLDIERS OF PHYTOPHTHORA ROOT ROT

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Text

Phytophthora cinnamomi is a globally important oomycete pathogen and the causal agent of Phytophthora root rot in *Persea americana* (avocado). Our understanding of the mechanisms *P. cinnamomi* utilises to infect and successfully colonise avocado is currently lacking, especially pertaining to how the pathogen can maintain its biotrophic and necrotrophic lifestyles respectively during infection. Crinkling and necrosis effectors (CRNs) are a class of cytoplasmic effectors in oomycetes known to manipulate plant cell death during infection. The current study aimed to identify the full repertoire of *P. cinnamomi CRNs* genes and subsequently classify them as either putative cell death inducers or suppressors based on their expression profile during avocado infection. A total of 25 full-length and 1 partial/CRN-like sequences were identified, of which seven are suspected to either induce or suppress cell death. Interestingly, *CRN53* and *CRN95* were shown to have two different alleles. This was confirmed in two separate *P. cinnamomi* isolates. The proteins encoded by these alleles are hypothesised to have contradictory functions during infection. Functional characterisation studies need to be performed to confirm the roles these effectors play in manipulating cell death during infection. This work has enhanced our understanding of how *P. cinnamomi* is able to maintain the different stages of its hemi-biotrophic lifestyle to successfully ensure colonisation of the host plant.

P5.2-003

HOST PATHOGEN INTERACTION BETWEEN EUROPEAN STRAINS OF RED ALGAE ‘BANGIA’ AND OOMYCETE OLPIDIOPSIS PORPHYRAE VAR. SCOTIAE

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Text

Abstract

Marine oomycetes of the genus Olpidiopsis (sensu lato) are one of the main disease-causing agents affecting Neoporphyra (commonly known as Nori) sea farms. Mechanisms of host resistance remain largely unknown. A recent study described a Scottish variety in the Scottish water Olpidiopsis porphyrae var. scotiae. We studied the resistance of 11 red algae (‘Bangia’) strains against this new described variety by using brightfield and fluorescence microscopy and RTqPCR assay. Results from microscopy inspection showed a significant effect for ‘Bangia’ strain identity on incubation period (days from inoculation until observation of the first symptom) and the percentage of infection (the number of infected cells as percentage). qPCR assay showed also significant effect for the ‘Bangia’ strain identity

suggesting different levels of resistance for different 'Bangia' strains. These results open up novel possibilities to investigate underlying mechanisms of resistance in the model.

P5.2-004

PHYTOPHTHORA PARASITICA "CORE" RXLR EFFECTOR PROTEINS INDUCE HOST DEFENCES

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Text

Phytophthora species are eukaryotic pathogens that are harmful to a wide variety of plants, many of which are important in agriculture and forestry. During the infection process, *Phytophthora* spp. secrete hundreds of RxLR effector proteins (including the conserved RxLR effectors (CRE)) into the cytoplasmic region, as putative virulence factors. The goal of this study was to determine the functions of CRE in *P. parasitica*. Towards this end, *in silico* analyses revealed that *P. parasitica* INRA 310 secretes 71 RxLR effectors that are conserved across 14 *Phytophthora* spp. Twenty-six CRE were found to be shared by more than five different *Phytophthora* spp. Two of these, PpRxLR1 and PpRxLR6 were selected from among the 26 CRE for further functional characterization. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analyses of the inoculated leaves showed that *P. parasitica* PpRxLR1 and PpRxLR6 are expressed during the biotrophic phase, suggesting their importance in virulence. *Agrobacterium tumefaciens*-mediated transient expression of PpRxLR1 and PpRxLR6 in *Nicotiana benthamiana* revealed potential mechanisms of *P. parasitica* PpRxLR1 and PpRxLR6 in promoting disease development. This includes inducing reactive oxygen species as well as callose deposition. Additionally, PpRxLR1 and PpRxLR6 induce phytohormones (SA, ET, and JA) and MAPKs (MPK3 and MPK6). These data indicate that both PpRxLR1 and PpRxLR6 are important virulence factors of *P. parasitica*.

P5.2-005

MEMBRANE-ASSOCIATED NAC TRANSCRIPTION FACTORS – VERSATILE ROLE IN PLANT STRESS ADAPTATION

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Text

Membrane-associated NAC transcription factors have important functions in plant biotic and abiotic stress adaptation. These transcription factors require cleavage from the membrane before they can translocate to the nucleus, where they regulate gene expression. Data show that the translocation mechanism is a prominent target of effector proteins from a variety of

pathogens. Here we focus on oomycete RxLR effector proteins and their respective host targets, the Arabidopsis NAC13 cluster, which also contains lettuce LsNAC069 and potato NTL1/2. This particular cluster is known to be involved in retrograde signalling associated to cellular reactive oxygen species, linking a multitude of abiotic and biotic stresses. We have shown that effectors and NAC targets co-localize at the endoplasmic reticulum; CoIP and Y2H experiments confirmed interaction of the proteins. Interestingly, co-expressed effectors hinder nuclear translocation of NACs induced by osmotic stress and pathogen associated molecular patterns. In a similar manner Ser/Cys-protease inhibitor TPCK reduced their translocation significantly. Utilizing targeted point mutations, structural biology and computational modelling we identified regulatory elements important for the translocation mechanisms of this NAC cluster. Our results indicate conservation of the NAC13 cluster translocation mechanism, which associates to their role in immunity, water acquisition, as well as to effector targeting by oomycetes.

P5.2-006

THE ROLE OF PHYTOPHTHORA PLUVIALIS RXLR EFFECTORS DURING EARLY INFECTION OF PINUS RADIATA

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Text

Phytophthora pluvialis, the causative agent of Red Needle Cast, is a destructive foliar disease of pine. Like other oomycetes, it utilises host-translocated effector proteins to promote successful infection. An RNA sequencing approach was chosen to elucidate differential expression effector genes in mycelium and pine needles of susceptible and resistant pine lines infected with P. pluvialis at two different time points (3- and 5-days post infection). Overall, 311 effector genes were identified to be differentially expressed (136 were Crinklers, 115 RxLRs, and 60 Elicitins). Further down-stream analysis focused on RxLR effectors expressed during the early stage of infection. First we employed a computational approach to acquire understanding on protein sequence and structural conservation. As expected, conservation on sequence level was limited. Interestingly, structural modelling showed that more than 90% of the early expressed RxLR effectors contained at least one WY-motif. Transient expression in *N. benthamiana* for nine selected candidates showed diverse localisation in planta. Successful protein expression was confirmed by Western blotting. Three WY-motif containing effectors candidates, PpR03, PpR06, PpR07 were expressed in *E. coli*, purified and characterised in detail. Infiltration assays with purified proteins in pine confirmed that PpR03 is not recognised, whereas PpR06 and PpR07 infiltrated samples showed browning of needles, indicating induced cell death.

P5.2-008

PHENOTYPIC AND GENOTYPIC DIVERSITY OF PHYTOPHTHORA INFESTANS POPULATIONS IN ALGERIA

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Text

Potato and tomato are important crops in Algeria. Every year, late blight caused by the oomycete Phytophthora infestans, is responsible for important losses on these two crops. A total of 208 isolates were collected from the main production regions during (2010 to 2016). This collection included 77 isolates collected from tomato and 131 isolates were collected from potato samples and 92 DNA fingerprints were captured on FTA cards. Isolates were phenotypically characterised for mating type, metalaxyl resistance, aggressiveness and host adaptation on potato and tomato. Genotypic diversity was analysed with 17 microsatellite loci. Results showed that both A1 and A2 mating type were found on potato isolates, but A1 mating type was only found on tomato isolates. All potato isolates were resistant to metalaxyl, except one. However, all isolates collected from tomato were susceptible. SSR markers revealed the prevalence of EU_13_A2 lineage on EU_2_A1 and EU_23_A1 in potato isolates, but in tomato, EU_23_A1 was the most important lineage which was mainly found in late season crops. This investigation showed that although tomato and potato are grown in the same production areas, the difference in population structure is clearly observed between the two hosts. The Knowledge of this pathogen diversity will contribute to the development of a sustainable control strategy for late blight of potato and tomato through a sustainable rotation of these crops.

P5.2-009

PHYTOPHTHORA EXPLOITS HOST TREHALOSE METABOLISM TO ACQUIRE CARBON AS A NUTRIENT SOURCE

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Text

Successful infection by pathogenic microbes requires the effective acquisition of nutrients from their hosts. Root and stem rot (RSR) caused by Phytophthora sojae is one of the most important diseases of soybean (Glycine max). However, the specific form and regulatory mechanisms of carbon acquired by P. sojae during infection remain unknown. Here, we show that P. sojae boosts trehalose biosynthesis in soybean through the virulence activity of an effector PsAvh413. PsAvh413 interacts with soybean trehalose-6-phosphate synthase 6 (GmTPS6) and increases its enzymatic activity to promote trehalose accumulation. P. sojae

directly acquires trehalose from the host and exploits it as a carbon source to support primary infection and development in plant tissue. Importantly, GmTPS6 overexpression promoted P. sojae infection, whereas its knockdown inhibited the disease, suggesting that trehalose biosynthesis is a susceptibility factor that can be engineered to manage RSR in soybean.

P5.2-010

TRANSCRIPTOME ANALYSIS OF CAPSIDIOL-MEDIATED DEFENSE IN PEPPER AGAINST ADAPTED AND NON-ADAPTED PHYTOPHTHORA PATHOGEN

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Text

Capsidiol is a bicyclic sesquiterpenoid phytoalexin synthesized by pepper and tobacco in species-specific manner. A significant decrease in capsidiol accumulation by virus-induced gene silencing compromises nonhost resistance in pepper against *Phytophthora infestans*, the Irish potato famine pathogen. However, adapted *Phytophthora capsici* causes severe disease on pepper. We have investigated the mechanism of *P. capsici* to overcome capsidiol activity different to non-adapted *P. infestans*. Different to *P. capsici*, mycelial growth of *P. infestans* on the media containing 5 μM capsidiol was inhibited and then the mycelia of both pathogens were harvested for transcriptome analysis. We found 2,113 differentially expressed genes (DEGs) in capsidiol-treated *P. capsici*, including 652 up-regulated and 1,461 down-regulated DEGs. KEGG pathway results showed that up-regulated DEGs were significantly enriched in membrane transport related to ABC transporters. Various ortholog groups of ABC transporters were identified in both *P. capsici* and *P. infestans* using Orthofinder. In particular, the ortholog group of OG0000284 existed in both genomes of two pathogens. Only *P. capsici* DEGs in the ortholog group was up-regulated, while those of *P. infestans* was down-regulated. Host-induced gene silencing of *P. capsici* ABC transporter gene displayed a significant symptom reduction by *P. capsici*. This study provides an insight to understand chemical defense in host and nonhost resistance.

P5.2-011

TRAFFIC MANIPULATION: A CONSERVED PHYTOPHTHORA EFFECTOR TARGETS A HOST RABGAP PROTEIN TO SUPPRESS DEFENSE-RELATED SECRETION

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Text

Plant innate immunity is characterized by the proper placement of immune components at the appropriate time and location. A cascade of transport regulators works together to achieve this by directing vesicular defense cargoes to the infection site. The emerging paradigm holds that *Phytophthora* species use effectors as a key virulence tactic to rewire host vesicle transport networks. Despite recent developments, little is understood about how vesicle trafficking is regulated in plant immunity. Here, we identified a novel strategy used by oomycete pathogens to subvert host vesicle trafficking. A host GTPase-activating protein (RabGAP) is targeted as a susceptibility factor by a conserved RXLR effector secreted by *Phytophthora infestans* and *P. palmivora* to subvert defense-related trafficking. Our proteomics screen, followed by biochemical and cell biology assays, suggests a model in which a RabGAP negatively regulates immunity by inactivating a host Rab GTPase that mediates defense-related vesicle transport towards the pathogen interface. Intriguingly, the RabGAP is guarded against effector manipulation by an NLR immune receptor. However, one of the *P. infestans* effector variants can evade and suppress activation of that NLR receptor. Our results shed light on the mechanism of defense-related secretion in plants by uncovering a negative regulatory mechanism controlled by the RabGAP-Rab association, that is guarded by host immune receptors against pathogen manipulation.

P5.2-012

PLACING THE SPOTLIGHT ON THE NPR1-DEPENDENT DEFENCE RESPONSE IN PERSEA AMERICANA (MILL.): INSIGHTS FROM A TIME COURSE DUAL RNA-SEQUENCING STUDY OF BOTH A SUSCEPTIBLE AND PARTIALLY RESISTANT ROOTSTOCK INOCULATED WITH PHYTOPHTHORA CINNAMOMI

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Text

In plants, the salicylic acid (SA)-signalling pathway is essential for defence responses that are effective against biotrophic and hemibiotrophic pathogens. Here, the nonexpressor of pathogenesis-related genes 1 (NPR1) is necessary for the majority of SA-related defence gene expression and the subsequent development of systemic acquired resistance (SAR). Therefore, to better understand pathogen defence, it is vital to understand the NPR1 pathway. *Phytophthora cinnamomi*, the causal agent of Phytophthora root rot (PRR) in avocado (*Persea americana*), is especially significant to the avocado industry which experiences significant financial losses each year as a result. It is reasonable to suggest that, given the hemibiotrophic lifestyle of *P. cinnamomi*, that the SA-signalling pathway and by extension the NPR1 pathway, could be vital to avocado's initial defensive response. Using

the *P. americana* West-Indian pure accession rootstock genome (Avocado Genome Consortium) we identified 88 NPR1 pathway-associated orthologs. Dual RNA-sequencing data was then used to investigate their expression following *P. cinnamomi* inoculation, in both a susceptible (R0.12) and partially resistant rootstock (Dusa®) at 6, 12, 24 and 120 hours post-inoculation. To date, this research is the most thorough analysis of the SA-induced, NPR1-dependent pathway in avocado, offering a fresh perspective on the potential mechanisms influencing *P. cinnamomi* resistance.

P5.2-013

XEG1: A CASE STUDY ON MICROBIAL ATTACK AND PLANT IMMUNITY IN THE APOPLAST

WANG Yan. (1), MA Zhenchuan. (1), **XIA Yeqiang. (1)**, WANG Yuanchao. (1)

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Text
The apoplast constitutes a major interaction niche in plant-microbe interactions. During infection, microbial pathogens secrete a large repertoire of effectors that act in the apoplast to modulate host conditions for infection. Plants respond to microbial attack via perception of conserved molecular patterns or apoplastic effectors using cell surface immune receptors to mount defense. The apoplastic effector XEG1 is a glycoside hydrolase 12 protein secreted by the soybean root rot pathogen *Phytophthora sojae*. XEG1 displays hydrolase activity toward xyloglucans and essential for *Phytophthora* infection. As a countermeasure, soybean secretes the inhibitor GmGIP1, which binds directly to XEG1 and inhibits its hydrolase activity, to increase soybean resistance. *P. sojae* secretes a paralogous XEG1-like protein, XLP1, with no enzyme activity. XLP1 binds GmGIP1 more tightly than XEG1, and acts as a decoy protecting XEG1 from the inhibitor GmGIP1. XEG1 is degraded by host aspartic protease GmAP5 in the apoplast. However, XEG1 undergoes N-glycosylation, which protects XEG1 from GmAP5 degradation. In addition, XEG1 can be recognized by a plant membrane-localized receptor-like protein RXEG1 to mount defense. Structural analyses revealed that RXEG1 inhibits the hydrolase activity of XEG1 and plays a dual immunogenic role in plant defense. Together, these studies revealed that co-evolutionary arms race tailored the multi-layered defense and counter-defense in plant-microbe interactions.

P5.2-014

XEG1: A CASE STUDY OF MICROBIAL ATTACK AND PLANT IMMUNITY IN THE APOPLAST

WANG Yan. (1), MA Zhenchuan. (1), XIA Yeqiang. (1), **WANG Yuanchao. (1)**

(1) Nanjing Agricultural University, Nanjing, CHINA

Text
The apoplast constitutes a major interaction niche in plant-microbe interactions. During

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P5.2-015

A PHYTOPHTHORA SOJAE RXLR EFFECTOR IMPACT HOST DEFENSE-ORIENTED TRANSCRIPTOME REPROGRAMMING BY TARGETING SOYBEAN MEDIATOR SUBUNIT 21

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Text

Upon pathogen infection, plant-activated defense systems undergo dramatic transcriptome reprogramming. In eukaryotes, conserved mediator complex bridges transcription factors and RNA polymerase II to regulate the transcription of specific genes. Mediator complex affects almost all stages of transcription and plays an essential role in the transition from normal development to immune response. However, pathogens may manipulate host transcription by delivering effectors into plant cells. Here, we report that the nucleus-localized RxLR effector PsAvh109 of *Phytophthora sojae* regulates plant immunity by interacting with the soybean (*Glycine max*) mediator subunit 21 (GmMED21). We further showed that GmMED21 is a positive regulator of plant resistance to pathogen. Silencing of *MED21* in *Nicotiana benthamiana* suppresses the expression of salicylic acid (SA) signaling pathway genes, leading to increased pathogen infestation. Consistent with this, over-expression of *PsAvh109* in soybean also suppressed the genes response to SA signaling pathway and significantly enhanced the invasion by *P. sojae*. In addition, we found that the nuclear localization of the effector PsAvh109 is crucial for its action. The further research reveals Avh109 blocks the function of host core Mediator. As a result, our study identifies a regulatory mechanism by which pathogen effectors target the mediator complex to regulate the transcription of plant defense genes.

P5.2-016

UNDERSTANDING THE EARLY EVENTS OF PLANT INFECTIONS BY OOMYCETES, AT NEW SPATIO-TEMPORAL SCALES: FROM ATTRACTION AND AGGREGATION OF ZOOSPORES TO HOST PENETRATION

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Text

Plant pathogens have evolved a wide range of strategies enabling surface colonization and invasion of host despite the plant defense mechanisms. Current knowledge of small spatio-temporal scales on mechanisms allowing attraction toward hosts and progression across each first plant cell layer remains sparse. To characterize which host signals and plant cell functions regulate zoospore attraction and penetration, we developed a multidisciplinary study of the rhizospheric dialogue between the telluric oomycete, *Phytophthora parasitica* and Arabidopsis. On the one hand, we generated new phenotyping tools dedicated to the short time-scale quantification of both zoospores behavior swimming in the presence of ionic signals and aggregation on the root surface. On the other hand, we defined the transcriptome of roots and zoospores during attraction of *P. parasitica* and the translatome of each root cell layer during the penetration of zoospores. Thus, we showed that (1) the zoospores aggregated on root in the first minute after inoculation, (2) both roots and zoospores stimulated transcriptomic changes during attraction, and (3) when *P. parasitica* penetrated the rhizodermis, the translatomes were also modulated beyond in the cortex, the endodermis and the stele while these cell layers are not yet colonized. The implication of these results in understanding the early stages of infection, at short spatio-temporal scales, and their use for disease control will be discussed.

P5.2-017

DIFFERENCES IN AVR-VNT1 ALLELES AND AGGRESSIVENESS IN THREE EUROPEAN PHYTOPHTHORA INFESTANS LINEAGES

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Text

Late blight, caused by the oomycete pathogen *Phytophthora infestans*, is one of the most damaging diseases of potato worldwide. During infection, *P. infestans* secretes effector

proteins that manipulate host cell function and enable infection by hampering basal resistance of the plant. The pathogen reproduces both asexually and sexually, resulting in constant development of new clonal lineages with varying aggressiveness. The lineages are routinely defined by analysis of simple sequence repeat markers and mating types. Twelve *P. infestans* isolates, representing three recently emerged clonal linages (genotypes) in Europe, EU13_A2, EU37_A2 and EU41_A2, were tested for aggressiveness by inoculating the three potato cultivars Craigs Royal, Irys and Tarpan that are susceptible to *P. infestans.* Aggressiveness was measured using three values: latent period, lesion diameter and sporulation. To find out whether the different genotypes were similar in functional genes, the conserved effector gene *Avr-vnt1* was sequenced for all isolates. One isolate of the EU13_A2 had all three alleles (V1, V2, V3) of the *Avr-vnt1* effector gene while three isolates had the V1 and V3 alleles. All isolates of the EU37_A2 had the V1 and V2 alleles, and all isolates of the EU41_A2 genotype had all three alleles. The EU41_A2 genotype was least aggressive as it showed significantly lower sporulation and smaller lesion diameter compared to the other genotypes according to ANOVA followed by Tukey's test.

P5.2-018

ADAPTING TO SURVIVE: THE KEY TO THE SUCCESS OF THE BIOTROPHIC PATHOGEN PLASMOPARA VITICOLA

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Text

Biotrophic pathogens have an absolute dependence on their hosts. Adaptation is an imperative for their success since it also allows to endure changing conditions, as in the case of *Plasmopara viticola*, the grapevine downy mildew agent. It originates from North America, where it adapted to climate and autochthone host species, thus resulting in a complex pattern of coevolved traits. Several QTLs (*Rpv*) have been found in resistant American grapevines. Despite the high susceptibility of the host (*Vitis vinifera* L.), when first introduced in Italy *P. viticola* caused limited damages, due to its appearance in autumn. Soon, *P. viticola* adapted to climate causing serious losses, for the overlap with the susceptible stages of the grape, suggesting that adaptation also includes a temporal component. Adaptation to the host resistance and phenology have been investigated with a multidisciplinary approach. Transcriptomic analysis revealed unique genes encoding effectors modulated by *P. viticola* to evade resistance to *Rpv3-1*, the major QTL used for introgressing resistance traits in *V. vinifera*. The analysis of oospore maturation and germination dynamics showed a great adaptation of the oospore, that are able to modulate their behavior in correlation with plant phenology to maximize the infection possibilities. The results provide new insight into host-pathogen interaction that could improve the development of durable disease control by preventing the selection of adapted strains.

P5.2-019

REVEALING PRINCIPLES OF PHYTOPHTHORA ZOOSPORES SENSING AND MOTION PROPERTIES THROUGH A BIO-PHYSICAL APPROACH

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Text

In the soil, early infection events of *Phytophthora* species are mediated by sensory and propulsion capabilities of biflagellate unicellular zoospores, orchestrated by rhizospheric guidance factors. Lack of detailed information on zoospores plasma membrane proteins prevents a comprehensive understanding of how they contribute to the perception of rhizospheric environment, particularly during migration toward host plant. A bio-physical approach was developed to identify the molecular key-players mediating host-driven taxis. At first, the membrane protein repertoire of *Phytophthora parasitica* zoospores was investigated through LC-MS/MS approach, resulting in a distinct peptide signature between zoospores cell body and flagella plasma membranes. Then, using a microfluidic set-up, functional biomechanics analyses were developed to quantify both zoospore motion (velocity, trajectory and cell rotation) and flagella beating (frequencies and oscillation amplitude) in response to distinct rhizospheric stimuli. The set-up further enabled to discriminate zoospores specific stimuli response among other rhizospheric microbial species. Altogether, the obtained results contribute to elucidate the mechanism of protein-mediated sensing and motion response of *Phytophthora* zoospores and improve the understanding of the complex rhizospheric interaction network driving oomycete dispersal.

P5.2-020

PHASE-SPECIFIC TRANSCRIPTIONAL PATTERNS OF THE OOMYCETE PATHOGEN PHYTOPHTHORA SOJAE UNRAVEL GENES ESSENTIAL FOR ASEXUAL DEVELOPMENT AND PATHOGENIC PROCESSES

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Text

Oomycetes are filamentous microorganisms easily mistaken as fungi but differ in physiology, biochemistry, and genetics. These differences are also reflected in the large variations in functional genes targeted by conventional fungicides. Thus, identifying novel functional genes for precisely controlling oomycete diseases is necessary. Therefore, this study reboots the analysis from available transcriptome data in a model oomycete pathogen, Phytophthora sojae, to excavate more functional genes. A set of expression matrix of 10,953 genes across ten stages were applied to recognize the transcriptional changes underlying critical stages for disease spreading. Based on hierarchical clustering, specification, and diversification analyses, we focused on the developmental stages due to their more remarkable transcriptional plasticity than the infection stages, and eventually these genes were resolved into six modules with their expression distinctness. Detailed analysis of these six modules identified candidate genes with stage-specific expression, including a serine/threonine phosphatase expressed in mycelial and sporangium stages, a histidine kinase expressed in zoospore and cyst stages, and a bZIP transcription factor exclusive to cyst germination.

Finally, diversiform gene editing tools were applied to obtain mutants of these three genes, and they were functionally verified with multiple phenotypes covering phase-specific developmental and infection stages.

P5.2-021

THE NON-CANONICAL AND OOMYCETE-SPECIFIC BAG IS ESSENTIAL FOR THE MAINTENANCE OF PROTEOSTASIS IN PHYTOPHTHORA SOJAE CYST GERMINATION

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Text
The maintenance of protein homeostasis is vital for the survival of all living organisms, and this process is carried out by molecular chaperones. The Bcl-2-associated athanogene (BAG) family is a conserved group of co-chaperones that helps maintain proteostasis in animals, plants, and fungi. This family functions by binding to heat shock protein 70 (HSP70) through its C-terminal BAG domain (BD). In this study, researchers discovered an unconventional subfamily of BAGs in oomycetes, which contain an N-terminal BD with a short, species-specific α1 helix and a unique C-terminal small heat shock protein (sHSP) domain. These BAGs are crucial for the germination of cysts and the pathogenicity of the oomycete pathogen Phytophthora sojae. Moreover, researchers found that PsBAGs play a role in the unfolded protein response, assisting in the degradation of misfolded proteins through the 26S proteasome. Both the BD and sHSP domains are necessary for the proper function of Phytophthora sojae, and these proteins form a homodimer through the unique α1 helix instead of the classical BAG-HSP70 complex. This study highlights a new mechanism for the protective role of oomycete BAGs, providing a potential target for disease control.

P5.2-022

AN AGO PROTEIN IS REQUIRED FOR AVIRULENCE GENE SILENCING IN AN OOMYCETE PLANT PATHOGEN

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Text
Successful pathogens can rapidly overcome host resistance through epigenetic silencing, but the underlying mechanisms of epigenetic variation are largely elusive. Based on genome-wide association study, we identified a natural allele of an Argonaute protein in *Phytophthora sojae* that confers adaptability to resistance soybean cultivar. Knockout of *PsAGO2* impaired avirulence gene *Avr1b* silencing and the *psago2* mutants were recognized by soybean cultivar carrying *Rps1b*. Further data revealed that PsAGO2 can bind 24-26 nt sRNAs and recruit the histone methyltransferase complex PRC2 to establish H3K27me3 at *Avr1b* loci. Our finding supports a model in which H3K27me3 formation is mediated by sRNA in

oomycete, highlighting the role of a new function of AGO protein in epigenetic gene silencing in a plant pathogen.

P5.2-023

SLIMS GO BIG: ROLE OF SHORT LINEAR MOTIFS (SLIMS) IN PHYTOPHTORA PARASITICA "CORE" PPRXLR1 EFFECTOR

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Text

The relationship between microbial pathogens and their host plants is epitomized by an endless arms race in the battle for survival. Successful plant pathogens, including *Phytophthora* spp. are known to secrete specific effector proteins to manipulate host immune responses. Examples of such effectors are the RxLR effector proteins, named after conserved Arg?any amino acid?Leu?Arg (RxLR) motif at the N-terminus. Our understanding of specific functions of RxLR effectors is limited by lack of knowledge concerning the motifs that facilitate functioning of these effectors at the cellular and molecular level. In this study, a highly conserved PpRxLR1 effector from *P. parasitica* was shown to encode a six aa long short linear motif (SLiM). We established that the SLiM mediates cell death inducing activity of PpRxLR1 effector that promotes the infection of P. parasitica. Similarly, the SLiM was shown to facilitate the interaction between PpRxLR1 effector and its host target protein. However, it was shown to be dispensable for effector subcellular targeting into the host cell. Together, our findings indicate that PpRxLR1 could be an important virulence RxLR effector of *P. parasitica,* promoting the pathogen's infection with the help of its SLiM. Uncovering the mechanisms of effector interference with targeted host functions is a critical step towards understanding host-pathogen interactions. Ultimately, this can be harnessed to breed for durable resistance in plants.

P5.2-024

PECTIN METHYLESTERASES INHIBITOR MODULATE PLANT HOMOGALACTURONAN STATUS IN DEFENSES AGAINST THE PHYTOPHTHORA SOJAE

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Text

Pectin Methylesterases Inhibitor Modulate Plant Homogalacturonan Status in Defenses against the *Phytophthora sojae*

Yeiqiang Xia et al., Yan Wang, Yuanchao Wang*

Hosts and pathogens are engaged in a continuous struggle for physiological dominance that drives the evolution and specialization of key defense and virulence proteins. A major site on the struggle is the plant cell wall. Here, we show the involvement of the dynamic remodeling pectin methylesterification of cell wall in the co-evolutionary struggle between host and microbe. Pathogen-secreted apoplastic pectin methylesterases, PsPME1, that loosening the plant cell wall and synergizing the activity of pathogen secreted endo-polygalacturonases by decreased the degree of pectin methylesterification. However, GmPMEI, a soybean produced pectin methylesterases inhibitor protein, expression controlled by PME-related damage-associated molecular patterns that binds to both soybean and *P. sojae* pectin methylesterases and inhibits them enzyme activity to remodeling the pectin to high methylesterification status for protecting themselves from enzymatic degradation. Totally, our work highlights that plants exploit induced defense mechanisms based on biochemical modification on the cell wall in shaping the balance of the arms race in the co-evolutionary conflict between host and microbe.

P5.2-025

INVESTIGATION OF THE ROLE IN VIRULENCE OF PHYTOPHTHORA INFESTANS EFFECTOR PI06099.

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Text

Plant pathogens secrete many effector proteins which are translocated inside plant cells and act to suppress host defences and promote pathogen colonisation. The RxLR effector Pi06099 from potato late blight pathogen *Phytophthora infestans* interacts with the plant red light receptor Phytochrome B (PhyB). Red light promotes plant immunity by accelerating cell death in response to the *P. infestans* MAMP INF1. Silencing the *Pi06099* effector using Host Induced Gene Silencing (HIGS) and stable RNAi transgenic lines demonstrated that it contributes to the virulence of *P. infestans* on Potato and *Nicotiana benthamiana*. Furthermore, domain swapping and mutagenesis of Pi06099 has been used to investigate the disruption of the interaction with PhyB and phenotypes associated with effector virulence.

P5.2-026

FUNCTIONAL DIVERGENCE OF A GLYCOSIDE HYDROLASE AND ITS DECOY PARTNER IN PHYTOPHTHORA EVOLUTIONARY CONTINUUM

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Text

Plants and pathogens constantly adapt to each other. In turn, the adaptation pressures impose on pathogens shape their variations and the fitness towards corresponding hosts. Our study shows that the XEG1/XLP1 gene pair originated from Phytophthora, and they had functioned differently through the evolution of Phytophthora, which is caused by the dependence of Phytophthora on its virulence function. Based on the distribution, phylogeny, collinearity, and gene arrangement, it appears that the XEG1/XLP1 gene pair originated with the Phytophthora. As well, Different Phytophthora XEG1/XLP1 paralogs exhibit different abilities to induce cell death, induce the expression of defense genes, and produce reducing sugars. Actually, XEG1/XLP1 ancestor genes produced reducing sugars less efficiently and induced immunity less effectively. A further analysis revealed that XEG1/XLP1 displayed different selection pressures, and these pressure sites were crucial to its ability to produce reducing sugars and induce immune. Meanwhile, the replacement experiments with ancestral genes indicate XEG1/XLP1 virulence is closely related to its ability to produce reducing sugar, and as virulence increases, immune activation increases as well. Overall, the results show that XEG1 and its decoy partner XLP1 continuously adapted to their hosts through functional divergence during Phytophthora evolution.

P5.2-027

SCREENING OF ALFALFA VARIETIES RESISTANT TO PHYTOPHTHORA CACTORUM AND RELATED RESISTANCE MECHANISM

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Text

Alfalfa is one of the most important legume forages in the world. Root rot caused by soil-borne pathogens severely restricts the production of alfalfa. The knowledge of the interaction between alfalfa and root rot-pathogens is still lacking in China. Phytophthora cactorum was isolated from symptomatic seedlings of an alfalfa field in Nanjing with high levels of damping-off. We observed the different infection stages of P. cactorum on alfalfa, and found that the purified P. cactorum strain was aggressive in causing alfalfa seed and root rot. By evaluating the resistance of 37 alfalfa cultivars from different countries to P. cactorum, we found Weston is a resistant variety, while Longdong is a susceptible variety. We further compared the activities of various enzymes in the plant antioxidant enzyme system between Weston and Longdong during P. cactorum infection, as well as gene expression associated with plant hormone biosynthesis and response pathways. The results showed that the disease-resistant variety Weston has stronger antioxidant enzyme activity and high levels of SA-responsive PR genes, when compared to the susceptible variety Longdong. These findings highlighted the process of interaction between P. cactorum and alfalfa, as well as the mechanism of alfalfa resistance to P. cactorum, which provides an important foundation for breeding resistant alfalfa varieties, as well as managing Phytophthora-caused alfalfa root rot.

P5.2-028

RNASEQ VS ENRICHMENT SEQUENCING TECHNIQUES: LIFTING THE LID ON THE POTATO-P. INFESTANS INTERACTIONS

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Text

Late blight, caused by an oomycete; Phytophthora infestans continues to be the primary disease of concern for potato farmers. The pathogen virulence is attributed to the presence of effectors (pathogenicity factors), which if recognised by plant resistance (R) genes, triggers the immune response. Not surprisingly P. infestans has evolved accordingly, which has driven effector diversity in P. infestans populations. Yet, our understanding of this adaptive evolution remains poor. Therefore, tracking effector diversity in light of disease resistance gene deployment is critically important if robust IPM strategies are to be developed in support of Farm to Fork (F2F) goals.

Enrichment sequencing techniques enable cost effective, high-confidence identification of functional R genes and effectors. Although merits of enrichment sequencing over WGS has been previously demonstrated, but its comparison with gene expression remains elusive. In this regard, P. infestans infected potato plants were subjected to RNAseq, PenSeq and RenSeq at different time points. The analysis reveals the presence of some unknown effectors (PITG_08949, PITG_14932, PITG_02900, etc.) with 100% gene coverage at very early stages of infection which was otherwise not possible with RNAseq.

This information combined with R gene expression during infection have potential to widen our current knowledge of potato-P. infestans interactions, and can be used as a platform for introducing informed phase of potato breeding.

P5.2-029

THE STRAWBERRY-PHYTOPHTHORA CACTORUM INTERACTION

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Text

Phytophthora cactorum causes crown rot, a devastating disease of strawberry plants, by attacking its roots and rhizome. Many commercial octoploid strawberry cultivars are susceptible, while many accessions of the wild diploid species *Fragaria vesca* are resistant to *P. cactorum*. To gain insights into the strawberry defence mechanisms and virulence mechanisms of *P. cactorum*, comparative transcriptome profiles of two resistant and one susceptible genotype of *F. vesca* were analysed by RNA-sequencing after inoculation with *P. cactorum*.

Differential gene expression analysis identified thirty-one putative disease resistance genes in *F. vesca* that were highly expressed in the resistant genotypes relative to the susceptible genotype after inoculation with *P. cactorum*. These included genes encoding receptor-like

proteins, receptor-like kinases, and leucine-rich repeat proteins with nucleotide binding sites. Furthermore, approx. 4600 *P. cactorum* genes were detected, of which 544 were predicted to encode secreted proteins that are potential effectors of importance to the pathogen's virulence.
Selected differentially expressed *F. vesca* genes and effector genes from *P. cactorum* were studied by transient expression in the model plant *Nicotiana benthamiana*. Some of these induced cell death or hypersensitive response, including accumulation of reactive oxygen species and callose deposition in the plant cell wall, indicating functional relevance in the plant-pathogen interaction.

P5.2-030

PUNCHING TRANSIENT SMALL HOLES INTO PLANT PLASMA MEMBRANE: THE UNIQUE CASE OF AN OOMYCETE NLP CYTOLYSIN

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Text
Necrosis and ethylene-inducing peptide 1-like proteins (NLPs) are produced by a variety of phytopathogens. Many NLPs cause cell death and tissue necrosis by disrupting the plant plasma membrane. Glycosylinositol phosphorylceramides (GIPC), the most abundant class of plant sphingolipids, are targets for NLP binding to membranes. Just recently, it was shown that this lipid recognition is electrostatic-driven and leads to shallow membrane binding, protein aggregation, and transient pore formation.
We are exploiting various model lipid systems, composed of plant-isolated GIPC, e.g. cell-sized vesicles, which are conveniently followed with confocal microscopy. Visual information after NLPPya – membrane interaction about localized toxin binding, changes in the morphology of the vesicles, and differential leakage of different-sized probes allows making predictions about membrane-damaging mechanisms. Furthermore, molecular dynamics simulations revealed that the C-terminal α-helix of NLPPya undergoes conformational rearrangements during membrane interactions. We are tackling this clue by designing mutants of NLPPya, among which cysteine ones are of special interest. Introduced cysteines are labeled with IANBD to monitor the insertion of these residues into the lipid bilayer by changes in its fluorescence.
Our study will contribute specific molecular insights into the toxic NLPs- plant membrane interaction which is crucial for the development of better strategies for crop protection.

P5.2-031

RENSEQ AS A ROBUST TOOL TO IDENTIFY NOVEL DISEASE RESISTANCE GENES IN WILD AND CULTIVATED POTATOES

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Text
Late Blight (caused by Phytophthora infestans), leads to an annual loss of ~€6 bn across the globe. Successful introgression of disease resistance (R) genes, mostly belonging to the nucleotide-binding, leucine-rich-repeat (NLRs) gene family, from wild species into commercial potato varieties provide a means to protect crops from the emerging virulent P. infestans isolates. NLRs account for only 0.25% of the entire genome, thus WGS for functional NLRs identification is a costly process. Resistance gene enrichment and sequencing (RenSeq), if used in a diagnostic mode (dRenSeq) can be utilised as a cost-effective, robust R gene identification tool. It led to the discovery and annotation of about 755 NLRs from Solanum tuberosum group Phureja clone DM.
The present study was focused on identifying broad-spectrum novel R genes from important potato species. The resistant accessions found through detach leaf assay were subjected to dRenSeq to gain insight into NLR profile. Our analysis revealed that although the cultivar Athlete is highly resistant to P. infestans, the source of resistance remains unknown. Therefore, two F1 progenies of Athlete X Gemson and Athlete X Ivory Russet were subjected to bulk segregant analyses in combination with enrichment sequencing to identify and map underlying novel resistances. The identified R gene(s) can be further deployed in potato cultivars through breeding programmes to reduce crop loss and thereby contribute towards sustainable food security.

Molecular drivers of plant bacterial interactions

C2.1-1

THE MAKING OF A PATHOGEN: HOW XANTHOMONAS ADAPTS TO PLANT ENVIRONMENTS

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Text
Xanthomonas campestris pv *campestris* (*Xcc*) is a phytopathogenic bacterium which causes black rot disease on cultivated and wild Brassicaceae. During its disease cycle, *Xcc* experiences changing environments from the leaf surface to distinct endophytic compartments (hydathode, xylem and mesophyll), seeds and plants residues. So far, genetic screens have only identified major determinants important for pathogenicity. Mechanisms of pathogen entry, vascular immunity suppression, metabolic adaptation and microbial fitness in the distinct plant environments, are essentially unknown. In order to identify bacterial determinants of plant adaptation we have performed high-throughput RB-TnSeq screens during colonization of different plant compartments. The results of these screens yields important insights into the physiological state of the bacterium during the infection process and to the identification and characterization of virulence determinants.

C2.1-2

ROLE OF PHLOEM MEMBRANE CONTACT SITES ON CANDIDATUS LIBERIBACTER ASIATICUS INFECTION

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Text
Synaptotagmin A (SYTA) is a protein responsible, together with NET3C and VAP27, for the formation of ER-PM membrane contact sites (MCSs). MCSs are regions in which two membranes come into close contact without fusing. MCSs are characterized as sites for membrane trafficking and Ca^{2+} fluxes, being Ca^{2+} dependent, and resealing membranes after damage. Besides its role in the MCSs formation, SYTA plays a role in the movement and replication of plant viruses, and plants with downregulated SYTA showed enhanced resistance to the pathogens. Here we studied the role of SYTA during *Candidatus* Liberibacter asiaticus (*C*Las) infection, an unculturable gram-negative bacterium that inhabits the sieve elements of Citrus *spp*. In Arabidopsis, SYTA::RFP co-localized with SUC2::GFP in the phloem. Moreover, in *syta-1* mutants, the cisterna shape of the sieve element reticulum (SER) was abnormal and detached from the PM. In Citrus, the downregulation of phloem SYTA with citrus tristeza virus (CTV) VIGS delayed the onset and severity of the symptoms in CTV-syta plants. Phloem plugging genes were downregulated, and the total area of callose deposits was lower. We suggest that the disruption of the SER could play a pivotal role in delaying the infection: this could affect the attachment of the pathogen to the SER and, therefore, disturb the trophic host-pathogen interaction.

C2.1-3

FOR BETTER OR WORSE - THE EFFECT OF INTERSPECIES MICROBIAL INTERACTIONS ON DISEASE SEVERITY IN PLANTS

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Text

Bacteria have evolved a large array of signaling pathways that allow them to reprogram behavior in response to environmental conditions. One such pathway is the carbon catabolite repression (CCR) pathway, in which the presence of glucose results in the inhibition of specific genes. In this regard we found that when *Paenibacillus* spp. bacteria are grown on glucose their motility is inhibited, thereby restricting them to a favorable environment. However, when these colonies are grown in proximity to other bacterial species, such as the plant pathogens *Xanthomonas perforans* or *Acidovorax citrulli*, the CCR pathway is overridden and the *Paenibacillus* cells start migrating towards their neighbors. Notably, a directional swarming induction of *Paenibacillus* cells by neighboring colonies was observed even when the colonies were inoculated on media without glucose. We further show that, when inoculated on plants, the interaction of *Paenibacillus* swarms with these phytopathogens could have two opposing effects on plant health. The outcome could either be harmful or helpful to the plant, depending on the characteristics of the *Paenibacillus* species in the swarm. Our results suggest that in mixed populations, interspecies interactions can affect the community's spatial organization and significantly impact disease outcomes in plants.

C2.1-4

CRISPR/FNCAS12A-MEDIATED EFFICIENT MULTIPLEX AND ITERATIVE GENOME EDITING IN BACTERIAL PLANT PATHOGENS WITHOUT DONOR DNA TEMPLATES

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Text

CRISPR-based genome editing technology is revolutionizing prokaryotic research, but it has been rarely studied in bacterial plant pathogens. Here, we have developed a targeted genome editing method with no requirement of donor templates for convenient and efficient gene knockout in *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), one of the most important bacterial pathogens on rice, by employing the heterologous CRISPR/Cas12a from *Francisella novicida* and NHEJ proteins from *Mycobacterium tuberculosis*. FnCas12a nuclease generated both small and large DNA deletions at the target sites as well as it enabled multiplex genome editing, gene cluster deletion, and plasmid curing in the *Xoo* PXO99^{A} strain. Accordingly, a non-TAL effector-free polymutant strain PXO99^{A}D25E, which lacks all 25 xop genes involved in *Xoo* pathogenesis, has been engineered through iterative genome editing. Whole-genome sequencing analysis indicated that FnCas12a did not have a noticeable off-target effect. In addition, we revealed that these strategies are also suitable for targeted genome editing in another bacterial plant pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*). We believe that our bacterial genome editing method will greatly expand the CRISPR study on microorganisms and advance our understanding of the physiology and pathogenesis of *Xoo.*

C2.1-5

HOW PROTEOSTASIS SHAPES PLANT-BACTERIA INTERACTIONS

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Text

Protein homeostasis is epitomized by a tight equilibrium of protein biosynthesis and degradation; the 'life and death' of proteins. Approximately one-third of newly synthesized proteins are degraded. As such, regulated protein turnover is required to maintain cellular integrity and survival. Autophagy and the ubiquitin-proteasome system (UPS) are the two principal intracellular degradation pathways in eukaryotes. Both degradation pathways orchestrate many cellular processes during plant development and upon environmental stimuli. As such, both pathways play a major role during plant-microbe interactions. We have recently identified that autophagy and the proteasome system are exploited by bacterial pathogens to reprogram host cellular pathways. By studying this intimate interplay, we can utilize plant pathogenic bacteria as tools to understand host cellular degradation machineries and to decipher novel components and functions. In my presentation, I will not only cover our recent work on the role of autophagy and the proteasome in plant-microbe interactions but will report on our attempts to identify new autophagy regulators and new functions of known UPS components. I will highlight different examples and discuss our recent advances.

C2.1-6

CRISPRI AS A TOOL FOR THE FUNCTIONAL STUDY OF GENE FAMILIES IN XANTHOMONAS

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Text

The *Xanthomonas* genus includes several plant pathogens responsible for significant crop losses worldwide. Most of the functional studies of virulence in this genus have been carried out by directed mutagenesis through homologous recombination. However, this strategy is

cumbersome for the study of gene families. CRISPR interference (CRISPRi) allows precise silencing of target genes by using a catalytically dead Cas9 (dCas9) which interferes with gene expression. Because of its RNA-directed nature, this technology can be used for silencing several genes in a single experiment in bacteria and other organisms. We implemented a CRISPRi strategy to silence several members of the Transcriptional activator-like effectors (TALE) gene family at once in four different species of *Xanthomonas*. Our results underscore the importance of the activation of the *SWEET* gene family in cassava upon infection by *Xanthomonas phaseoli* pv. *manihotis*. Remarkably, we report the importance of this gene family in the infection of this host by the non-vascular pathogen X. cassavae. In addition, we successfully silenced several TALE genes in a total of five species, including *X. oryzae* pv. *oryzae*, *X. citri* pv. *citri* and *X. campestris* pv. *campestris* using CRISPRi, confirming the importance of this gene family in these pathosystems. The CRISPRi tool can be further modulated to silence sets of genes within a gene family for functional studies in *Xanthomonas* and other plant pathogenic bacteria.

F2.1-1

PLANT-ENCODED ARTIFICIAL SMALL RNAS DIRECT GENE SILENCING IN PSEUDOMONAS SYRINGAE AS WELL AS DISEASE PROTECTION

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Text

Plant small RNAs (sRNAs) can trigger non-cell autonomous RNA interference (RNAi) in interacting eukaryotic pathogens or parasites possessing canonical RNAi factors. However, it is currently unknown whether a similar process could operate against a phytopathogic bacterium, which lacks a eukaryotic-like RNAi machinery. We recently demonstrated that Arabidopsis-encoded artificial sRNAs can trigger the sequence-specific silencing of a virulence factor from *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto* DC3000). However, the sRNA species that are implicated in this phenomenon remain elusive. In the present study, we identified and characterized two populations of apoplastic sRNAs that orchestrate antibacterial gene silencing. The first one involves sRNAs that are associated with extracellular vesicles (EVs), and presumably incorporated in ribonucleoprotein complexes. Intriguingly, the second one involves sRNA duplexes that are in a free form, and thus referred to here as extracellular free small RNAs or efsRNAs. Here, I will present the experimental data supporting these findings. I will also discuss the relevance of these findings in the understanding of how plants regulate transcriptome, community composition and genome evolution of associated bacteria.

F2.1-2

SA-INDEPENDENT MECHANISM IN THE TOMATO DIAGEOTROPICA (DGT) MUTANT ENHANCE ROOT-MEDIATED RESISTANCE TO RALSTONIA SOLANACEARUM K60

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Text

Bacterial Wilt is among the most devastating plant diseases in the world. This disease is caused by the soilborne *Ralstonia solanacearum* (*Rs*) and affects more than 200 species of plants. In tomato, resistance to *Rs* is quantitative and the result of many genes and no resistance genes to the US *Rs* strain K60 have been identified in tomato. Transcriptomic analysis of resistant tomato roots showed that at 48 hours post inoculation with *Rs*K60, genes involved in auxin transport and signaling pathways are downregulated. A tomato mutant defective in auxin transport and signaling, known as *diageotropica* (*dgt*) has enhanced resistance to *Rs*K60. Auxin acts antagonistically with the plant hormone Salicylic Acid (SA), and we found that *dgt* roots have endogenously higher levels of SA and a strain of *Rs* that can degrade SA is partially virulent on *dgt*. However, after inoculation with *Rs*K60, the expression of SA-dependent response genes is not activated and the SA-deficient double mutant *dgtxNahG* is still resistant to *Rs*K60. Inoculation with *Pseudomonas syringae* pv *tomato* showed that *dgt* is susceptible to this foliar pathogen. Our research suggests that the resistant response of the *dgt* mutant to *Rs*K60 may be due a SA-independent mechanism in roots and that the *DGT* gene and proper auxin transport and signaling are important for susceptibility to *Rs*K60 in tomato roots. Understanding the role of DGT and auxin in defense responses to *Rs* in tomato is important for Solanaceae crop improvement.

F2.1-3

GENETIC STRUCTURE OF XANTHOMONAS ORYZAE PV. ORYZAE POPULATIONS AND DIVERSITY OF THEIR TAL EFFECTOR REPERTOIRES IN BURKINA FASO

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Text

Bacterial Leaf Blight of rice (BLB) caused by Xanthomonas oryzae pv. oryzae (Xoo) is a major threat for food security in many rice growing countries including Burkina Faso where the disease was reported first in the 1980's. In line with the intensification of rice cultivation in West-Africa, BLB has been on the rise along the last 15 years. West-African strains of Xoo differ from their Asian counterparts as they (i) are genetically distant, (ii) belong to new races and, (iii) contain reduced repertoires of Transcription Activator Like (TAL) effector genes. In order to investigate the evolutionary dynamics of Xoo populations in Burkina Faso, 177 strains were collected from 2003 to 2018 in three regions where BLB is occurring. Multilocus VNTR Analysis (MLVA-14) targeting 10 polymorphic loci enabled to discriminate 24

haplotypes and showed that Xoo populations were structured according to their geographical localization and year of collection. Considering their major role in Xoo pathogenicity, we next surveyed the TAL effector repertoires of the 177 strains upon RFLP-based profiling. Surprisingly an important diversity was revealed with up to eight different RFLP patterns. Finally, comparing neutral vs. TAL effector gene diversity allowed to suggest scenarios underlying the evolutionary dynamics of Xoo populations in Burkina Faso, which could be helpful to guide the deployment of BLB resistant varieties in the country.

P2.1-001

INCREASING THE RESILIENCE OF PLANT IMMUNITY TO A WARMING CLIMATE

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Text

Extreme weather conditions associated with climate change affect many aspects of plant life, including the response to infectious diseases. Production of salicylic acid (SA), a central plant defense hormone, is particularly vulnerable to suppression by short periods of hot weather above the normal plant growth temperature range. We recently found that suppression of SA production in Arabidopsis thaliana at 28 °C is independent of PHYTOCHROME B (phyB) and EARLY FLOWERING 3 (ELF3), which regulate thermo-responsive plant growth and development. Instead, we found that formation of GUANYLATE BINDING PROTEIN-LIKE 3 (GBPL3) defense-activated biomolecular condensates (GDACs) was reduced at the higher growth temperature. The altered GDAC formation in vivo is associated with impaired recruitment of GBPL3 and SA-associated Mediator subunits to the promoters of CBP60g and SARD1, which encode master immune transcription factors. Unlike many other SA signaling components, including the SA receptor and biosynthetic genes, optimized CBP60g and SARD1 expression was sufficient to broadly restore SA production, basal immunity and NLR-mediated immunity at the elevated growth temperature without significant growth trade-offs. CBP60g family transcription factors are widely conserved in plants. These results have implications for understanding the concept of the “plant–pathogen–environment” disease triangle and the emergence of new disease epidemics in a warming climate.

P2.1-002

THE RALSTONIA PSEUDOSOLANACEARUM TYPE III EFFECTOR RIPL DELAYS FLOWERING AND PROMOTES SUSCEPTIBILITY TO PATHOGEN IN PLANTS

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Text

The plant defense responses to microbial infection are tightly regulated and integrated with the developmental program for optimal resource allocation. Several types of association between disease susceptibility or resistance and the floral transition have been reported. The functional characterization of pathogen virulence factors, termed effectors, provides molecular insights into plant disease susceptibility. Ralstonia pseudosolanacearum injects tens of effectors in the host cells that collectively promote bacterial proliferation inside the vascular tissues and cause bacterial wilt in many solanaceous crops. Here, we characterized the function of the broadly conserved effector RipL, through heterologous expression in Arabidopsis thaliana. RipL-expressing transgenic lines presented delayed flowering which correlated with a low expression of flowering regulator genes. In parallel, RipL promoted plant susceptibility to virulent strains of Pseudomonas syringae in the effector-expressing lines or when delivered by the type III secretion system. Although the SA-dependent immune signaling was not significantly affected by RipL expression, RNA-seq analysis of infected RipL-expressing lines revealed that the overall amplitude of the transcriptional response was dampened compared to the control line. Together, our results indicate that RipL could contribute to the pathogen virulence in an SA-independent manner.

P2.1-003

SSTF, A NOVEL SULFORAPHANE SENSING TRANSCRIPTION FACTOR OF XANTHOMONAS CAMPESTRIS, IS REQUIRED FOR SULFORAPHANE TOLERANCE AND VIRULENCE

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Text

Avoiding the host defence system is necessary for the survival of pathogens. However, the mechanism by which pathogenic bacteria sense and resist host defence signals is still unknown. Sulforaphane (SFN) is a secondary metabolite of crucifers. It not only plays an important role in maintaining the local defence response but also directly inhibits the growth of some pathogens. In this study, we identified a key SFN tolerance-related gene, *saxF*, in *Xanthomonas campestris* (*Xcc*), the causal agent of black rot of crucifers. More interestingly, we found that the transcription of *saxF* was regulated by the novel transcription factor SFN sensing transcription factor (SstF). As a LysR family transcription factor, SstF can sense SFN and regulate the expression of *saxF* cluster genes to increase SFN resistance by directly binding the promoter of *saxF*. In addition, we found that SstF and *saxF* also play an important role in positively regulating the virulence of *Xcc*. Collectively, our results illustrate a previously unknown mechanism by which *Xcc* senses the host defence signal SFN and activates the expression of SFN tolerance-related genes to increase virulence. Therefore, this study provides a remarkable result, that is, during pathogen-plant coevolution, new functions of existing scaffolds are activated, thus improving the proficiency of the pathogenic mechanism.

P2.1-004

IDENTIFICATION OF GENES REQUIRED FOR PTR1-MEDIATED IMMUNITY IN NICOTIANA BENTHAMIANA

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Text

The *Ptr1* (*Pseudomonas tomato race 1*) gene confers resistance to race 1 strains of *Pseudomonas syringae pv. tomato* and to *Ralstonia pseudosolanacearum* by recognizing the type III effector AvrRpt2 or RipBN, respectively. To identify host proteins that play a role in Ptr1-mediated immunity, we performed virus-Induced gene silencing (VIGS) on *Nicotiana benthamiana* plants, which naturally expresses Ptr1, to knock down the expression of various immunity-associated proteins and protein kinases. Cell death assays using *Agrobacterium* carrying AvrRpt2 revealed that plants silenced for the *RAR1*, *SIPK*, *SIPKK*, *MKK1*, and *Epk1* genes showed less or no Ptr1-associated cell death in response to AvrRpt2, indicating their involvement in the Ptr1 response. Interestingly, the *Nrc2/3* genes, which are required for the function of the Pto/Prf complex, showed no involvement in the Ptr1 response to AvrRpt2. The *SIPK*, *MKK1*, and *Epk1* genes play a role in both the Ptr1 and the Pto/Prf responses, whereas the *RAR1* and *SIPKK* genes only appear to play a role in the Ptr1 response. These results provide the foundation for investigating the role of different well-studied signaling kinases and immunity-associated proteins and their function in the Ptr1 pathway in response to AvrRpt2.

P2.1-005

A NOVEL PSEUDOMONAS CYCLIC LIPOPEPTIDE INDUCES PLANT IMMUNITY THROUGH CELL WALL PERCEPTION AND CYTOPLASTIC SIGNALING

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Text

Cyclic lipopeptides (CLPs) are multifunctional secondary metabolites produced by a large variety of bacteria and have emerged as an important category of plant immunity elicitors. Pseudomonas-CLPs (Ps-CLPs) are extremely diverse in structure and biological activity. However, current understanding of CLP-plant structure–function interactions remain elusive. Here, we identified medpeptin, a novel CLP from P. mediterranea, which consists of 22 amino acids and is synthesized by a non-ribosomal peptide synthase gene cluster and regulated by a quorum-sensing system. Further research indicates that medpeptin does not exhibit antimicrobial activity but instead induces plant cell death immunity and confers resistance to bacterial infection. Comparative transcriptome analysis and virus-induced gene silencing revealed a set of immune signaling candidates involved in medpeptin perception. Silencing of a cell wall leucine-rich repeat extensin protein (NbLRX3) or a receptor-like protein kinase (NbRLK25), but not BAK1 or SGT1, compromises medpeptin-triggered cell death and resistance to pathogen infection in Nicotiana benthamiana. Our findings point to a non-canonical mechanism of CLP sensing and suggest perspectives for the development of plant disease resistance.

P2.1-006

A UBIQUITIN FAMILY PROTEIN PLAYS A ROLE IN THE PATHOGENICITY OF ACIDOVORAX CITRULLI

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Text

Acidovorax citrulli is a plant pathogenic bacterium that causes bacterial fruit blotch (BFB) disease of cucurbits. Symptoms of BFB include seedling blight, necrotic spots in cotyledon and leaves, and blotch on the fruit that can result in significant yield losses. In this study, bioinformatic analysis was applied to rank genes according to their potential contribution to pathogenicity of plant-pathogenic Acidovorax species. BlastP analysis of one of the selected genes suggested it encodes a ubiquitin family protein. Signal peptide prediction tools indicated an N-terminal fused signal peptidase I (SPI) signal sequence, suggesting its secretion to the extracellular environment. We applied a marker-free mutagenesis approach to delete the entire ORF of this gene and found the mutant to be severely compromised in virulence on melon plants in a seed-to-seedling transmission assay. Furthermore, in-vitro growth curves indicated that the mutant differed from the wild type during the late-lag phase of growth and during the stationary phase, where it persisted for much longer than the wild type before the population declined. This is in line with much larger colonies formed by the mutant, with greater number of cells per colony and significantly longer cells than the wild type. We are currently investigating how this protein influences bacterial growth and virulence.

P2.1-007

DUAL RNA-SEQUENCING OF CHILI PEPPER CULTIVARS AND XANTHOMONAS EUVESICATORIA PV. EUVESICATORIA CAUSING BACTERIAL LEAF SPOT (BLS)

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Text

Chili pepper (*Capsicum annum*) is a globally important crop, but its production is impacted by Bacterial Leaf Spot (BLS), caused by *Xanthomonas euvesicatoria* pv. *euvesicatoria*. The

fine-scale interaction between the pathogen and host remains unknown. Utilizing stranded total RNA sequencing and the Illumina NovaSeq6000, high-depth Dual RNA-Sequencing was undertaken to investigate the host and pathogen gene expression profiles for a resistant and a susceptible cultivar over a time course of 0, 7, and 14 days after infection. A customized host-pathogen tailored ribosomal RNA (rRNA) depletion step removed >95% rRNA in all samples. The remaining sequences were mapped into the host (65-85%) and pathogen (0.01-8.9%) genomes. Differentially expressed genes (DEGs) were identified using fold change in expression and Manhattan distance of each treatment, compared to controls. The up-and-down-regulated genes among the resistant and susceptible chili pepper cultivars were identified, and gene ontology (GO) functional classes and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were assigned. Obtained KEGG pathways suggest that resistant and susceptible cultivars showed different responses against *X. euvesicatoria* pv. *euvesicatoria* infection. This study provides a comprehensive representation of the genes involved in disease progression from both the host and pathogen which presents as a model for the exploration of genetic factors in other plant-microbe interactions.

P2.1-008

NICOTIANA BENTHAMIANA AS A SURROGATE HOST OF TWO PLANT-PATHOGENIC CLAVIBACTER SPECIES

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Text

Clavibacter michiganensis and *C. capsici* are Gram-positive bacterial pathogens that cause bacterial canker in tomato and pepper, respectively, as host plants. In terms of detail symptoms, both bacterial pathogens cause canker in stems, blisters in leaves, and bird's eyes in fruits, and *C. michiganensis* additionally cause wilting in whole plants. Here we show that *Nicotiana benthamiana*, a commonly used model plant for studying molecular plant–pathogen interactions, is a surrogate host of *C. michiganensis* and *C. capsici*. When *C. michiganensis* and *C. capsici* were infiltrated into leaves of *N. benthamiana* with low concentration ($5x10^4$ cfu/ml), blister-like lesions were observed on the infiltrated sites, and their growth was dramatically increased about 10^4-fold like natural pathogens. Blister-like lesions were closely associated with cell death and the generation of reactive oxygen species. Infiltration with a high concentration (10^8 cfu/ml) of two *Clavibacter* species caused strong necrosis in leaves. When both pathogens were injected into *N. bethamiana* stems, they caused typical canker like in tomato and pepper, and *C. michiganensis* eventually caused wilting. These results suggest that both *Clavibacter* bacteria can cause all symptoms in *N. benthamiana* similar to those developed in tomato and pepper. We propose that *N. benthamiana* is a surrogate host of these *Clavibacter* pathogens and it can be used for identifying novel virulence factors.

P2.1-009

COMPARISON OF THE SIGNAL TRANSDUCTION EFFICIENCY IN THE VFM QUORUM SENSING SYSTEM OF THE GENUS DICKEYA ACCORDING TO THE POLYMORPHISM OF THE VFM GENES

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Text

The Vfm quorum sensing (QS) system is preponderant for the virulence of different species of the phytopathogenic bacteria of the genus *Dickeya*. In the model strain *D. dadantii* 3937, the Vfm QS system was shown to be responsible for the control of the production of plant cell wall degrading enzymes (PCWDEs). The transduction of the Vfm QS signal results into the activation of the promoter of the gene *vfmE* encoding a transcriptional regulator of the AraC family which itself activates the promoter of PCWDE genes. The *vfm* gene cluster includes 26 genes involved in the biosynthesis, sensing or transduction of the QS signal. It encodes several nonribosomal peptide synthetases (NRPS), indicating that the Vfm QS signal is a complex short peptide. To date, the Vfm QS signal has escaped detection by analytical chemistry methods. Using a strain-specific polymorphism in the NRPS genes *vfmO* and *vfmP* was shown to determine the production of different analogs of the Vfm QS signal. By analogy with the Agr QS system of *Staphylococcus aureus*, the production of different analogs of the signal is expected to be related to variations in the signal transduction activity of the Vfm QS system, resulting in variations in the level of activation of the promoter of the regulator gene *vfmE*. To explore this hypothesis, we used a reporter gene fused to the promoter of the gene *vfmE* to compare the activity of the *vfmE* promoter among strains of *Dickeya* producing different analogs of the Vfm QS signal.

P2.1-010

A PUTATIVE MULTI-SENSOR HYBRID HISTIDINE KINASE, BARAAC, INHIBITS THE EXPRESSION OF THE TYPE III SECRETION SYSTEM REGULATOR HRPG IN ACIDOVORAX CITRULLI

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Text

Bacterial fruit blotch, caused by *Acidovorax citrulli*, severely damages watermelon, melon, and other cucurbit crops worldwide. Although many virulence determinants have been identified in *A. citrulli*, including swimming motility, twitching motility, biofilm formation, and the type III secretion system (T3SS), research on their regulation is lacking. To study virulence regulation mechanisms, we found a putative histidine kinase $BarA_{Ac}$ that may

be related to the T3SS regulator HrpG in *A. citrulli*. We deleted and characterized $barA_{Ac}$ in *A. citrulli* Aac5 strain. Compared to the wild-type Aac5, virulence and early proliferation of $barA_{Ac}$ mutant in host watermelon cotyledons were significantly increased, and induction of hypersensitive response in non-host tobacco was accelerated, while biofilm formation and swimming motility were significantly reduced. In addition, the transcriptomic analysis revealed that the expression of many T3SS-related genes was upregulated in the Δ$barA_{Ac}$ deletion mutant when cultured in KB medium. Meanwhile, the Δ$barA_{Ac}$ deletion mutant showed increased accumulation of the T3SS regulator HrpG in KB medium, which may account for the increased deployment of T3SS. This suggests that the putative histidine kinase $BarA_{Ac}$ is able to repress the T3SS expression by inhibiting HrpG in the KB medium, which appears to be important for rational energy allocation. In summary, our research provides further understanding of the regulatory network of *A. citrulli* virulence.

P2.1-011

DDI1 – A NOVEL REGULATOR OF THE 26S PROTEASOME AND IMMUNITY IN PLANTS

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Text

The yeast DNA-damage inducible protein 1 (Ddi1, mammalian DDI1/DDI2) has been previously shown to be a ubiquitin-dependent protease, acting synergistically with the 26S proteasome protein degradation machinery. One of the characterized targets of DDI2 in mammals is NRF1, a transcription factor that is translocated to the nucleus and activates transcription of proteasome subunit genes upon proteotoxic stress. Here, we identify DDI1 as a potential important regulator of the plant 26S proteasome, as well as plant immune responses. Proteomics analysis revealed DDI1 as a putative interactor of the NRF1 functional Arabidopsis analogs NAC53 and NAC78, suggesting a similar role of DDI1 in plants. Its role as a novel regulator of proteasome-dependent processes is further validated by additional proteomics and TurboID proximity labeling experiments with DDI1. Moreover, virus-induced gene silencing (VIGS) of *DDI1* in *N. benthamiana* enhances proteasome activity upon infection with the plant pathogen *Pseudomonas syringae pv. tomato* (*Pst*), further supporting a synergistic function between DDI1 and the 26S proteasome in plants. VIGS of *DDI1* also leads to a higher susceptibility of *N. benthamiana* towards *Pst*, revealing that DDI1 has a positive regulatory role during plant immune responses. As such, DDI1 might be a novel component at the nexus of proteolytic degradation and plant immunity. We will discuss different approaches to unravel the mode of action of DDI1 regulating both processes.

P2.1-012

PROCESSING BODIES: NOVEL REGULATORS OF PLANT IMMUNITY TARGETED BY BACTERIAL EFFECTORS

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Text

Compartmentalization of transcripts in membraneless aggregates allows rapid and cost-efficient responses to stimuli. Processing bodies (PBs) are dynamic ribonucleoprotein aggregates formed by phase separation in the cytosol. PBs are involved in translational arrest and mRNA decay and regulate several developmental processes and responses to stresses, including plant-pathogen interactions. Basal plant defense responses trigger a quick disassembly of PBs, possibly deregulating the expression of immunity genes. Here, we show that upon *Pseudomonas syringae* (*Pst*) infection, PB assembly is enhanced in an effector-dependent manner. Moreover, a PB-defective mutant is more tolerant to bacterial infection. Counterintuitively, this mutant is not affected in canonical defense responses such as salicylic acid or ROS production, suggesting alternative mechanisms contributing to susceptibility currently being studied through transcriptomic approaches. We identified two *Pst* effectors that associate with PB components and induce their formation. Interactomic studies allowed us to identify new PB-associated components upon infection. Among these, we found translational regulators, proteasomal subunits and ubiquitin-binding proteins, connecting RNA metabolism with protein homeostasis in the context of compatible plant-pathogen interactions. Altogether, this work reveals PBs as novel negative regulators of plant immunity directly targeted by bacterial effectors to promote infection.

P2.1-013

ANALYSIS OF PSA EFFECTOR AND ACTINIDIA IMMUNITY GENES

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Text

Pseudomonas syringae pv. *actinidiae* (Psa) is a causative agent of bacterial canker of kiwifruit (*Actinidia chinensis*) and its pandemic lineage has caused considerable damage in kiwifruit orchards worldwide. While there is growing understanding of how pathogens evolve in agricultural environments subsequent to their emergence, far less is understood about how pathogens and wild crop relatives coevolve prior to pathogen spillover to crops. To better understand how plants and pathogens coevolve in the wild, we collected samples from nearly 200 wild kiwifruit relatives across South Korea for dual pathogen virulence and host immunity enrichment sequencing (PenSeq + RenSeq). A complementary analysis of the genus-wide diversity of nucleotide-binding leucine-rich repeat proteins in 43 previously sequenced *Actinidia* genomes was performed, for comparison with a detailed examination of

Psa effector dynamics and evolution. This work enables us to uncover the coevolutionary dynamics of effectors and immunity genes circulating in wild populations.

P2.1-014

EVALUATION OF DEFENSE INDUCTION IN GRAPEVINE PLANTS (VITIS VINIFERA L.) BY PSEUDOMONAS PROTEGENS, THROUGH QPCR.

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Text

In Chile, the production of wine grapes has annual growth rates of 7.4%. However, there is a great threat to this growth associated with the development of vineyard diseases that can lead to low yields and loss of competitiveness in the market. Disease control is based on the application of chemical fungicides and bactericides that affect the environment and limit the target markets. Induction of resistance in plants through biological inducers appears as an alternative to be include in integrated disease management programs. The objective of this study was to evaluate the induction of resistance genes in grapevines plants cultivar Chardonnay by applying two inducers based on Pseudomonas protegens (Taniri® WP; 1 g L^{-1}, MaxGrowth 0,1 mL L^{-1}), by qPCR, through the ΔΔCt method. The expression of the genes coding for the PR1 (Pathogenesis-related protein 1), PR2 (Beta-1,3-glucanase), PR10 (Pathogenesis-related protein 10), PAL (Phenylalanine ammonia-lyase), SUB (Protease-Subtilisin), and LOX (Lipoxygenase) proteins was studied at 24 hours, 7 days, and 14 days after application of the bioinductors. Bacterial formulation induced the expression of this genes at a level equal to or greater than a chemical inducer (Acibenzolar-S-metil), evidencing their effectiveness in inducing resistance in grapevines plants.

P2.1-015

CHARACTERIZATION OF DIFFERENTIAL COLONIZATION ABILITIES OF PSEUDOMONAS AMYGDALI PV. LACHRYMANS, THE CAUSAL AGENT OF CUCUMBER BACTERIAL DISEASES

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Text

Pseudomonas amygdali pv. *lachrymans* (*Pal*) causes two different diseases on cucumber: bacterial stem wilt and bacterial angular leaf spot. Under field conditions, bacterial stem wilt of cucumber infects leaf veins and stems, while bacterial angular leaf spot of cucumber does

not infect leaf veins and occasionally infects stems. The aim of this study was to characterize the colonization ability of *Pal* strains of the two cucumber diseases. Representative *Pal* strains were labeled with an ultraviolet green fluorescent protein (GFPuv) for the bacterial stem wilt pathogen (A2-GFPuv) and the angular leaf spot pathogen (psl8-GFPuv). Cucumber tissues (leaf, stem, and fruit) were inoculated with strains A2-GFPuv and psl8-GFPuv, and observed under ultraviolet light and by a fluorescent microscope. The results showed that *Pal* strain A2-GFPuv infected leaf veins in the seedling spray-inoculation assay, and colonized up to the true leaves and down to the roots in the stem inoculation assay. However, *Pal* strain psl8-GFPuv could not infect leaf veins, and the stems showed relatively mild symptoms near the inoculation point at 7 days post inoculation. On cucumber fruit tissues, the water-soaked spots were larger and more obvious for fruits inoculated with A2-GFPuv compared with those of psl8-GFPuv. Overall, the ability of bacterial stem wilt pathogen *Pal* A2-GFPuv to infect cucumber leaves, stems, and fruits was more aggressive than that of bacterial angular leaf spot pathogen *Pal* psl8-GFPuv.

P2.1-016

TYPE III-SECRETED EFFECTORS AND TOXINS PLAY A TISSUE-SPECIFIC ROLE IN THE PATHOGENICITY OF PSEUDOMONAS SYRINGAE PV SYRINGAE

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Text

Pseudomonas syringae pv *syringae* (Pss) has a reduced type III effector repertoire, but often produces syringomycin, syringopeptin, and syringolin A toxins. Comparative genomics was used to categorize the effector abundance in Prunus-infecting Pss strains. In addition to the conserved effector locus (CEL) identified in most *P. syringae* (Ps) pathogens (*hopAA1*, *hopM1*, and *avrE1*), a Core of effectors was found to be common in Ps phylogroup 2 (*hopAG1*, *hopAH1*, *hopAI1*, and *hopI1*). A set of Prunus-specific effectors is associated with cherry pathogenicity (*hopAR1*, *hopH1*, *hopA2*, *hopAE1*, and *avrRpm1*) and a Flexible set (*hopAF1*, *hopAZ1*, and *hopBE1*) varies among isolates. RNA-seq of Pss9644 validated effector gene expression in Hrp-Inducing Minimal Media. Effectors were then deleted group-by-group using the Flexible, Prunus, Core, CEL, and toxin gene sets. The pathogenicity of the mutants was tested on wood, leaves, and fruits of sweet cherry (*Prunus avium* L.). Toxins were found to play a key role in disease development in wood and fruit. The effectorless mutant had a zero-pathogenicity phenotype in leaves and CEL played an important role in the early stages. *HopAF1* in an otherwise effectorless background was found to be required for virulence in wood, despite deletion having no phenotype when more conserved effectors were present. Our results highlight the niche-specific differential roles of toxins in cherry tissues and the complexity of effector redundancy in cherry pathogens.

P2.1-017

CHEMOTAXIS AND AEROTAXIS ARE REQUIRED FOR PLANT INFECTION OF PSEUDOMONAS SYRINGAE PV. TABACI 6605

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Text

Pseudomonas syringae pv. *tabaci* 6605 (*Pta*6605) is a causal agent of wildfire disease in host tobacco plants. Although chemotaxis has been shown to be necessary for *Pta*6605 for tobacco infection, the chemoattractants at the site of infection are unclear. *Pta*6605 was attracted to the apoplastic fluid from host tobacco leaves, which contain abundant amino acids, including γ-aminobutyric acid (GABA). *Pta*6605 has 54 potential chemoreceptor genes. Among them, we investigated 5 dCache_1 type chemoreceptor genes and identified a GABA chemoreceptor gene, *mcpG*, and three amino acids chemoreceptor genes, *pscA*, *pscB*, and *pscC2*. Although, the deletion of *mcpG* retained surface motility and chemotaxis ability to amino acids, the mutant abolished chemotaxis to GABA and reduced the ability to cause disease. The *pscA*, *pscB*, and *pscC2* mutant strains reduced chemotaxis to most amino acids. A mutant of another dCachs_1 type chemoreceptor, *pscC1* lost motility. Among these mutant strains, *pscB*, *pscC1*, and *pscC2* remarkably reduced surface motility and virulence. *Pta*6605 also shows positive aerotaxis ability. We found at least two aerotaxis receptor genes, *aerA* and *aerB*. Although *aerA* and *aerB* mutant strains showed wild-type levels of surface motility and chemotaxis ability to yeast extract, these mutant strains showed less colonization in the early stage of plant infection. These results indicate chemotaxis and aerotaxis contribute to the successful plant infection of *Pta*6605.

P2.1-019

CONTACT DEPENDENT INHIBITION IN RALSTONIA PSEUDOSOLANACEARUM

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Text

Ralstonia pseudosolanacearum is a deadly bacterial plant pathogen known to infect many plant species. Comparative genomics of a South Korean population of R. pseudosolanacearum revealed that recombination frequently targets secreted gene products including type III secreted effectors and CdiA proteins, similar to hemagglutinin. CdiA proteins vary in the presence of a C-terminal toxin domain that inhibits the growth of adjacent cells lacking cognate immunity protein CdiI, a phenomenon called contact-dependent growth inhibition (CDI). CDI mediates both antagonistic and cooperative contact-dependent interactions, contributing to the formation of populations sharing identical CDI loci. R. pseudosolanacearum carries an expanded set of CDI loci compared to Burkholderia spp., where their function is better characterized. My work examines whether these loci mediate contact-dependent interactions and contribute to phenotypes associated with virulence and cooperative behavior in R. pseudosolanacearum.

P2.1-020

QTL-BOUND TALES: BACTERIAL EFFECTOR ASSOCIATION WITH RESISTANCE QUANTITATIVE TRAIT LOCI OF ORYZA SATIVA

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Text

Durable resistance to Xanthomonas oryzae pathovars oryzae and oryzicola is highly sought after in rice due to the pathogens' ability to impact crop yield. Regions of the rice genome known as quantitative trait loci (QTL) were previously identified using a multi-parent advanced generation intercross (MAGIC) rice population. These QTL are associated with decreased lesion lengths by X. oryzae on rice. What remains unknown is the molecular basis for induction of rice genes within QTL during pathogen infection. Upon infection, X. oryzae injects the host with transcription activator-like effector (TALE) proteins. These effectors bind to plant promoters to induce gene transcription. We hypothesize that differential binding of TALE to promoters of rice genes under QTL leads to the varied phenotypes exhibited across varieties. We designed a pipeline that predicts TALE-regulated candidate genes involved in quantitative resistance. This pipeline identifies genes under QTL that have binding sites for X. oryzae TALEs in their promoter and a strong correlation between binding site presence and disease phenotype. We used this pipeline with data for the eight MAGIC founders to identify candidate genes involved in resistance against seven X. oryzae strains. We then analyzed transcriptomes of plants inoculated with X. oryzae to validate candidate genes. Here, we propose a method that could streamline the identification of genes involved in quantitative resistance to TALE-harboring Xanthomonas.

P2.1-021

FLAVONOIDS AND FATTY ACIDS, KEY METABOLITES IN PSEUDOMONAS SYRINGAE DEVELOPMENT ON CHERRY SHOOTS

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Text

Pseudomonas syringae pv. *syringae* (*Pss*) and pv. *morsprunorum* (*Psm*) are the causal agent of the bacterial canker of cherry trees. Both pathogens infect woody tissues but exhibit different pathogenicity towards their host, which may be related to their adaptation to this environment. In this study, we aimed to characterize (*in vitro*) the changes in *Pss/Psm* metabolism in the presence of their host using mass-spectrometry-based untargeted metabolomics. After five days of inoculation on sterile cherry shoots, *Pss* and *Psm* caused the accumulation of several flavonones, such as naringenin, suggesting the degradation of their respective glycosylated forms. This hypothesis was supported by the reduction in the abundance of several o-glycosylated flavonoids (e.g. Naringenin-7-O-glucoside), which

remained stable on the control shoots. Additionally, a significant decrease of epicatechin was observed in *Pss*, and *Psm* inoculated shoots. *Pss* seemed to be more efficient at degrading cherry flavonoids than Psm, which could explain why this bacterium grows better while using cherry as the sole source of nutrients. Interestingly, only *Psm* accumulated Rhamnolipid-related backbone fatty acid chains while growing on cherry shoots. Despite its role in the infection process remains unclear; a relationship with *Psm* motility is suggested. Further research will be done to understand the differences between *Pss* and *Psm* interaction with cherry and to confirm the observed results under in vivo conditions.

P2.1-022

CHARACTERIZING ENVIRONMENTAL SENSING MECHANISMS IN DICKEYA DADANTII

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Text

Bacteria sense their surrounding environment and move accordingly via chemoreceptor proteins in a process known as chemotaxis. These proteins play essential roles during the disease cycle. Members of the *Dickeya* genus, that cause disease on numerous crops and ornamental plants, present notoriously more methyl-accepting chemoreceptors (MCPs) than other closely related bacteria. However, the functions and signals of many of these MCPs remain unknown. Interestingly, long untranslated regions exist upstream of the coding regions of MCPs in *Dickeya*. We hypothesized these regions harbor small non-coding RNAs. Transcription start sites were identified using Cappable-seq and found to align well with the areas being transcribed, which were detected via RNAseq and validated via qRT-PCR, *in vitro* and *in planta*. Using biocomputational methods we identified potential promoters, putative regulatory sequences, and terminators in these regions. Together these results showed that such intergenic regions and MCP genes are actively transcribed *in planta*. Mutants lacking these regions, as well as lacking the MCP genes, showed differences in their ability to swim and swarm, compared to wild-type. Opposite patterns of motility were noticed among some MCP mutants and their corresponding upstream deletion mutants. Some mutants displayed altered symptoms in potato stems. Our results provide new insight into the sensing and signaling mechanisms used by *Dickeya* and provide targets for disease control.

P2.1-023

THE XANTHOMONAS TYPE III EFFECTOR NUDX4 IS AN NADH/ADP-RIBOSE PYROPHOSPHORYLASE THAT MANIPULATES PLANT IMMUNITY

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Text

Bacterial blight caused by Xanthomonas oryzae pv. oryzae (Xoo) severely affects rice yield. Transcription activator-like (TAL) effectors and non-TAL effectors play key roles in Xoo pathogenicity. The TAL effectors were reported to regulate gene expression by combining with host target gene promoters. However, the study on non-TAL effectors virulence mechanism is very limited. Here, we report a new non-TAL effector NUDX4 of Xoo that contains a Nudix hydrolase motif. The NUDX4 knockout mutant displays lower virulence in rice than wild-type Xoo. Ectopic expression of NUDX4 suppressed reactive oxygen species (ROS) burst and pathogenesis-related genes expression in transgenic rice plants, which are more susceptible to Xoo infection. The biochemical assays showed that NUDX4 possesses NADH/ ADP-ribose pyrophosphorylase activity. Mutation of key residue in Nudix hydrolase motif significantly impaired NUDX4 catalytic activity and virulence function. Upon further investigation, we found NUDX4 is a dimer in vitro and in vivo. We then built the homodimer model of NUDX4 through the three-dimensional structure predicted by AlphaFold 2. Based on structure-guided mutagenesis, we demonstrated that homodimerization of NUDX4 is essential for catalytic activity and virulence function. Taken together, our results indicate that Xanthomonas could produce Nudix hydrolase effector to manipulate host immunity.

P2.1-024

A MICRO- AND MACRO-PERSPECTIVE OF BACTERIAL PATHOGENS AFFECTING ONION IN GEORGIA, USA

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Text

Onion bacterial diseases are caused by diverse pathogens belonging to Pantoea, Pseudomonas, Burkholderia, Rouxiella, Rahnella, and Enterobacter. Interestingly, virulence factors employed by these bacteria to infect onion are diverse. For example, Pantoea ananatis and some onion-adapted strains of P. stewartii subsp. indologenes utilize distinct phosphonate biosynthetic gene clusters, “HiVir” and “Halophos”, respectively, to infect onion. The thiosulfinate tolerance cluster “alt” in Pantoea spp. aids in colonization of the thiosulfinate-rich environment in onion bulbs. Two closely related Pseudomonas species, P. viridiflava and P. alliivorans are also associated with onion. While an “alt” gene cluster is present in both, alt makes greater contributions to symptom development in P. viridiflava than in P. alliivorans. Onion pathogenic strains of Rouxiella badensis utilize a lipopeptide gene cluster, the “rot” cluster, to infect onion. Management of these bacterial pathogens is based primarily on applications of copper-based bactericides at susceptible crop growth stages. Cultural practices, e.g., use of a chain digger for mechanical harvest, also reduced internal bacterial bulb rot in field trials. Clipping onion necks at least 5 cm above the bulb reduced bacterial rot in storage compared to shorter necks. Together, advances in onion-pathogen interactions and applied aspects of onion production are aiding long-term sustainable strategies to reduce losses to bacterial rots.

P2.1-025

CHARACTERIZATION OF THE INFECTION PROCESS IN BACTERIAL RICE BLIGHT BY XANTHOMONAS ORYZAE PV. ORYZAE.

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Text
The plant pathogen *Xanthomonas oryzae pathovar oryzae* (*Xoo*) is responsible for the so-called bacterial leaf blight in *Oryza sativa*. Infestation of rice plants by *Xoo* not only causes monetary losses of several billion U.S. dollars annually, but also threatens global food security, as rice is considered a staple food in Africa and Asia. *Xoo* is mainly spread during the typhoon season. The storms ensure that the rice leaves are wounded, offering a potential entry point into the plant. However, even without wounding, *Xoo* can invade the plant through natural openings. After invasion of the hydathodes or the wound, *Xoo* colonizes into the xylem, where it spreads rapidly throughout the plant. Controversial here is the direction of the colonization from the leaf tip toward the stem, while the flow in the infested xylem is the opposite direction from the root through the stem to the leaf tip.

P2.1-026

DEVELOPMENT OF A SOIL INOCULATION METHOD COUPLED WITH BLOCKER-MEDIATED 16S RRNA GENE AMPLICON SEQUENCING REVEALS THE EFFECT OF ANTIBACTERIAL T6SS ON AGROBACTERIA TUMORIGENESIS AND GALLOBIOME COMPOSITION

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Text
The type VI secretion system (T6SS) is deployed by many proteobacteria for interbacterial competition or pathogenesis against the host. Agrobacteria, a group of soil-borne phytopathogen causing crown gall disease, use the T6SS to attack closely- and distantly-related bacteria *in vitro* or *in planta*. Current evidence suggests that the agrobacterial T6SS is not essential for pathogenesis, but its influence on natural disease incidence and gall-associated microbiota (i.e., gallobiome) is unknown. Here, we established a soil inoculation method for wounded tomato seedlings that mimics natural infections, and optimized a blocker-mediated enrichment method for bacterial 16S rRNA gene amplicon sequencing to address these questions. By comparing the *Agrobacterium* wild-type strain C58 with two T6SS mutants, we demonstrated that the T6SS promoted disease occurrence and influenced on gallobiome composition. Also, the season of inoculation played a more important role than

the T6SS in shaping the gallobiome. The influence of T6SS was evident in summer, in which two Sphingomonadaceae species and the family Burkhoderiaceae were enriched in the gallobiome induced by the mutants. In vitro competition and plant colonization assay showed T6SS-mediated antagonism to Sphingomonas sp. R1 strain isolated from tomato rhizosphere. In conclusion, this work demonstrates that the Agrobacterium T6SS promotes tumorigenesis in infection process and provides competitive advantages in gallobiome.

P2.1-027

GENE EXPRESSION PROFILING IN SOILS OF DICKEYA DADANTII, A CAUSAL PATHOGEN OF QUICK DECLINE DISEASE OF FRUIT TREES

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Text

The plant pathogen *Dickeya dadantii* is known as a soft-rot disease pathogen of various plants. Many economic crops and vegetables are affected by production losses due to this pathogen. In Japan, *D. dadantii* has been known to exist on farmlands throughout the country. Additionally, it has been reported that the diseases caused by this pathogen occur not only in annual crops but also in some fruit trees such as peach, Japanese apricot, apple, and Japanese pear. These diseases of fruit trees have been reported as “the quick decline”.

Typical symptoms of “the quick decline” are the sudden leakage of reddish-brown sap from the trunks and branches, and are that the trees decline rapidly. In addition, *D. dadantii* seems to invade the roots and grafting parts of rootstocks from the soil and proliferate inside the trees.

To elucidate the pathogenicity of *D. dadantii* to fruit trees, especially the virulence factors working during soil-to-fruit tree invasion, our group analyzed gene expression in orchard-like soils.

The analysis by RNA-seq and qRT-PCR revealed that *D. dadantii* in soils expressed *hrpN* gene more strongly than pectinase genes, and expressed the related genes to iron ion chelation. These results will help to clarify the ecology of *D. dadantii* in soils and understand the pathogenicity to fruit trees.

This work was supported partly by MAFF Commissioned project study on “Development of stable cultivation technology in young stage of fruit trees” Grant number JPJ008720.

P2.1-028

ELUCIDATING THE ROLE OF THE 'CANDIDATUS PHYTOPLASMA MALI' PROTEIN PME10 DURING PHOTOSYNTHESIS AND SYMPTOM DEVELOPMENT OF APPLE PROLIFERATION DISEASE

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Text

Apple Proliferation is an economically important and widespread disease in apple growing regions in Europe. It is caused by an infection with the plant pathogen 'Candidatus Phytoplasma mali' (P. mali). The infection leads to the production of low-quality fruits and disease outbreaks cause high economic losses in affected regions. Phytoplasma release different types of effector proteins which are involved in changing the plant host metabolism and play a pivotal role in symptom development. Thus, unravelling their function is an important step towards a better understanding of the disease and an indispensable prerequisite for developing specific control strategies against Apple Proliferation disease. PME10 is a novel, potential P. mali effector. It is expressed during infection and binds different Malus × domestica proteins, such as RuBisCo- Activase (RCA) and E3 ligases. RCA plays an important role during photosynthesis by activating RuBisCo. E3 ligases are a class of enzymes that are involved in the regulation of proteasomal protein degradation. A direct interaction between the PME10-interacting RCA and E3 ligases could not be demonstrated. It is hypothesized that PME10 acts as an adaptor that mediates an interaction between E3 ligases and RCA and thus drives the proteasomal degradation of the latter one. The aim of this project is to elucidate the role of the PME10 on photosynthesis during infection and disease development.

P2.1-029

TYPE III EFFECTORS OF PSEUDOMONAS SYRINGAE REGULATE THE NLR TRANSCRIPTS' STABILITY BY TRIGGERING UPF3 DEGRADATION IN ARABIDOPSIS

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Text

Plants have evolved two layers of immunity: the first layer is pattern-triggered immunity (PTI) triggered by the recognition of microbe-associated molecular patterns via pattern recognition receptors, and the second layer is effector-triggered immunity (ETI) activated through interaction between pathogen-derived effectors and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). Nonsense-mediated mRNA decay (NMD), a conserved eukaryotic mRNA surveillance mechanism that degrades aberrant RNAs, has been shown to play a role in the plasticity between PTI and ETI. Recently, our group reported that NMD controls the expression of a subset of NLR genes. However, the role of bacterial effectors in manipulating NMD activity during infection remains unclear. Infection of *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000) dramatically impacted on accumulation of UP-FRAMESHIFT 3 (UPF3) protein, while degradation of UPF3 was minimized in leaves infected with effector-depleted derivatives of *Pst*DC3000. The transcripts of NLRs carrying NMD-sensitive features were more stable in infected plants with *Pst*DC3000 than those with mutated *Pst*DC3000. Further analysis of the total RNAome revealed transcriptional changes under NMD-defective conditions, such as pathogen-infected conditions and mutant plants. These results propose a possibility that plants may use the NMD machinery, especially UPF3 protein, as a decoy-like thing to enhance the level of NLR transcripts during infection.

P2.1-030

THE SECRETED SERINE PROTEASE CHPG OF CLAVIBACTER MICHIGANENSIS ACTS AS A HOST SPECIFICITY DETERMINANT THAT RESTRICTS IT FROM EGGPLANTS

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Text
Clavibacter michiganensis (Cm) is the causal agent of bacterial canker of tomato. Data regarding the host range of Cm outside of tomato is not well established. We found that eggplant is resistant to Cm strain 382. A screen of a Cm mutant library for HR elicitation found that a marker exchange mutant of the secreted serine protease chpG failed to elicit HR in eggplant. In addition, the chpG mutant was able to cause disease in eggplant and the mutant bacterial populations in eggplant were 1000-fold higher than Cm 382, indicating that chpG acts as an avirulence gene. Complementation analysis conducted with wild-type and catalytically inactive chpG variants showed that immune recognition of ChpG is dependent on its catalytic activity. To examine whether chpG acts as a host specificity determinant within Cm populations, we screened 50 Cm isolates for virulence and HR elicitation in eggplant. We identified that only two isolates, C47 and C48, were pathogenic on eggplant, and both failed to elicit HR. We sequenced chpG regions of Cm isolates and found that eggplant-pathogenic clones harbor a unique allelic variant of chpG. Introduction of the chpG variant of C48 into the 382 chpG mutant failed to complement it while the introduction of the chpG variant of 382 into C48 abolished its virulence in eggplant. Our data shows that chpG is an avirulence gene of Cm in eggplant, and that natural allelic variations in chpG act as a host range determining factor in different Cm isolates.

P2.1-031

PHENOTYPIC TESTING OF CANDIDATE VIRULENCE GENES IN PSEUDOMONAS SYRINGAE PV. AESCULI ASSOCIATED WITH ADAPTATION AND INFECTION IN HORSE CHESTNUT

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Text
The bleeding canker of European horse chestnut is a recently emerged disease caused by *Pseudomonas syringae* pathovar *aesculi* (*Pae*). The major virulence mechanisms driven by this pathogen to infect woody hosts are scarcely investigated. However, genome comparison of the *Pseudomonas syringae* complex revealed conserved regions only present in *Pae* which are implicated in the metabolism of aromatic and phenolic compounds, in sucrose uptake and utilization and in fatty acid biosynthesis. The *hopAB1* effector and HrpL regulon are essential for suppressing and/or triggering defences in different plant species and for symptom development, respectively. However, their contribution to the virulence of *Pae* is also important to analyse.
Our goal was to investigate the main components contributing to *Pae* virulence on horse chestnut. Therefore, we conducted functional analyses through mutagenesis and

complementation experiments to evaluate the genetic adaptations to infection of the woody parts of the tree. Our study revealed the important role of *hopAB1* and hrpL in suppressing the plant immune response and causing disease in tobacco plants and horse chestnut. The deletion of the genes encoding for the catabolism of anthranilate and catechol reduced virulence in horse chestnut as well as those encoded within sucrose and fatty acid clusters. Our results highlight the role of the enzymatic activities encoded within *Pae* and their implication on its evolution and adaptation to woody hosts.

P2.1-032

IMPROVED METHOD FOR ISOLATION OF CLAVIBACTER SEPEDONICUS FROM POTATO EXTRACTS USING SOLANUM MELONGENA

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Text
Clavibacter sepedonicus is the causal agent of bacterial ring rot characterized by wilting of potato plants and rotting of tubers and is known from Europe, North-America and Asia leading to substantial economic losses. Isolation of C. sepedonicus from material with a low level of infection is required for confirmation of a positive diagnostic test. Limited information is available on the minimum inoculum concentrations needed to confirm Koch's Postulates. Inoculation of Solanum melongena is used for isolation of low concentrations of C. sepedonicus from potato extracts. Here we present an improvement of this method by adding sodium pyruvate and/or D-mannitol to the inoculation solution. C. sepedonicus suspensions were mixed with potato extracts to obtain concentrations ranging from 1,6x102 – 1,4x104 cfu ml-1 and injected into the axil of the first grown leaves. The inoculation solutions were supplemented with sodium pyruvate or D-mannitol. After 2 wks the inoculated stem parts were harvested, processed and plated on MTNA+Natamycine agar plates. Plates were evaluated for the presence of C. sepedonicus after 6d of incubation at 22 °C. Isolation of C. sepedonicus with sodium pyruvate or D-mannitol was 10-100 times more sensitive compared with isolation without the additives. Concentrations of C. sepedonicus as low as 1,6x102 cfu ml-1 in potato extract have been successfully isolated with the improved method.

P2.1-033

INTERACTIONS AND GENOME BIOLOGY OF DICKEYA FANGZHONGDAI: A POTENTIAL THREAT TO POTATO INDUSTRY

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Text
Soft Rot *Pectobacteriaceae* (SRP) incurred significant losses globally to vegetable, ornamental and fruit industries. *Dickeya fangzhongdai* strain PL145 was isolated from

infected taro corm (Hawaii, USA), and found highly pathogenic to potato. The strain (peritrichous; confirmed using TEM) was identified using qPCR and MLSA, and phenotypically characterized using Biolog GEN III microplate and in vitro assays. High-quality hybrid assembly (5.26 Mb; 56.4% GC) was prepared using Oxford Nanopore MinION and Illumina NovaSeq data. The ANI and dDDH values were 97.1 and 73.3% with DSM10197^T *D. fangzhongdai*, respectively. The genome showed high heterogeneity—the presence of CWDE, Out-type T2SS, Type III secretion system, flagellar type secretion system (except Pilus cluster I) were similar to DSM10197^T. *vir* gene Type IV secretion system was present in PL145 but not in DSM10197^T. Sixty-four genomic islands, prophage, and CRISPR-Cas 1E and 1F were present in PL145. Significant differences (p=0.05) were observed in motility, and found significantly (P=0.05) aggressive, macerating entire taro corm but produced no symptoms on aerial parts of the plants. On potato, a typical severe black leg symptoms leading to death of the plants were observed. The Tn7 generated mScarlet-I mutant of PL145 rapidly colonized the potato plants, while no symptoms were observed with DSM10197^T, demonstrating differences in virulence and potential threat to the vegetable industry.

P2.1-034

LARGE–SCALE TRANSPOSON MUTAGENESIS REVEALS TYPE III SECRETION EFFECTOR HOPR1 IS A MAJOR VIRULENCE FACTOR IN PSEUDOMONAS SYRINGAE PV. ACTINIDIAE

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Text

Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa3) is a serious threat to kiwifruit production worldwide. To investigate the molecular mechanism of Psa3 infection, we developed a rapid and reliable high-throughput flood-inoculation method using kiwifruit seedlings. Using this inoculation method, we screened 3000 Psa3 transposon insertion mutants and identified 91 reduced virulence mutants and characterized the transposon insertion sites in these mutants. We identified seven type III secretion system mutants, and four type III secretion effectors mutants including *hopR1*. Mature kiwifruit leaves spray-inoculated with the *hopR1* mutant showed significantly reduced virulence compared to Psa3 wild-type, indicating that HopR1 has a critical role in Psa3 virulence. Deletion mutants of *hopR1* in Psa1, Psa3, Psa5, and Psa6 revealed that the type III secretion effector HopR1 is a major virulence factor in these biovars. Moreover, *hopR1* mutants of Psa3 failed to reopen stomata on kiwifruit leaves, suggesting that HopR1 facilitates Psa entry through stomata into plants. Furthermore, defense related genes were highly expressed in kiwifruit plants inoculated with *hopR1* mutant compared to Psa wild-type, indicating that HopR1 suppresses defense-related genes of kiwifruit. These results suggest that HopR1 universally contributes to virulence in all Psa biovars by overcoming not only stomatal-based defense, but also apoplastic defense.

P2.1-035

RECENT XANTHOMONAS TRANSLUCENS PV. UNDULOSA ISOLATES ARE MORE VIRULENT AND POSSESS A DISTINCT TAL EFFECTOR REPERTOIRE COMPARED TO OLDER ISOLATES

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Text

Xanthomonas translucens, the bacterial pathogen that causes leaf streak disease in cereals, has become increasingly destructive around the world. While climate change and other challenges likely contribute to increased disease incidence and severity, many isolates collected within the past decade appear to be more virulent, and we hypothesize that genetic variation may be contributing to the increased aggressiveness in recent years. We characterized the virulence phenotypes of two isolates collected in 2018 from Colorado in the US and found that both caused more disease than older isolates from our collection. One isolate was collected from barley (CO236) and the other from wheat (CO237). We then sequenced the whole genomes of these isolates using ONT long-read sequencing and compared these to previously sequenced genomes in GenBank. While we did not observe any major genomic rearrangements between genomes, the analysis grouped CO236 and CO237 with all other translucens and undulosa pathovar genomes, respectively. We then analyzed the Type III Effectors of the pathovar undulosa and found that while the putative effectors were highly conserved, there were differences between isolates in their encoded Transcription Activator-Like (TAL) effectors, suggesting that more aggressive isolates had a similar TALE repertoire, distinct from less aggressive ones. We are currently conducting mutagenesis studies to determine the contribution of these TALE classes to the pathogen virulence.

P2.1-036

HOST RECOGNITION OF CLAVIBACTER SECRETED SERINE PROTEASES

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Text

Clavibacter michiganensis (*Cm*) is a Gram-positive, xylem-colonizing bacterial pathogen of tomato. Worldwide, *Cm* is one of the most important tomato bacterial pathogens, causing bacterial canker. *Cm* is known to secrete serine protease effectors that contribute to bacterial virulence. Recently, two *Cm*-secreted protease effectors, Pat-1 and ChpG, were found to elicit host-specific immune responses in *Nicotiana tabaccum* (tobacco) and *Solanum melongena* (eggplant), respectively. Using comparative genomics, serine protease effectors of the chp family including Pat-1 and ChpG were found to be highly conserved in Clavibacter species causing disease on Solanaceous hosts, indicative of candidates mediating host range. The purified Pat-1 effector elicits cell death in *N. tabacum* varieties and one of two tobacco progenitors, but not in *N. benthamiana*. The ChpG effector elicits cell death in 15 cultivated S. melongena varieties, but not in the eggplant progenitor *Solanum incanum*. Because Cm lacks machinery to secrete proteins into host cells, we

hypothesize surface-localized immune receptors may recognize Pat-1 and ChpG. We seek to discover these receptors and will report progress on our screen of receptor candidates. If immune receptor candidates can be identified, their transfer into tomatoes would mark an important first step in breeding for *Cm* resistance.

P2.1-037

SERRATIA FONTICOLA, A PUTATIVE BACTERIA ASSOCIATED WITH ABNORMAL VERTICAL GROWTH SYNDROME IN MACADAMIA

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Text

Abnormal vertical growth is a disease syndrome that affects macadamia, an evergreen tree nut crop. The syndrome is expressed as physiological and morphological aberrations causing significant yield losses to farmers. Based on the symptoms including abnormal elongation and reduced lateral branching and flowering, this study hypothesised that a vascular-limited pathogen, which modulates plant hormone production, is likely the cause of the syndrome. Two approaches were used to profile the assemblage of bacteria in macadamia trees with or without the symptoms. Conventional media culturing techniques revealed that regardless of the host phenology, Bacillus species were the most abundant, followed by Serratia and Paenibacillus. Serratia fonticola was exclusively isolated from the roots of all symptomatic trees sampled. DNA metabarcoding revealed 15 bacterial taxa only occurred on symptomatic trees, but only S. fonticola was consistently identified from the roots of affected trees. Based on the fact that S. fonticola strains are known to alter the hormone balance in plants, we suggest it is a likely candidate responsible for abnormal vertical growth syndrome in macadamia.

P2.1-038

GENOMIC DIVERSITY AND PATHOGENICITY OF AUSTRALIAN AGROBACTERIUM SPECIES IN HORTICULTURE CROPS

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Text

Agrobacterium is a genus of bacteria that interact with and alter the growth of host plants that may be beneficial or pathogenic in nature. Where this interaction is pathogenic, the presence of Agrobacterium species with tumour-inducing plasmids causes uncontrolled growths in plant tissue that may affect growth and yield in commercial crops. Detection and isolation of pathogenic Agrobacterium species in a diagnostic context is complicated by the limited distribution of the bacteria in the host plant and the potential diversity of these populations.

While detection of the Ti (tumour-inducing) plasmid is often used as an indicator of the presence of pathogenic Agrobacterium, the presence and content of these plasmids in Australian Agrobacterium populations requires more work to elucidate. The implications of Agrobacterium plasmid number and content and how this affects pathogenicity will allow for a more in-depth understanding of these interactions and their impact on Australian crops. This work utilised genomic analyses of genome and plasmid content in conjunction with pathogenicity assays to investigate the diversity of pathogenic Agrobacterium in Australia.

P2.1-039

PLANT-ASSOCIATED BACTERIA EXTRACELLULAR VESICLES: CHARACTERIZATION AND POTENTIAL ECOLOGICAL ROLES

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Text

Bacterial extracellular vesicles (BEV) are lipidic shuttles allowing the export of cellular material at a great distance from the cell. Mostly studied in animal-bacteria interactions, BEV were shown to play a role in virulence, inter-species competition and induction of the host immune response. Little is yet known about phytobacterial BEV. Recently, biotic factors have been shown to regulate BEV production such as in Pseudomonas putida where lignin derivatives influence BEV size and cargo (1). Hydroxycinnamic acids like ferulic acid, are largely released in plant environment, where they can modulate the ecology of numerous phytobacteria (2 ,3) . We hypothesized that ferulic acid would influence the production of BEV in the phytopathogen *Agrobacterium fabrum* C58 and the phytobeneficial *Azospirillum* sp. B510. Conversely, we also hypothesized that BEV from bacteria would have an influence on the host plant metabolites. In both *A. fabrum* and *Azospirillum* sp., we assessed the effect of ferulic acid on the BEV production and their cargos using microscopy and LC-MSn analyses respectively. Finally, after an exposure to BEV, we compared the specialized metabolite of plants using UHPLC-MS/QTOF. Our first results support the view that the plant environment influences the production of BEV in plant-associated bacteria and provide insight on their potential ecological role in plant-bacteria interactions.

(1) 10.1073/pnas.1921073117
(2) 10.1094/MPMI-10-17-0236-R
(3) 10.1111/jipb.12810

P2.1-040

LEAF MARGIN MORPHOLOGY DEEPLY AFFECTS PATHOGEN FITNESS AND ECOLOGY

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Text

Xanthomonas campestris pv. *campestris* (*Xcc*) is a vascular pathogen of Brassicaceae naturally infecting leaf hydathodes to initiate black rot disease. Hydathodes are plant organs located at leaf margin and mediate guttation, ie. release of fluid derived from xylem sap. We conducted two parallel genetic screens on the Arabidopsis HEM (Homozygous EMS mutants) mutant collection searching for changes in hydathode numbers per leaf and bacterial multiplication in plant tissues upon hydathode infection conditions. Surprisingly, mutants with increased number of hydathodes did not sustain higher *Xcc* titers while plants with less hydathodes showed significantly more *Xcc* multiplication. Infection of Arabidopsis mutants with lower hydathode densities or infection of leaves with sealed hydathodes confirmed this phenotype. These conditions that promote watersoaking of leaf mesophyll link a developmental phenotype with an immune defect and suggest that water availability inside leaf tissues is critical for pathogen fitness and plant immunity.

P2.1-041

BIOINFORMATICS APPROACH FOR IDENTIFYING POSSIBLE PHYTOPLASMA EFFECTOR PROTEINS

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Text

Phytoplasmas, which are plant pathogens belonging to the class Mollicutes, are transmitted by insects feeding on plant phloem. These pathogens manipulate host cell functions through the effector proteins they secreted and cause various anomalies in the plants. Our current aims to identify effector proteins, which cause damage to various agricultural crops.

In order to determine candidate effector proteins, N-terminal signal peptides containing candidate effector proteins were identified by using SignalP 4.1, TMHMM v2.0 and Phobius tools. The results were compared with the the signal peptides and genes organization of previously reported effectors. To examine the relationship of the orthologs and to predict the function, Conserved Domain Database (CDD), PSI-BLAST, and HMMER tools used. Second step, candidate protein structures were predicted for structure/function analysis with the AlphaFold 2 tool, then investigated in Protein Data Bank (PDB) and AlphaFold Protein Structure Database by DALI server tool.

Our results showed that one of the most potential effector candidates was SAP55 which has a metalloendopeptidase-like structure and contains the peptidase family M41 like domain. Interestingly, SAP55 does not have ATPase domain, but rather an additional α-helix at its C-terminus. As a result of our *in silico* study, an effector showing possible protease activity was discovered for the first time in Phytoplasmas.

P2.1-042

GENOMIC IDENTIFICATION OF THE NOVEL AGROBACTERIAL GENES CONTRIBUTING TO PLANT TRANSFORMATION

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Text

Agrobacterium tumefaciens is a tool for genetic engineering based on its ability of transferring a specific segment of DNA on its tumor-inducing plasmid (pTi) to the host genome. Although *A. tumefaciens* strains are genetically diverse and can be classified into > 10 genomospecies, the disarmed strains used in *Agrobacterium*-mediated transformation are mostly derived from one genomospecies 8 strain, C58. In our characterization of wild-type *A. tumefaciens* strains from different genomospecies, we found that one genomospecies 1 (G1) strain, 1D1108, has strong virulence while another G1 strain, Ach5, has weak virulence against several Fabaceae hosts. To further characterize agrobacterial virulence, we expanded the sampling to allow for comparisons of diverse G1 strains. A total of 30 G1 strains are selected for phenotyping and comparative genomics. Our result indicates that virulence, as measured by transient transformation efficiency in *Nicotiana benthamiana*, has no obvious link to overall gene content or the pTi type. We also found that 1D1108 has the highest transient transformation efficiency among all tested strains. Transcriptomic analysis of 1D1108 identified ~400 genes exhibiting differential expression in planta. Further investigations of these candidates are required to confirm their roles and to understand the mechanisms. The knowledge may be used for future synthetic biology work to improve *Agrobacterium*-mediated transformation.

P2.1-043

COMPARATIVE GENOMIC ANALYSIS OF SPIROPLASMA CITRI IN NATURALLY INFECTED CITRUS SAMPLES AND IN VITRO CULTURES

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Text

Citrus stubborn disease (CSD) is one of the most important vector-borne diseases in most citrus growing regions worldwide. *Spiroplasma citri*, the causal agent of CSD, is a phloem-limited, wall-less bacterium, belonging to reduced genome, high A-T content bacteria group, Class Mollicutes.

In the present study, *S. citri* was detected and characterized from naturally infected citrus trees and *in vitro* cultures obtained from periwinkle, sesame, turnip and cicadellids by PCR-based detection techniques focusing on spiralin , P58 putative adhesin-like multigene, and P89 putative adhesin genes of *S. citri*. The detection rate of *S. citri* was consistently higher in the fruit columella than in the leaf midribs for naturally infected field samples. For cultured samples,P89 were more sensitive in recognizing *S. citri* in field samples than those based on the spiralin gene and P58 gene. Furthermore, the obtained isolates showed 99.75% identity with *S. citri* GII3-3X strain which was originally isolated from the leafhopper *Circulifer haematoceps*, collected in Morocco (1980) and 99.25 % identity with

BLH-MB strain which was originally isolated from a Navel orange tree in Riverside, California (1972). In terms of the current situation of *S. citri*, this study presents an overview of the disease's spread in the citrus-growing regions and compare the genomic features of *S. citri* in culture and naturally infected citrus.

P2.1-044

INVOLVEMENT OF TALES IN XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS PATHOGENICITY IN CAULIFLOWER

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Text

Xanthomonas bacteria cause many diseases affecting commercially important crops. Most *Xanthomonas* species translocate Transcription Activator-Like Effectors (TALEs) into plant cells via their type III secretion system. TALEs are a unique class of bacterial effector acting as eukaryotic transcription factors to upregulate the expression of specific plant genes called susceptibility genes (S genes) for the bacteria benefit. According to recent studies, *Xanthomonas* species-specific disease management techniques can be developed by manipulating the host S genes.

SWEET genes are S genes targeted by *Xanthomonas* species, which encode for sugar transporters that are key to susceptibility in rice, cassava and citrus. Upregulation of these transporters in response to TALEs is speculated to accelerate disease development by increasing the amount of nutrients supplied to pathogens and/or by contributing to sugar signaling for disease resistance.

We identified the repertoire of *Xanthomonas* tal genes from *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of blackrot disease in *Brassicaceae*. To determine the transcriptome modifications induced by the *Xcc* TALEs, we did RNAseq experiments in cauliflower (*Brassica oleracea*). Interestingly, we demonstrated that Tal12a contributes to *Xcc* virulence on cauliflower, possibly by inducing the expression of *BoSWEET* sugar transporters.

We will present our latest results on the contribution of *SWEET* genes to susceptibility in cauliflower.

P2.1-045

IDENTIFICATION OF VIRULENCE RELATED GENES REGULATED BY A BACTERIAL CARBONIC ANHYDRASE

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Text
cynT (PSPTO_5255) encodes a carbonic anhydrase whose expression is induced by calcium in *Pseudomonas syringae* pv. *tomato* DC3000. Previously we showed *cynT* contributes to growth of DC3000 and symptom development in tomato plants. To understand the mechanism of how *cynT* impacts the virulence of DC3000, global transcriptome analysis (RNA-Seq) was performed for the *cynT* mutant and wild-type (WT). The expression of many virulence-related genes was impacted when *cynT* was deleted. On rich media (nutrient broth agar) supplemented with calcium, 73 genes were upregulated, and 56 genes were downregulated in the Δ*cynT* strain compared to the WT. On minimal media (mannitol-glutamate agar) supplemented with calcium, 495 genes were upregulated, and 253 genes were downregulated in the Δ*cynT* strain compared to the WT. Comparing the datasets, we found that the regulation of 31 genes by *cynT* was independent of the growth condition, however many of these genes showed opposite patterns of expression, including PSPTO_2870 and PSPTO_2871. PSPTO_2870 and PSPTO_2871 are predicted orthologs of putative virulence genes, *srfA*, and *srfB* respectively in *P. syringae* pv. *syringae* B728. Little is known about the functions of these genes in DC3000. Knockout mutants of srfABC operon were constructed in DC3000 and currently being characterized. The results indicate that the carbonic anhydrase, CynT is a key regulator for bacterial factors related to virulence.

P2.1-046

DEVELOPMENT OF GENETIC TOOLS FOR STUDY OF HOST-PATHOGEN INTERACTIONS IN XANTHOMONAS

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Text
Several significant plant diseases are caused by members of the genus *Xanthomonas*, including bacterial leaf streak on small grains and forage grasses caused by *X. translucens*. Despite the importance of these diseases, genetic tools to understand the molecular basis of disease and host-pathogen interactions remain limited. Implementation of high-throughput sequencing, including comparative genomics, has facilitated discovery of many genes associated with virulence that are candidates for further molecular characterization. Functional characterization of candidate genes is currently limited by challenges to reverse genetics in this genus. In our work, we have created a series of broad-host-range plasmids that allow for inducible expression of phage lambda red-recombinase genes, which have been successfully used for recombineering in various bacterial genera, such as *Escherichia*, and *Pseudomonas*. Using these plasmids, we have developed protocols for an implementation of recombinase-based mutagenesis in *X. translucens*. We have successfully replaced target DNA sequences in the chromosome with an antibiotic cassette, creating gene knockouts for various proof-of-concept genes. Our approach allows use of PCR products directly for gene replacement without need of cloning. We anticipate that these tools and approaches will be broadly applicable to the genus of *Xanthomonas* and will facilitate molecular studies in the genetic and physiological basis of host-pathogen interactions.

P2.1-047

ROLE OF CHEMOTAXIS CLUSTER II IN WOODY AND HERBACEOUS PLANTS PATHOGEN BACTERIA.

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Text

Chemoreceptors are essential proteins able to detect environmental changes for bacterial adaptation to the environment. The genes encoding these proteins are found individually in the genome or forming clusters with other genes related to chemotaxis. In plant-pathogenic bacteria, about 82 thousand chemosensory sequences have been described. In the *Pseudomonas syringae* complex, important groups of plant-pathogenic bacteria, four chemotaxis-related clusters have been described. However, cluster II is absent in some bacteria of this complex infecting woody hosts of the *Apocinaceae* family. Therefore, the aim of this work focuses on the functional characterization of cluster II, not only in bacteria isolated from woody hosts, but also in strains infecting herbaceous plants.

First, we constructed knockout mutants of genes encoded in cluster II, i.e. *cheA*, *cheB*, *cheD, cheY* and two genes coding for chemoreceptors in Psn23 strain and in *P. syringae* pv. tomato DC3000. Motility and virulence assays performed in oleander, dipladenia and tomato plants revealed that cluster II is involved in both phenotypes. In addition, bioinformatic analysis of the ligand-binding domain (LBD) of the two chemoreceptors encoded in cluster II showed that only one of them has an LBD domain. To characterise this chemoreceptor in strain Psn23, capillarity chemotaxis assays are being performed, and its LBD domain has been purified. The purified domain will be used in protein-ligand interaction assays.

P2.1-048

GENOME BIOLOGY AND EVOLUTION OF CLAVIBACTER MICHIGANENSIS

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Text

Gram-positive bacterium Clavibacter michiganensis (Cm) is a high consequence pathogen and causal agent of tomato canker. The electronic microscopic and genomic studies were conducted to understand the pathogenicity determinants and evolution of this seed-borne pathogen. Total 17 genomes were sequenced using either Pacbio or Illumina and Oxford Nanopore (hybrid assembly); genomes were also retrieved from the NCBI GenBank. Non-

pathogenic strains showed either no or truncated clusters of pathogenicity islands (tomA and chp), and no colonization inside the tomato plant (absence of chpG). The Scanning Electron Microscopy (SEM) and qPCR analyses suggested that pathogenic EPS-producing strain moved and colonized faster than EPS non-producing strain. In evolutionary studies, phylogeny and recombination events were identified using ClonalFrameML—4,080 recombination sites were predicted with the largest recombination fragment size of 1.7kb. The genome analyses indicated 1,801 core genes, 4,44 soft core genes, 1,305 shell genes and 3,120 cloud genes. The fastGear was used to detect recent (1,494) and ancestral (169) recombination events—strains from lineage VI were predicted as major donor. The recombination events indicated that C. michiganensis is continuously adapting and evolving.

P2.1-049

IMPACT OF A CARBONIC ANHYDRASE ON EXPRESSION OF VIRULENCE-RELATED GENES IN PSEUDOMONAS SYRINGAE

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Text

Pseudomonas syringae pv. *tomato* DC3000 (*Pto* DC3000) is a plant pathogenic bacterium that infects tomato and *Arabidopsis.* During infection bacteria produce a number of proteins, including those of the Type III secretion system (T3SS) to suppress the host defense response and promote disease. Previously we found that deletion of a *Pto* DC3000 carbonic anhydrase, *cynT*, reduced disease symptoms and bacterial population in tomato and resulted in a delay in a hypersensitive response (HR) in *Nicotiana benthamiana*. To further investigate the role of the *cynT* in *Pto* DC3000, we performed a global transcriptome analysis for the Δ*cynT* and wild-type (WT) strains in different media. The analysis revealed opposite patterns of expression for some T3SS genes in the *cynT* mutant compared to the WT when calcium was present. The RNA-Seq data also showed that many genes that encode proteins involved in motility were down regulated in the *cynT* mutant compared to WT. To investigate regulatory mechanisms involved in expression of *cynT*, we evaluated expression of *cynT* in multiple mutant backgrounds. T3SS-related genes such as *hrpS*, *hrpL*, *hrpJ*, and *hrpP*, were found to impact expression of the *cynT* gene, suggesting that CynT is a key regulator for interaction with host plants. More research is being conducted to determine how CynT affects the pathogenesis.

P2.1-050

DIVERSITY OF POTENTIAL MOBILE UNITES IN ‘CANDIDATUS PHYTOPLASMA SOLANI’ GENOMES – IMPLICATIONS OF SPECIFIC TRANSPOSON-LIKE ELEMENTS IN PHYTOPLASMA PATHOGENICITY AND EVOLUTION

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Text

Although not well comprehended and recognized, phytoplasmas (genus '*Candidatus* Phytoplasma') are bacteria that infect many plant species causing substantial losses in agriculture. These uncultivable endocellular wall-less pathogens have a specific life-style including colonization of plant phloem and insects. Their genomes are small and diverse (0,53-1,35 Mbp), but often repetitive, prone to rearrangements and characterized by the presence of putative transposon-like elements named potential mobile units (PMUs). So far, there are only a few that are fully sequenced and assembled. Within this study we sequenced two '*Ca*. P. solani' strains (ST19 and STOL) from infected periwinkle on Illumina MiSeq and Nanopore platforms. *De novo* assemblies generated 2 draft genomes with total size of 707,036 bp (ST19) and 656,141 bp (STOL) in 28 and 19 contigs, respectively. Detailed genome analyses revealed the presence of PMU-like regions and elements (14 in ST19 and 6 in STOL strain) of a different composition and size, up to 12 kbp. In STOL strain some of the PMUs were more complete and resembled those already described in '*Ca.* P. solani' and '*Ca*. P. asteris', while in ST19 more diversification in PMU-like elements was revealed. In both, effector and putative secreted protein genes were frequently found within PMU-like regions. Molecular phylogeny of selected PMU genes demonstrated different origin and horizontal gene transfer suggesting their role in host adaptation and pathogenicity.

P2.1-051

TEMPERATURE PLAYS A DECISIVE ROLE IN THE ABILITY OF PSEUDOMONAS SYRINGAE TO TRIGGER ETI AND DRIVES IN PLANTA BACTERIAL GROWTH DYNAMICS

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Text

The *P. syringae* species complex has a very important economic impact, causing diseases on many plant species. Considerable research effort is being invested to understand how effector repertoires could determine the host-range of a given strain. Recently, we showed that the incapacity of *P. syringae* pv. *actinidiae* to induce the hypersensitive response (HR) in *A. thaliana* is due to its inability to inject effectors rather than the absence of a recognized effector. In this context, we compared several *P. syringae* strains carrying the same plasmid-borne avirulence gene for their ability to induce an HR in *A. thaliana* Col-0, at different temperatures. *Pto* DC3000 and *Pma* M6 consistently triggered a strong HR while other strains induced it at different intensities significantly depending on temperature. Moreover, the strains were also compared for their growth dynamics at different temperatures in about ten plant species from various botanical families. Although temperature influenced bacterial growth dynamics *in planta*, the differences observed among the strains were partially correlated to their ability to activate their type-three secretion system. Together, these results

indicate that (i) *Pto* DC3000 is a reliable model strain but not representative of the *P. syringae* complex, (ii) temperature plays a crucial role in bacterial fitness, and (iii) deployment of molecular virulence factors may be influential but not sufficient to predict the outcome of plant-bacteria interactions.

P2.1-053

IDENTIFICATION AND CHARACTERIZATION OF XANTHOMONAS ARBORICOLA PV. JUGLANDIS FROM BACTERIAL BLIGHT AND BROWN APICAL NECROSIS OF WALNUTS IN TÜRKIYE

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Text

Walnut bacterial blight (WBB) is one of the important bacterial disease in Türkiye. Walnut immature fruit drops caused by Brown Apical Necrosis (BAN) also threatened walnut production in Aegean and Marmara Region. *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) is the causal agent of WBB and BAN. Alternaria/Fusarium are secondary agents of BAN. The purpose of this study was to determine the prevelance and causal agents of WBB and BAN in Manisa, Bal?kesir, Çanakkale. 31 isolates were identified as *X.arboricola* based on classical diagnosis. For WBB, in pathogencity tests on immature walnuts only 11 of 31 *Xaj* isolates evaluated as negative. Otherwise, 4 bacterial x fungal isolates caused BAN symptoms. Furthermore, molecular identification of *Xaj* strains was performed using 16S rRNA sequence and showed the highest homology to *Xaj* (%99.83-100). rep-PCR and MLSA using 7 housekkeping genes showed genetic diversity between *Xaj* strains. According to MLSA, *Xaj* grouped in 3 different clades. *Xaj* strains obtained from WBB symptoms in Bal?kesir were grouped in same clade. Although *Xaj* strains from BAN symptoms in Manisa and Çanakkale were grouped in same clade but different grouped with WBB strains. Variability between groups resulted different pathogenicty and virulence that are still being adressed. This is the first study of genetic diversity for *Xaj* strains in Türkiye which provides evidence for genotypic variability and provide a better understanding of WBB and BAN epidemiology.

P2.1-054

MOLECULE POLYMORPHISM OF G-X-Y INTERRUPTIONS IN COLLAGEN TRIPLE HELIX PROTEIN OF CANDIDATUS LIBERIBACTER ASIATICUS

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Text
Candidatus Liberibacter asiaticus, currently an unculturable phloem-limited bacterium, is associated with the most devastating disease of citrus Huanglongbing worldwide. Collagen-like proteins involved in a key aspect of pathogenicity, are an important source of genetic diversity in pathogenic bacteria. In the present study, 358 samples collected from different areas and hosts in China and 52 samples from USA were analyzed. Full length amplicon of 1652 bp were got only from USA isolates. Sequences ranging from 941 bp to 1588bp were got both from Chinese and USA isolates. A typical 63-amino-acid terminal domain with a potential transmembrane helix domain, a central collagen-like region containing Gly-Xaa-Yaa (G-X-Y) repeats, and a 7-amino-acid carboxy-terminal domain were found. Both the N' and C'-terminals of CthP were always conserved among different isolates. Yet hypervariable (Gly-X-Y) n repeats were identified. Phylogenetic analysis indicated most of USA isolates were grouped into one independent clade indicating a complex origination. The rest were grouped with gene-variants from Jiangxi, Yunnan and Fujian. Yunnan isolates contains most gene-variants and differ greatly from all the others. There's no correlation between symptoms and gene-variants. Based on our results, it can be concluded that the population of CLas contains different gene-variants which resulted in a complex citrus pathosystem for the study of interactions of phenotype and virulence.

P2.1-055

COLONIZATION OF THE LEAF VASCULATURE BY XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS

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Text
Xanthomonas campestris pv. campestris (Xcc) causes systemic infection of the leaf vasculature after initial colonization of hydathodes, water pores at the margin of leaves. By contrast, the closely related pathovar Xc pv. raphani (Xcr) colonizes the leaf mesophyll by entering stomata. The genetic basis of this striking difference in virulence strategy is not well understood. Here, we report about the genome sequence of 96 Xanthomonas campestris isolates and use comparative genomics to determine which genes exclusively occur in Xcc, while being absent in Xcr. This Xcc-specific set of genes contains four plant cell wall degrading enzymes (CWDEs), which are likely to be secreted by the type 2 secretion system. We show experimentally that this secretion system is required for colonization of the leaf vasculature after colonization of hydathodes, whereas it is dispensable for initial hydathode colonization, vascular mobility or multiplication in the leaf apoplast. Single CWDE knockouts show a similar loss of vascular colonization. Taken together, these results demonstrate that a hydathode-specialized pathovar uses an adapted repertoire of cell wall degrading enzymes to colonize the vasculature of its host plant.

P2.1-056

COMPLETING KOCH'S POSTULATES FOR THE CITRUS HUANGLONGBING BACTERIUM, CANDIDATUS LIBERIBACTER ASIATICUS

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Text

'*Candidatus* Liberibacter asiaticus' (Las) is one of the causal agents of huanglongbing (HLB), the most devastating disease of citrus worldwide. Due to the intracellular lifestyle and significant genome reduction of Las, it is extremely challenging to culture Las *in vitro*. In this study, we developed semi-selective media with the optimized growth conditions Las *in vitro,* and methods for re-inocuation of the cultured Las back to citrus. Under the optimized conditions we were able to culture Las to a growth peak in 2-3 weeks in the liquid medium, which was estimated as 10^6-10^7 cells per/ml with approximate 500-fold increase. The cultured Las bacteria remained in a dynamic growth for over 24 months and displayed limited growth in subcultures. We further confirmed the presence and growth of the Las bacterial cells using fluorescence *in situ* hybridization, gene expression profiling and metagenomic sequencing. It is worth noting that Las growth in the medium relied on the presence of a helper bacterium that co-exists both in citrus and psyllid hosts. Cultured Las was inoculated back to citrus sedlings via psyllid feeding. Although only a relatively low percentage of the fed psyllids and inoculated plants became positive, this is the first demonstration of significant growth of Las *in vitro* and successful inoculation of cultured Las back to psyllids and citrus plants. Factors that affect Las growth *in vitro* and the completion of "Koch's postulates" for Las are discussed.

P2.1-057

REVEALING BARELY SUSCEPTIBILITY GENES RESPONDING TO BACTERIAL LEAF STREAK

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Text

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *translucens* (Xtt), is a major disease of barley in the world. Our understanding of the barley-Xtt interaction is very limited, making it difficult to develop resistant cultivars for disease control. Xanthomonads are known to utilize transcriptional activator like (TAL) effectors to cause disease by upregulating specific host susceptibility genes, such as *SWEET* genes. In this work, we conducted a genome wide transcriptomic analysis to reveal barley susceptibility genes in response to Xtt infection. The barley cultivar Morex was inoculated with a local strain or buffer using infiltration and clipping methods. Leaf samples were taken at different time points with each having three biological replicates. Transcriptional analysis revealed slightly over half of barley genes expressed during infection for both inoculation methods. A total of 1037 and 23 differentially expressed genes (DEG) were identified between bacterial and mock inoculation

for infiltration and clipping method, respectively, and among them only one gene was shared by both methods. Most *SWEET*-like genes in barley had either low level of expression or no significant difference in expression between treated and mock samples for all time points. Using TAL effector RVD sequences, we identified five highly upregulated DEGs that could be targeted by Xtt TAL effectors. Those genes can be edited to investigate their role in disease susceptibility to BLS.

P2.1-058

GENOMIC AND PHENOTYPIC BIOLOGY OF NOVEL STRAINS OF DICKEYA ZEAE

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Text

Dickeya zeae causes soft rot of *Colocasia esculenta* and bacterial heart rot of *Ananas comosus. Dickeya zeae* is responsible for a wide range of diseases and significantly reduces crop production. In this study, we used Pacific Biosciences SMRT sequencing to sequence two high-quality complete genomes of *D. zeae*: PL65 (4.74997 MB) and A5410 (4.779) isolated from taro and pineapple, respectively. Additional complete genomes of D. zeae representing three additional hosts (philodendron, rice and banana) were included in the comparative analyses. The truncated T3SS and T4SS were observed in taro strain, which only harbors 1 and 2 genes of T3SS and T4SS, respectively, and showed high heterogeneity in T4SS. Unlike strain EC1, which was isolated from rice and recently reclassified as *D. oryzae*, neither the PL65 nor the A5410 genome harbors the zeamine biosynthesis gene cluster, which plays a key role in virulence of other Dickeya species. In this study, we compared major virulence factors produced by *D. zeae* strains and evaluated virulence on taro corms and pineapple leaves. Both strains produced proteases, pectate lyases and cellulases but no significant quantitative differences were observed among the strains. All the strains produced symptoms on taro corms and pineapple leaves, but strain PL65 produced symptoms more rapidly than the others.

P2.1-059

DNA SUPERCOILING AS A GLOBAL TRANSCRIPTIONAL REGULATOR: A COMPLEX MECHANISM INVOLVED IN THE INFECTION PROCESS OF THE PHYTOPATHOGEN DICKEYA DADANTII ?

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Text

Dickeya dadantii is a bacterium causing soft rot disease in a wide range of plant species. During the infection process, *D. dadantii* undergoes environmental stresses: acidic stress during penetration in the apoplast, oxidative stress due to the plant immune response, and osmotic and oxidative stresses during plant maceration and disease generalization. Interestingly, these stresses affect DNA supercoiling (SC): acid and oxidative stresses lead to DNA relaxation and osmotic stress induces an increase in SC level. Deciphering the mechanisms of SC-related transcriptional regulation in that species is thus crucial for our understanding of the mechanisms of virulence. As far as we know, the SC level is mainly regulated by topoisomerase I and DNA gyrase. Inhibiting either of these enzymes with antibiotics leads to global SC modifications and subsequent changes in global gene expression. We analyzed the first transcriptomic response of a Gram-negative bacterium to topoisomerase I inhibition by an antibiotic. We detected distinct patterns of expression level and spatial organization along the chromosome. Particularly, we found that all SC variations affect the expression of two major virulence genes, encoding pectate lyases (destroyer of the cell wall, causing the soft rot symptom), *pelE* and *pelD*.

P2.1-060

FUNCTIONAL CHARACTERIZATION OF THE XANTHOMONAS CAMPESTRIS TALOME

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Text

Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot disease, which is the most important and destructive bacterial disease of Brassica crops. Most *Xanthomonas* species translocate Transcription Activator-Like Effectors (TALEs) proteins inside plant cells using their type III secretion machinery. TALEs are a unique type of bacterial effector proteins that help the pathogen to exploit and modify the plant environment by directly altering the expression of plant genes to the benefit of the bacteria. TALEs play an essential role in many plant diseases caused by *Xanthomonas* spp. but the contribution of TALEs to virulence of *Xcc* has not been characterized. We identified a rich repertoire of *tal* genes (TALome) in *Xcc* and studied its functional role in disease development. First, we successfully silenced the *tal* genes in two *Xcc* strains using CRISPRi technology, demonstrating the role of TALEs for *Xcc* pathogenicity. In addition, the contribution of individual *Xcc* TALEs to bacteria virulence was tested by gain and loss-of-function experiments. Our data provide a platform to explore the roles of TALEs in black rot disease. We will present our latest results on the mechanisms of TALE-mediated susceptibility in Brassicaceae.

P2.1-061

CASSAVA BACTERIAL BLIGHT IS PROMOTED BY TALE-INDEPENDENT ACTIVATION OF THE MESWEET10E SUGAR TRANSPORTER

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Text

Sugars Will Eventually be Exported Transporters (*SWEETs*) genes are key susceptibility factors in several *Xanthomonas*-plant pathosystems. *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*), the causal agent of Cassava Bacterial Blight (CBB), transcriptionally activates the susceptibility (*S*) gene *MeSWEET10a* via the highly conserved Transcription Activator-Like Effector (TALE) TAL20. Until this study, *MeSWEET10a* was the only *S* gene described for CBB, and its transcriptional activation was shown to be necessary to develop watersoaked symptoms and to enhance *Xpm* growth. In this study, exploration of *Xpm* genomes and TALomes across a world-wide collection of strains revealed an alternative virulence-promoting mechanism based on activation of the gene *MeSWEET10e*, which encodes another clade-III SWEET sugar transporter. Here we validated the role of *MeSWEET10e* as a new cassava *S* gene and provided evidence that its transcriptional activation is TALE-independent, indirectly induced upon the action of a so far unknown host transcription factor. Our study provides the first report of a TALE-independent *SWEET* activation in the *Xanthomonas* genus, implying that non-TAL effectors may also determine *SWEET*-based host susceptibility.

P2.1-062

RALSTONIA TALE-LIKE PROTEINS TARGET HOST ARGININE DECARBOXYLASE GENES AND CAN ACT AS HOOKS FOR PLANT RESISTANCE AGAINST BACTERIAL WILT DISEASE

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Text

Transcription-activator-like effectors (TALEs) are a well-known class of effectors from plant-

pathogenic *Xanthomonas* bacteria but are also present in *Ralstonia solanacearum*, the causal agent of bacterial wilt disease in many crop plants. Within the genetically diverse *R. solanacearum* species complex (Rssc) TALE-like proteins (RipTALs) are found in all four phylotypes of the pathogen. Typically, TALEs are injected into plant cells by the bacterial type-III-secretion system and translocated into the plant nucleus where they specifically bind to host promoters in order to activate target genes that either promote disease or trigger plant resistance. In *Xanthomonas*, TALEs show a remarkably amount of diverse plant target genes and mechanisms of disease promotion or resistance. However, here we report that despite of significant genetic variation within the Rssc, RipTALs from different *Ralstonia* phylotypes all target *arginine decarboxylase* (*ADC*) genes in their host plants. While clear bacterial benefits from *ADC*-activation remain mostly elusive, this constraint on the part of *Ralstonia* enabled us to engineer transgenic tobacco plants with resistance to bacterial wilt in a RipTAL-dependent manner, showing that the conservation of RipTAL target specificity can be used as a hook for enhanced bacterial wilt control in crop plants.

P2.1-063

COMPLETE GENOME SEQUENCES AND CHARACTERIZATION OF XANTHOMONAS ARBORICOLA, THE NOVEL CAUSAL AGENT OF BACTERIAL LEAF BLIGHT OF BLUEBERRY

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Text
The cultivation of blueberry (Vaccinium corymbosum L.) is becoming increasingly important. For many years this plant species remained rarely infected by bacterial pathogens. Hitherto described, were tumorigenic Agrobacterium spp., Burkholderia andropogonis, Xylella fastidiosa and Pseudomonas spp. Recently, new pathogenic bacteria – the subject of this study] – were discovered. In 2013, on the blueberry cv. Toro and Duke growing in a nursery located in Central Poland russet brown, irregular spots on leaves were observed. From these leaf spots, fluorescent and yellow bacteria were isolated. Two yellow isolates, named 1311a and 1314c, were positive in a PCR assay using primers X1 and X2 specific for bacteria belonging to the genus Xanthomonas. Based on partial sequences analysis of gyrB, fuyA and rpoD, the strains were placed within the strains of Xanthomonas arboricola. Their complete genomes were determined. The genomes size of the strains 1311a and 1314c are 4,889,189 bp and 4,891,143 bp, respectively. Whole genome-based taxonomic analysis using the Type (Strain) Genome Server confirmed the affinity of these two strains to X. arboricola. It is the first report on the occurrence of bacterial leaf blight on blueberries caused by a Xanthomonas species.

This work was financed by the National Science Centre, Poland, Grant UMO-2017/25/B/NZ9/01565
Based upon work from COST Action CA16107 EuroXanth supported by COST (European Cooperation in Science and Technology)

P2.1-064

DECIPHERING OF THE PROXIMAL PROTEOME OF TWO YOPJ FAMILY ACETYLTRANSFERASES FROM TWO PLANT VASCULAR PATHOGENIC BACTERIA

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Text

Gram-negative bacterial pathogens inject variable repertoires of effectors into host cells to interfere with defense responses. Among those, the Yersinia outer protein J (YopJ) effector family of acetyltransferases, produced by diverse animal and plant bacterial pathogens, promote pathogen virulence by acetylating specific host components. However, the range of host processes that YopJ effectors can interfere with remains elusive. Thus, we developed a proximity-dependent protein labeling involving the Turbo biotin ligase for identifying the interactomes of two well-characterized YopJ members: the *Ralstonia pseudosolanacearum* PopP2 and its close homolog, XopJ6 from *Xanthomonas campestris*. Both of them are recognized by the Arabidopsis RRS1/RPS4 pair through manipulation of an integrated WRKY domain that mimics effector primary targets, the WRKY transcription factors. Interestingly, a single residue substitution in a XopJ6 natural variant disrupts physical interaction with WRKY proteins, enabling XopJ6 to avoid host recognition while retaining XopJ6 virulence functions, likely through interference with components other than WRKYs. The different PopP2 and XopJ6 variants fused with Turbo will be expressed in both *N. benthamina* and *Arabidopsis*. Alternatively, we developed an approach consisting in *Pseudomomas fluorescens*-mediated delivery of the Turbo fusion proteins to remain as close as possible to the level of effectors injected in plant cells by pathogenic bacteria.

P2.1-067

OPTIMIZED YEAST 2-HYBRID PLATFORM TO DISCOVER PLANT-BACTERIA INTERACTIONS

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Text

Yeast two-hybrid (Y2H) protein interaction screening has proven instrumental to identify countless plant-pathogen interactions (1). However, interaction map completeness has been limited using full-length proteins and C-terminal polypeptide fragments which result in significant false negative rates.

To circumvent these limitations, we used a domain-based strategy to build 35 random-primed cDNA libraries from 21 plant species: from model plants and crops, but also from exotic plants (*G. barbadense, K. laxiflora*, Morning glory, Cacao Tree…), fruit-giving plants

(Melon, oranges…), root vegetables (sugar beets, manioc…) or numerous plant pests. Each library has a complexity of 10 million independent fragments, with an average 1000bp fragment size.
Using an optimized mating procedure, these libraries are screened to saturation, allowing to test on average 83 million interactions per screen. Therefore, multiple independent fragments are isolated for each interactant, enabling to delineate the minimal interacting domains and to compute confidence scores (2).
These libraries have been integrated into our ULTImate Y2H high-throughput platform and are available for screening on a fee-for-service basis. **We will introduce how the platform recently supported the discovery of a mechanism by which *R. Solanacearum* bacteria hikacks plant immunity** (3).

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P2.1-068

LIVE VISUALIZATION OF TYPE III-MEDIATED HOST TRANSCRIPTIONAL REPROGRAMMING DURING XANTHOMONAS TRANSLUCENS INFECTION

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Text
During infection, Gram-negative plant-pathogenic bacteria use a Type III secretion system (T3SS) to deliver effector proteins into the host cells. Type III-secreted effectors (T3E) modulate a variety of host biological processes, including immunity, physiology, or gene expression, promoting host-susceptibility, and causing disease. For most pathosystems, studies have extensively focused on effector functions but the spatiotemporal dynamics of T3E activity during infection remain poorly understood. Here, we developed a method to visualize T3E activity of Xanthomonas translucens during live infection of barley. We engineered transgenic barley that only expresses green fluorescent protein (GFP) when a Type III-secreted artificial transcriptional activator-like effector (TALE), designated as dTALE-GFP, binds and activates the GFP promoter. X. translucens harboring dTALE-GFP also constitutively express a red fluorescent protein enabling simultaneous detection of bacteria and dTALE-induced GFP via confocal microscopy. With this system, we demonstrated that

stomatal guard cells and mesophyll cells, but not epidermal cells, are targeted by X. translucens T3Es during the internal leaf infection stage. This system provides new insights into the spatiotemporal dynamics of plant-bacteria interactions during infection.?

P2.1-069

A NATURAL SINGLE NUCLEOTIDE MUTATION IN THE SMALL REGULATORY RNA ARCZ OF DICKEYA SOLANI SWITCHES OFF THE ANTIMICROBIAL ACTIVITIES AGAINST YEAST AND BACTERIA.

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Text
The necrotrophic plant pathogenic bacterium *Dickeya solani* emerged in the potato agrosystem in Europe. All isolated strains of *D. solani* contain several large polyketide synthase/non-ribosomal peptide synthetase gene clusters. Analogy with genes described innother bacteria suggests that the clusters *ooc* and *zms* are involved in the production of secondary metabolites of the oocydin and zeamine families, respectively. A third cluster that we named ssm for solani secondary metabolite had an unknown function. In this study, we constructed mutants impaired in each of the three secondary metabolite clusters *ssm*, *ooc*, and *zms* to compare first the phenotype of the *D. solani* wild-type strain D s0432-1 with its associated mutants. We demonstrated the antimicrobial functions of these three PKS/NRPS clusters against bacteria, yeasts or fungi. The secondary metabolite cluster ssm, conserved in several other *Dickeya* species, produces a secondary metabolite inhibiting yeasts. Phenotyping and comparative genomics of different *D. solani* wild-type isolates revealed that the small regulatory RNA ArcZ plays a major role in the control of the clusters *ssm* and *zms*. A single-point mutation, conserved in some *Dickeya* wild-type strains, including the type strain IPO 2222, impairs the ArcZ function by affecting its processing into an active form.

P2.1-070

THE SELECTIVE AUTOPHAGY RECEPTOR NBR1 IS A CENTRAL HUB IN PLANT IMMUNITY

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Text

To increase virulence, plant pathogenic effectors disturb the ubiquitin-proteasome system and autophagy proteolytic pathways by interfering with host cellular functions. We studied the bacterial pathogen *Xanthomonas campestris pv. vesicatoria* (*Xcv*), a significant agricultural pest. We demonstrated that the *Xcv* type-III effector, XopL, inhibits host autophagy. XopL interacts with and ubiquitinates a component of the host autophagic machinery, SH3P2. This causes SH3P2 to be degraded by the proteasome, perturbing host autophagy and enhancing *Xcv* bacterial growth. In turn, the plant uses NBR1, a defense-related selective autophagy receptor, to defend itself triggering XopL degradation through a process termed “effectorphagy”. However, we discovered that this connection is driven by both ubiquitin-dependent and -independent interaction. This suggests another layer, apart from “effectorphagy”, in NBR1-mediated immunity. To unravel the tight regulation of this component, we aim to understand the dynamic of Ubiquitin-dependent and -independent NBR1 interactome in an infection context. In response to *Xcv* infection, we identified multiple post translational modifications as well as novel interactors involved in protein degradation, plant immunity, trafficking and cytoskeleton regulation. This supports the role of selective autophagy as a central plant immune mechanism and will permit to decipher its precise regulation in response to phytopathogenic bacteria.

P2.1-071

ALIEN PATHOGEN IMPACT IS DRIVEN BY THE LACK OF PLANT-PATHOGEN CO-EVOLUTION DYNAMICS: THE CASE OF OLIVE QUICK DECLINE SYNDROME CAUSED BY XYLELLA FASTIDIOSA SUBSP. PAUCA

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Text

Plant and pathogen are in eternal arms race. Longer the plant-pathogen get in touch and more sophisticated are the mechanisms that regulate the resistance and the virulence. Alien pathogens cannot be perceived by the host by co-evolved molecular patterns such as the zig-zag model. Early reactions to an alien pathogen include the production of free fatty acids (FFA) and oxylipins. Xylella fastidiosa (Xf) evolved into several hosts in the Americas and it is relatively new in Europe where can be considered as “alien”. Global trade shipped it in Italy causing in less than 10 years death of hundreds of thousands olive trees. In our study FFA and oxylipins signature characterise the plant reaction to Xf subsp. pauca (Xfp). Nicotiana benthamiana, Arabidopsis thaliana, Olea europea develop a specific lipid signature in response to Xfp infection: increase of monoenoic fatty acids (14:1; 16: 1; 18:1) and of linoleic and linolenic acid derived oxylipins (9/13-HpOD/TrE). These lipids are more abundant in symptomatic plants and less in positive-Xfp plants, symptomless or with a low rate of symptoms (i.e. olives treated with zinc-copper-citric acid biocomplex, resilient olive trees,

plant deleted in lipoxygenases). The modulation of FFA and oxylipin-forming enzymes may allow the plant-pathogen to co-exist and turn-down the “Xylella” emergency in Europe.

P2.1-072

THE ANALYSIS OF EXTRACELLULAR VESICLES MEDIATING THE INTERACTION BETWEEN PECTOBACTERIUM AND ARABIDOPSIS

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Text

Plants and bacteria release extracellular vesicles (EVs) that carry various molecules, nucleic acids, proteins, and lipids. Plant EVs contain defence-related proteins that may reduce the virulence of pathogens. Bacterial EVs contain effectors that participate in pathogenicity and modulate plant immunity. This study analysed the EVs produced during the interaction between *Pectobacterium* and *Arabidopsis*. The MALDI-TOF/TOF-MS analysis was conducted to identify the cargo of EVs.

Using electron microscopy, we showed that *Pectobacterium* and *Arabidopsis* secreted small amounts of EVs when cultivated separately in a liquid MS medium. The inoculation of *Arabidopsis* with bacteria resulted in increased production of EVs.

Proteomics analysis of EVs content showed that three times more proteins were secreted when plants were cultivated in the presence of bacteria than alone. Among 394 proteins identified in EVs isolated from plants inoculated with bacteria, 126 were also recognised for the monoculture of *Arabidopsis*. Only 36 proteins were unique for the non-inoculated plants. Among the plant proteins, the most abundant were those related to the stress response, protein synthesis and various transporters. In the case of bacterial proteins, virulence factors were detected. Our results indicate that EVs mediate the interaction between plants and bacteria, and their cargo consists of proteins that play a role in virulence and nutrient acquisition.

Funding: OPUS18-2019/35/B/NZ9/01973

P2.1-073

IDENTIFICATION OF EFFECTORS FROM PHLOEM-RESTRICTED BACTERIAL PATHOGENS, AND THEIR HOST TARGET

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Text

'Candidatus Liberibacter solanacearum' (Lso) is a phloem restricted bacterium infecting

potato and other Solanaceae in the USA, and carrots and other apiaceous crops in Europe and the Mediterranean basin. Liberibacter pathogens do not encode a Type III or Type IV secretion system therefore, effectors sec-dependent and non-traditionally secreted protein. Candidate effectors were selected from analysis of Lso proteins using Signal P and secretome. Our strategy for effector validation includes validation of the signal peptide and secretion in a surrogate system, followed by transient expression to evaluate if the candidates induce and reduce HRs, followed by identification of proteins targets using yeast-two hybrid, bimolecular fluorescent complementation, and co-immunoprecipitation. Using this strategy, we characterized five effectors, which suppressed the hypersensitive response induced by PrfD1416V. Lso-HPE1 interacts with Solanum lycopersicum RAD23c, d and e but not RAD23a. We also identified CKC_5770 which interact with APX-6. Both of these effectors have homologous gene in Ca. L. asiaticus (CLas, the pathogen responsible for citrus greening) which interact with the corresponding citrus proteins. This finding demonstrate that the Lso-tomato interaction can be used as a model for CLas – citrus system. Three other effectors specific of Lso are under investigation.

Mycotoxin producing fungi and their management: a serious challenge to attain the One Health goals

C1.4-1

PREDICTION OF CLIMATE CHANGE IMPACT ON OCCURRENCE AND PREVALENCE OF MYCOTOXIN PRODUCING FUNGI

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Text

Climate change (CC) has a significant impact on agricultural systems and threatens safety and security of food and feed. Increased temperature and CO2, along with uncertainties related to extreme weather events, such as heat waves, droughts, and heavy rains, are driving variables for fungi and mycotoxin occurrence. Europe has experienced unexpected outbreaks of aflatoxin contamination in maize since the early 2000s, particularly during extremely warm and dry seasons. Fungi showed a shift towards higher latitudes, and significant differences in mycotoxin contamination were noticed depending on the growing year. Fungi and mycotoxin co-occurrence were underlined in different crops, enhanced by extreme weather events, with relevant variation also in small geographic scales. CC has already impacted the cropping systems and the geographical distribution of crops. As a result, we can expect increased variability and new scenarios, therefore accurate predictions will be crucial for ensuring sustainable food systems. Big data, machine learning (ML), and decision support systems are rapidly advancing in agriculture, offering a new approach to farm management. ML can effectively combine qualitative data input, like cropping system information, with meteorological data, thereby improving the accuracy of predictions. To

address the adverse effects of CC, research needs to embrace predictive data-driven solutions for a more sustainable future.

C1.4-2

PATHOGENICITY, MYCOTOXINS, AND GENETICS OF THE TOXINOGENIC FUSARIUM PROLIFERATUM

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Text

Fusarium proliferatum, is a polyphagous plant pathogen of a wide range of agriculturally important plant hosts and produces a relevant number of mycotoxins, mainly the carcinogenic fumonisins B (FBs). Many studies on F. proliferatum mycotoxin profile according to the crop of origin and its genetic variability have been carried out, aimed to understand the capability of this fungus to perfectly adapt in a wide array of different ecological niches and express its pathogenicity. A wide amount of studies have shown that F. proliferatum is a major FB producing species worldwide. However, strains isolated from a number of crop plants worldwide (date palm, fig, sugarcane, wheat) lack capacity to produce FBs, although the data on gene occurrence, orientation and genome location of the FB gene cluster between producing and non-producing strains revealed no significant differences. Phylogenetic analyses for identifying possible clades within the species provided only slight differences. Therefore, genomic and metabolomics investigations were carried out and underpinned that a poly-omic approach is a powerful tool for unraveling the genetic and mycotoxin profile variability of F. proliferatum from different plants. The capability of F. proliferatum to adapt to such a wide number of ecological niches allows us to acknowledge it as a perfect example of fungal biodiversity. Studies performed in cooperation with several reasearch groups worldwide will be presented

C1.4-3

AFLATOXIN BIOCONTROL: A CLIMATE-SMART TECHNOLOGY PROTECTING STAPLE CROPS IN THE SAHEL

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Text

The risk of exposure to mycotoxins in countries in sub-Saharan Africa is high. The Sahel

region is particularly affected due to its adverse climatic conditions exacerbated by climate change impacts. Consequently, maize, groundnut, and sorghum grown in Burkina Faso, Mali, Niger, and Togo are frequently contaminated with aflatoxins biosynthesized by *Aspergillus* spp. Exposure assessments conducted in Burkina Faso, Mali and Niger revealed elevated aflatoxin levels in crops with estimated liver cancer cases up to 126 cancer cases/100,000 persons/year in some regions. About a decade ago, an aflatoxin biocontrol product named Aflasafe BF01 containing four atoxigenic isolates of A. flavus, widely distributed across Burkina Faso, was developed. The efficacy of the product in Burkina Faso (70 to 100% less aflatoxins in treated compared to untreated crops) paved the way for its registration with the Sahelian Pesticide Committee (CSP) of the Permanent Interstate Committee for Drought Control in the Sahel (CILSS) which currently has 13 member states. Similar efficacies were demonstrated in Mali, Niger, and Togo. Consequently, use of this nature-based solution is proving an effective pre-harvest management solution against aflatoxins. The technology can expand potential market outreach and provide a true area-wide strategy for sustainable solution to One Health and trade challenges posed by crop aflatoxin contamination in the region.

C1.4-4

IDENTIFICATION AND IMMOBILIZATION OF A PATULIN BIODEGRADING ENZYME CGSDR AND ITS APPLICATION IN DETOXIFICATION OF APPLE JUICE

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Text

Mycotoxin contamination of fruits and their derived products is a problem of great concern. Patulin, a secondary metabolite mainly produced by Penicillium expansum, is the most potent mycotoxin in fruits, especially apples and apple-based products. Traditional physical and chemical methods are currently limited because of chemical hazards and affecting nutritional quality of the products. Biological detoxification methods using microorganisms or enzymes represent fewer health and environmental risks and are emerging as promising alternatives. Recently, we identified a short-chain dehydrogenase/reductase CgSDR from a patulin biodegrading strain Candida guilliermondii. CgSDR could transform patulin into E-ascladiol with NADPH as a coenzyme. A molecular docking analysis and site-directed mutagenesis indicated that VAL188 and THR190 were the key active binding sites for patulin biodegradation. Further, we covalently linked CgSDR to magnetic nano-Fe_3O_4 particles to prepare an immobilized enzyme. The immobilized CgSDR combined the advantages of both the magnetic nanoparticles and the degrading enzyme, could completely remove 1 μg/mL patulin in phosphate-buffered saline within 4 h. It also exhibited a high detoxification rate in apple juice and did not cause adverse effects on juice quality. Immobilized CgSDR had characteristics of high efficiency, stability, safety, and easy separation, showing a good application prospect in controlling patulin contamination.

C1.4-5

EFFICACY OF THE ASPERGILLUS FLAVUS ATOXIGENIC STRAIN TECHNOLOGY TO REDUCE RISKS OF AFLATOXIN CONTAMINATION IN COMMERCIAL TREE NUT ORCHARDS IN CALIFORNIA

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Text

Aflatoxins are the most toxic mycotoxins, which are highly regulated worldwide. They are produced by fungi in the *Aspergillus* section *Flavi*, including *A*. *flavus* and *A*. *parasiticus* commonly found in California. Aflatoxins pose a highly economical threat to the tree nut (pistachio and almond) industries due to product rejection from markets. The *A*. *flavus* atoxigenic strain technology effectively reduces aflatoxins in crops by increasing the proportion of atoxigenic isolates and reducing the aflatoxin production potential of the population. The efficacy of *A*. *flavus* AF36 to reduce aflatoxins in commercial tree nut orchards has been evaluated for over a decade in California, including the effects of product rates, time of application, and irrigation method on both aflatoxin content, and displacement of toxigenic isolates by the biocontrol AF36. Aflatoxin content in 200-500 samples annually was determined by HPLC and displacement was determined from soil samples taken after harvest each year. Results indicate that the biocontrol AF36 reduces aflatoxin contamination by increasing the proportion of the atoxigenic strain AF36 in commercial orchards. However, the efficacy of the biocontrol applications in tree nuts in California has not reached its full potential with only an average 40% aflatoxin reduction compared to untreated controls, and 70% displacement of toxigenic isolates in both pistachio and almond orchards. The effect of treatments to improve efficacy will be discussed

C1.4-6

PREPARING THE OAT INDUSTRY FOR HT2 AND T2 MYCOTOXIN LEGISLATION

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Text

HT-2 and T-2 are related fusarium mycotoxins produced by several *Fusarium* species but are predominantly produced by *Fusarium langsethiae* on European cereals, with oats being particularly susceptible. European legislative limits for HT-2 and T-2 are currently in draft and expect to be introduced in 2024. Based on previous surveys of UK commercial oat crops, the proportion of samples exceeding the draft limit for unprocessed oats (1250 µg/kg combined HT2+T2 concentration) varied between 0 and 25% each year, with a mean of 13%.

Analysis of the impact of agronomic factors on the HT-2 and T-2 concentration of oat crops within the surveys identified significant differences between HT2+T2 concentration across production systems (organic v conventional), rotations and oat varieties. Field experiments have confirmed differences between varieties but have failed to show any differences

associated with conventional production (fertilisers, plant growth regulators and fungicides). When HT-2 and T-2 legislation is set then growers will need to minimise the risk of exceeding limits. Based on the known impact of agronomy on the HT2+T2 content of oats there are few economically viable options to reduce these mycotoxins. The most readily available option for growers to reduce the risk of exceeding HT2+T2 legislations is a change to more resistant oat varieties.

F1.4-1

EVALUATION OF WINTER WHEAT FOR FUSARIUM HEAD BLIGHT RESISTANCE AND DEOXYNIVALENOL LEVEL IN ONTARIO, CANADA

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Text

Development of wheat resistant to Fusarium head blight (FHB), while improving yield and maintaining quality requirements is important in Ontario, Canada. FHB is a serious disease of wheat and deoxynivalenol (DON) is the most common mycotoxin produced by Fusarium graminearum (FG). All wheat commercially grown in Ontario is entered in the Performance Trials, and tested for agronomy traits and FHB resistance, in nurseries spray inoculated with FG isolates, at anthesis. FHB symptoms are recorded as incidence (percent of heads infected) and severity (percent of spikelets infected). FHB index is calculated as severity x incidence, divided by 100. DON level is estimated using ELISA method. Wheat cultivars and checks are grouped, based on FHB visual symptoms and DON level, using historical and the most recent data (www.gocereals.ca). The categories are: moderately resistant (MR), moderately susceptible (S), susceptible (S) and highly susceptible (HS). Some cultivars have different category for FHB index and DON level. Phenotyping, genotyping and development of new cultivars, with increased level of FHB resistance and higher yield is in progress, in breeding program from University of Guelph, Ridgetown Campus. Correlation among morphological traits (plant height, heading date, awns presence), FHB related traits (severity, incidence, index, and DON level) and yield, will be presented. DON level of winter wheat populations, with different sources of FHB resistance, will be compared.

P1.4-001

CASE STUDIES OF MYCOTOXIN CONTAMINATION IN ORGANIC MAIZE STORED AND MILLED BY SMALLHOLDER FARMERS IN SOUTHWEST FRANCE

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Text

In Europe, there is a trend toward increased production of small grain cereals and maize by small-scale organic farmers storing and processing their production on-farm. This responds to consumer demand for local, healthy food. In this context, knowing what happens to

mycotoxins throughout the grain production chain, from the field to the final product, is a challenge for food safety and the resilience of this production system.
With the contribution of 12 organic farmers growing population varieties of maize for human consumption, we collected samples of grains at harvest and after storage for several months at the farms and the resulting flour and meal after milling, for two successive years. The samples were analysed for mycotoxin content. (Trichothecenes B and A, Zearalenone, Fumonisins, Aflatoxins) and contamination by different Fusarium species. The results of the analyses were interpreted in the light of the farmers' technical practices regarding cultivation, storage and processing.
Alarming levels of contamination have been measured in some samples and levers for action have been identified. Convincing farmers to adapt some of their practices that they associate with the typicality of their product is a new challenge.

P1.4-002

ENCAPSULATED ESSENTIAL OILS IN MESOPOROUS SILICA NANOPARTICLES TO CONTROL FUSARIUM AVENACEUM AND ITS ENNIATINS PRODUCTION

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Text
Essential oils (EOs) having antifungal activity and mycotoxins reduction ability are candidates to develop bioactive alternatives and environment friendly treatment against *Fusarium* species affecting cereals and other crops. However, their practical use is facing limitations such as high volatility, UV sensitivity, and oxidation. Nanoencapsulation techniques are supposed to provide protection to the EOs and control their release into the environment. We selected *Ammoides pusilla* essential oil (AP-EO) as an efficient inhibitor of *Fusarium avenaceum* growth and its Enniatins (ENNs) production. AP-EO was encapsulated, into mesoporous silica nanoparticles (MSNPs) with narrow slit pores (3.1 nm). In contact assays in an agar medium, the antifungal activity of AP-EO at 0.1 µL mL-1 was improved three times when encapsulated into MSNPs and the ENNs production was significantly inhibited. Controls of MSNPs also inhibited the ENNs production without affecting the mycelial growth. In fumigation experiments assessing the activity of the EO volatile compounds, encapsulation into MSNPs improved significantly both the antifungal activity and the ENNs inhibition. Encapsulation of an EO into MSNPs improving its antifungal and antimycotoxins properties is promising for the formulation of a natural fungicide to protect plant or food products from the contamination by *Fusarium spp*. and their potential mycotoxins, after risk assessment of the use of silica nanoparticles in agriculture.

P1.4-003

INVESTIGATION OF THE EFFECTIVE INHIBITION OF DON PRODUCTION BY BOTANICAL COMPOUNDS LA AND MEJA AND THEIR INHIBITION MECHANISM

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Text

Deoxynivalenol (DON) is one of the mycotoxins produced by Fusarium head blight, which not only seriously threatens human and animal health, but also is an important pathogenic factor. However, the number of DON-inhibiting fungicides is small, and there are problems with chemical fungicides such as resistance and environmental pollution. Here, botanical compounds that are environmentally friendly and difficult to develop resistance are screened to address DON contamination. Among dozens of botanical compounds, lauric acid (LA) and methyl jasmonate (MeJA) with the best DON-inhibiting effect were screened. The inhibition rates of LA (2.65 mM) and MeJA (1 mM) were about 65% and 75%, respectively. In terms of application potential, LA had protective activity against wheat coleoptile and exhibited synergistic effects when used in combination with metconazole. As for the mechanism of DON inhibition, LA was able to destroy the toxisome. Interestingly, LA also affected DON synthesis by influencing pathogen metabolism, as deletion mutants of the LA metabolism gene were inconsistent in DON production compared to wild-type. In contrast, MeJA did not affect toxisome, but was effective in reducing DON content in the seeds in field trials and reducing the pathogenicity of coleoptile by inhibiting DON. Collectively, all results suggest that LA and MeJA have a high potential to be developed as DON-inhibiting fungicides. This study provides new insights into the prevention and control of DON.

P1.4-004

POPULATION GENETIC STRUCTURE OF PATHOGENS CAUSING FUSARIUM CROWN ROT AND ITS MYCOTOXIN CONTAMINATION OF WHEAT IN CHINA

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Text

Fusarium crown rot (FCR) caused by *Fusarium* spp. has been prevalent in China in recent years, posing a serious threat to wheat production. In this study, a large investigation of the species composition of FCR pathogen in 375 sites of 13 provinces was conduct, covering all the main wheat growing areas in China. A total of 23 Fusarium species were isolated and identified. *F. pseudograminearum* and *F. graminearum* were predominant species in Winter Wheat Region in North China and Northeast Spring Wheat Region, respectively. *F. asiaticum* was the dominant species in the Middle-Lower Yangtze Valleys Winter Wheat Region. The *F. pseudograminearum* population had increased in North China in the recent years. The proportion of *F. graminearum* had also raised in the Middle-Lower Yangtze Valleys Winter Wheat Region. Simple sequence repeats analysis showed that the population diversity of *F. pseudograminearum* was low and asexual reproduction was dominant in all regions. Mycotoxin was mainly observed in lower internodes of infected wheat plants and a low amount

was detected in the upper parts above the 4th internode in artificial inoculation. However, under field conditions, high levels of trichothecene accumulation were detected in the upper segments. Trace amounts of mycotoxin appeared to be translocated to grains, indicating that FCR infection in natural fields poses a relatively small threat to contamination of grains, but a larger amount to plant parts that may be used as animal feed.

P1.4-005

ENDOPLASMIC RETICULUM-MITOCHONDRIA ENCOUNTER STRUCTURES (ERMES) REGULATE ENERGY METABOLISM: A NEW DRUG TARGET FOR PATHOGENIC FUNGI?

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Text

In fungal cells, the information exchange between mitochondria and endoplasmic reticulum (ER) is realized through the contact sites of organelle—ER-mitochondria encounter structure (ERMES) complex. ERMES complex consists of four subunits MMM1, MDM10, MDM12, MDM34. Our study found that ERMES complex plays a regulatory role in energy synthesis and secondary metabolism in *Fusarium graminearum*. In the ERMES mutants, the mitochondrial morphology significantly changed from normal filamentous to functional defect spherical, and ATP content decreased. This suggests that it may be involved in regulating mitochondrial morphology and function. Our further study that MDM10 interacts with the mitochondrial fission protein DNM1 confirms this result. DON produced by *F. graminearum* is a secondary metabolic product and a vital virulence factor. Our research shows that the generation of ERMES mutants leads to the decrease of DON and morphological structural disruption of Tri1-labeled toxisome, and all subunits of the ERMES complex were partially co-located with Tri1-labeled toxisome under confocal microscopy. These data indicates that the ERMES also plays a regulatory role in DON production and toxisome formation in *F. graminearum*. Although SDHIs fungicides can obviously reduce the transcription and protein expression of ERMES, whether SDHIs can reduce DON is related to ERMES remains unclear. ERMES was found only in fungal cells, making it a potential target for pathogen fungal control.

P1.4-006

EFFORTS TO DEVELOP MANAGEMENT STRATEGIES FOR THE AFLATOXIN CONTAMINATION OF HAZELNUT IN AZERBAIJAN

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Text

Hazelnut is of great cultural and economic importance in Azerbaijan. Apart from domestic consumption, a large portion of the production is exported. However, hazelnut trade has been recently affected by aflatoxin contamination by *Aspergillus* spp. FAO and various

partners are implementing a project to improve productivity and safety of hazelnut. However, in Azerbaijan, little is known about factors contributing to aflatoxin contamination of hazelnut. FAO and IITA met with farmers, partners, and key institutions affected by aflatoxin in hazelnut. Aflatoxin was determined in samples collected in farmers' stores in three regions using an *in-situ* quantification protocol. The processing and quantification were conducted in farmers homes/warehouses. Aflatoxin was found in all 33 examined samples (range = 1.1 to 7.2 ppb). Most farmers stored hazelnut sub optimally. Chances of increased aflatoxin throughout storage are thus high. Despite several challenges, there is a strong desire and political will of various stakeholders to reduce aflatoxin and improve trade in hazelnuts. Opportunities to reduce aflatoxin relatively rapidly in Azerbaijani hazelnut exist. There is a need to converge efforts of different stakeholders and continue to create awareness about aflatoxin and its repercussions. Challenges and opportunities for effective aflatoxin mitigation strategies to reduce contamination, reduce rejection of hazelnut from Azerbaijan, and increase trade in the region will be discussed.

P1.4-007

THE OCCURRENCE AND CONTROL OF OCHRATOXIN A FROM POSTHARVEST DISEASES

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Text

Ochratoxin A (OTA) contamination and the associated issues of food security, food safety and economic loss are widespread throughout the world. The occurrence of OTA depends on ochratoxigenic fungi, foodstuffs and their environment. In this report, natural occurrence and control strategy of OTA, with a focus on the impact of environmental factors, are summarized. First, this report introduces potentially contaminated foodstuffs, including the emerging ones which are not regulated in international legislation. Secondly, we gives an update of native producers based on foodstuffs and OTA biosynthesis. Thirdly, complicated environmental regulation is disassembled into individual factors in the research, such as pH and substrates, in order to clarify their regulatory effect and mechanism. Finally, we developed some strategies to control OTA, including biocontrol and effective detoxification.

P1.4-009

UNRAVELING TH HOST-SELECTIVE TOXIC INTERACTION OF CASSIICOLIN WITH LIPID MEMBRANES AND ITS CYTOTOXICITY

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Text

Cassiicolin (Cas), a toxin produced by *Corynespora cassiicola*, is responsible for Corynespora leaf fall disease in susceptible rubber trees. Currently, the molecular mechanisms of the cytotoxicity of Cas and its host selectivity have not been fully elucidated. Here, we analyzed the binding of Cas1 and Cas2 to membranes consisting of different plant lipids and their membrane disruption activities. Using high-speed atomic force microscopy and confocal microscopy, we reveal that the binding and disruption activities of Cas1 and Cas2 on lipid membranes are strongly dependent on the specific plant lipids. The negative phospholipids, glycerolipids, and sterols are more sensitive to membrane damage caused by Cas1 and Cas2 than neutral phospholipids and betaine lipids. Mature Cas1 and Cas2 play an essential role in causing membrane disruption. Cytotoxicity tests on rubber leaves of Rubber Research Institute of Vietnam (RRIV) 1, RRIV 4, and Prang Besar (PB) 255 clones suggest that the toxins cause necrosis of rubber leaves, except for the strong resistance of PB 255 against Cas2. Cryogenic scanning electron microscopy analyses of necrotic leaf tissues treated with Cas1 confirm that cytoplasmic membranes are vulnerable to the toxin. Thus, the host selectivity of Cas toxin is attained by the lipid-dependent binding activity of Cas to the membrane, and the cytotoxicity of Cas arises from its ability to form biofilm-like structures and to disrupt specific membranes

P1.4-010

POST-HARVEST SUSTAINABLE STRATEGIES TO REDUCE MYCOTOXINS CONTAMINATION AND PEST INFESTATION IN CHICKPEA (CICER ARIETINUM L.) STORED SEEDS

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Text

Mycotoxigenic fungi and pests represent a pivotal threat for global food safety, with crucial implications for human and animal health. Controlled atmosphere and oxidising agents (i.e., nitrogen, N_2; ozone, O_3) represent a sustainable strategy for the reduction of mycotoxin content and the growth of deleterious organisms in foodstuffs. In our work, O_3 treatment (500 ppb; 30, 60 or 90 min) and high N_2 concentration (98.5%; 21 days) were tested in the post-harvest storage of four batches of *Cicer arietinum* L. seeds to reduce the presence of mycotoxigenic fungi (i.e. *Penicillium* spp.) and their mycotoxins (i.e. aflatoxins and patulin), as well as pest (i.e., *Callosobruchus maculatus*) infestation. Ozone exposure critically decrease the presence of *Penicillium* spp. (−50% on average, independently to the time of exposure) and the patulin and aflatoxins amount after just 30 min from the beginning of the exposure (−85 and −100%, respectively). High N_2 concentrations remarkably reduced mycotoxins contamination (−94% on average) and induced *C. maculatus* mortality (100% after 5 days of exposure). These results confirm the promising potential of controlled atmosphere and oxidizing agents in post-harvest storage to drastically reduce the risk of

mycotoxin contamination and pests infestation. Further investigations are needed to extend these technologies on a large scale and to elucidate the effects of these treatments on the qualitative traits of seeds.

P1.4-011

QUEST FOR FUSARIUM: A SAMPLING STRATEGY TO PREDICT THE RISK OF CONTAMINATION IN IRISH CEREAL CROPS

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Text

Fusarium spp. are pathogenic fungi producing toxic secondary metabolites called mycotoxins; the most common mycotoxins in cereals are deoxynivalenol, zearalenone, T-2 and HT-2. As there is a concern regarding the health impact of emergent, modified and combination of mycotoxins present in cereals, the EU is considering imposing new limits for the mycotoxins commonly found in oats. In oats, mycotoxin contamination can occur in the absence of visual symptoms of fungal contamination, making it more difficult to assess. Mycotox-I aims to minimise the mycotoxin contamination in Irish cereals, focusing on oats. As the production of the mycotoxin starts in the field and continues during storage, the project will develop and standardise the technologies for predicting the mycotoxin potential in crops and mycotoxin content in cereal products.
To identify the spectrum of Fusarium species and mycotoxins in cereals, the level of infection is assessed based on field scale surveys developed to cover different production systems and key factors that might contribute to fungal infection. Further, the Fusarium species are isolated, identified and characterised. The impact of cropping systems on mycotoxin contamination of Irish grains will be also evaluated by conducting field scale trials.

P1.4-012

RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT: INFLUENCE OF THE FUNGAL MYCOTOXIN PROFILE

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Text

Fusarium head blight (FHB) is a destructive wheat disease which reduces yield and grain quality, and the associated mycotoxins threaten food safety. Host resistance especially resistance to tricothecenes (deoxynivalenol) is considered an important management strategy. The study aims to determine the correlation between FHB resistance and the FHB causal pathogen mycotoxin profile. Species-specific primers validated morphological results of 39 isolates from eight localities, identified as *F. graminearum* (*sensu lato*). TRI13 sequences were used for the genotypic characterisation of trichothecene gene clusters. All

isolates were deoxynivalenol (DON) producers. In cooler regions of South Africa, temperature < 35°C, a high frequency and concentration of 3-ADON was recorded. In contrast, warmer regions, temperature > 35°C, had a high frequency and concentration of 15-ADON isolates. PCR assays confirmed the presence of FHB resistance QTLs and genes in the evaluated wheat lines. Under inoculated conditions with three isolates, wheat lines with type I resistance had a lower incidence than those with type II resistance. A high disease incidence (~39%) in wheat spikes and low disease severity (~4%) was observed. Suggesting little need to breed for type III (resistance to DON accumulation) as type I and II (resistance to disease initiation and spread) can manage disease in South African wheat.

P1.4-013

EFFICACY OF THE ASPERGILLUS FLAVUS ATOXIGENIC STRAIN TECHNOLOGY TO REDUCE RISKS OF AFLATOXIN CONTAMINATION IN COMMERCIAL TREE NUT ORCHARDS IN CALIFORNIA

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Text

Aflatoxins are the most toxic mycotoxins, which are highly regulated worldwide. They are produced by fungi in the *Aspergillus* section *Flavi*, including *A. flavus* and *A. parasiticus* commonly found in California. Aflatoxins pose a highly economical threat to the tree nut (pistachio and almond) industries due to product rejection from markets. The *A. flavus* atoxigenic strain technology effectively reduces aflatoxins in crops by increasing the proportion of atoxigenic isolates and reducing the aflatoxin production potential of the population. The efficacy of *A. flavus* AF36 to reduce aflatoxins in commercial tree nut orchards has been evaluated for over a decade in California, including the effects of product rates, time of application, and irrigation method on both aflatoxin content, and displacement of toxigenic isolates by the biocontrol AF36. Aflatoxin content in 200-500 samples annually was determined by HPLC and displacement was determined from soil samples taken after harvest each year. Results indicate that the biocontrol AF36 reduces aflatoxin contamination by increasing the proportion of the atoxigenic strain AF36 in commercial orchards. However, the efficacy of the biocontrol applications in tree nuts in California has not reached its full potential with only an average 40% aflatoxin reduction compared to untreated controls, and 70% displacement of toxigenic isolates in both pistachio and almond orchards. The effect of treatments to improve efficacy will be discussed

P1.4-014

DISPLACEMENT OF AFLATOXIN PRODUCING FUNGI BY AN ASPERGILLUS FLAVUS ATOXIGENIC BIOCONTROL IN COMMERCIAL ALMOND ORCHARDS IN SEVERAL AREAS OF CALIFORNIA

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Text
Aflatoxins are toxic secondary metabolites produced by fungi in *Aspergillus* section *Flavi* and pose a highly economical threat to the tree nut industries, including almond, due to risks of product rejection from markets by government regulations. Avoiding insect damage, mainly by navel orangeworm (*Amyelois transitella*), does not completely prevent contamination. The *A. flavus* atoxigenic strain biocontrol technology effectively reduces aflatoxins in susceptible crops. The goal of this technology is to reduce the aflatoxin production potential of the fungal population by increasing the proportion of atoxigenic isolates. The efficacy of atoxigenic *A. flavus* AF36 applications to reduce the risk of aflatoxin contamination in almonds was evaluated in seven areas with a total of 5,000 treated acres in California. Soil samples were taken before application of the biocontrol and after harvest. Fungal isolates were recovered from the soil samples and characterized as *A. flavus* AF36, toxigenic strains and other non-toxigenic strains. Five of the 7 areas had 70% or higher of the biocontrol AF36. In two areas, although there was an increase of AF36, it was <50%. Results indicate that application of the *A. flavus* AF36 biocontrol in commercial almonds is effective to change the population structure of the aflatoxigenic fungi by displacing the toxigenic strains with atoxigenic strains, which will reduce the aflatoxin production potential and the risk of aflatoxin contamination in almonds.

P1.4-016

DEVELOPING POTENTIAL BIOCONTROL AGAINST MYCOTOXIGENIC FUNGI OF CEREALS

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Text
During postharvest storage, contamination of cereals, such as wheat and maize, by mycotoxigenic fungi affects the quality and produces toxic substances rendering the grains unsafe for consumption. *Aspergillus flavus*, and *Fusarium proliferatum* are considered major contaminants of stored grains that are usually controlled by chemicals applied by fumigation. There is an urgent need for sustainable and ecologically safe approaches to manage mycotoxigenic fungi. In this work, we focused on isolation of endophytic microorganisms and testing their antagonistic activity against both mycotoxin-producing fungi. Results showed that among the isolated endophytic microorganisms, bacterial strains belonging to Bacillus spp. were found effective in inhibiting the growth of *A. flavus* and F. *proliferatum*, both in vitro and on grains. The fungal DNA was reduced by 70.5% and 89.7% for the wheat grains that were inoculated with *A. flavus* and pretreated with *B. subtilis* or *B. amyloliquefaciens*, respectively, as compared to control. The fungal DNA was decreased by 41.6% and 62.7%, for the wheat grains inoculated with *F. proliferatum* and pretreated with *B. subtilis* or *B. amyloliquefaciens*, respectively, as compared to control. In addition, their secondary metabolites exhibited antifungal activity against *A. flavus* and *F. proliferatum* in a disc diffusion assay. Further, isolate VB24 significantly decreased the production of Aflatoxin B1 in liquid culture by *A. flavus* as compared to the control.

P1.4-017

NEW CHALLENGES IN THE APPLE CHAIN DUE TO MYCOTOXIN PRODUCING FUNGI AND MYCOTOXINS

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Text

Mycotoxin producing fungi are causing new challenges for safe and sustainable food systems due to climate change. Apples and their derived products are largely consumed by the European population. Patulin is the only regulated mycotoxin in apple derived products, but recently aflatoxin B1 and fumonisins were signaled in apple puree. Therefore, the aim of this study was to monitor mycotoxin producing fungi and mycotoxin in the apple chain. Apple fruits, cv Golden delicious and Imperatore, were collected in three orchards placed across three different production zones located in north Italy during harvesting. Stored fruits, same varieties, were sampled before processing together with the corresponding puree. Fruits were mashed using a portable blender before analysis. Alternaria spp and Penicillium spp were almost always isolated from field samples and the related toxins, patulin and Alternaria toxins too. In addition, aflatoxins were detected, even with differences between cultivars and growing areas. The same fungi and mycotoxins were detected in stored apples, with in addition the sporadic occurrence of Aspergillus section Flavi and Fusarium spp. In the puree, no fungi were isolated, due to the thermal treatment, but all the mentioned toxins occurred, with the addition of fumonisins. This study confirmed the co-occurrence of mycotoxins in apples fruits and puree at non neglectable levels, but further research is requested to contribute to a safer sustainable apple system.

P1.4-018

POTENTIAL FOR AFLATOXIN B1 AND B2 PRODUCTION BY ASPERGILLUS FLAVUS STRAINS ISOLATED FROM BAMBARA GROUNDNUT (VIGNA SUBTERRANEA (L.) VERDCOURT) SEEDS PRODUCED IN BURKINA FASO

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Text

In Burkina Faso, Bambara groundnut is the second most important food legume after cowpea, especially in rural areas. However, this crop, due to certain conditions of production,

storage or preservation is susceptible to be contaminated by potential mycotoxin producing fungi. The aim of this study was to evaluate the aflatoxinogenic capacity of Aspergillus flavus strains isolated from Bambara groundnut seeds produced in Burkina Faso. Thus, 198 strains of fungi belonging to section Flavi were isolated from 99 samples of Bambara groundnut seeds collected in the three agro-ecological zones of Burkina Faso. According to molecular identification, from the 198 isolates, 28 were identified as Aspergillus flavus. Then, we investigated their potential for aflatoxin B1 (AFB1) and B2 (AFB2) production by growing them on rice at 25°C for 7 days. AFB1 and AFB2 were extracted and quantified by liquid chromatography–mass spectrometry (LC-MS/MS). All of the strains (28) produced AFB1 at concentrations ranging from 46.987 µg kg–1 to 1080.320 µg kg–1. However, AFB2 was produced by 26 strains with concentrations ranging from 47.200 µg kg–1 to 1760.240 µg kg–1. Globally, most of the tested strains produced higher levels of AFB1 than AFB2. The results obtained point out the risk associated with post-harvest fungi and the need for the development of storage technics to control them, for food security.

P1.4-019

IDENTIFYING SORGHUM GRAIN FUNGAL COLONISERS, QUANTIFICATION OF MYCOTOXINS AND DEVELOPMENT OF WEATHER-BASED PREDICTIVE MODELS FOR FUSARIUM GRAMINEARUM

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Text

Sorghum grain mold negatively affects crop yield and quality across the globe, and associated mycotoxin contamination poses a risk to livestock and human health. In the study mycotoxigenic fungi in sorghum grain were identified, concomitant mycotoxins quantified and conducive weather to initiate disease during anthesis was explored. Grain harvested from field trials in KwaZulu Natal and Limpopo (South Africa) were used, from 2009-2013 and 2017-2019, respectively. Thirty-six fungi were morphologically and molecularly identified. Mycotoxin quantification was conducted on representative grain samples. Nine of the 24 representative samples contained deoxynivalenol (DON) and zearalenone (ZEA). One sample contained a ZEA concentration of 1250 µg/kg, exceeding the maximum legislative limit. All DON detected in samples was lower than 1000 µg/kg. Nonlinear regression analyses determined five conducive days of air temperature between 20 and 28°C (R2 = 0.81), 3 to 8 days of rainfall greater than 1 mm (R2 = 62) and six days of 70% relative humidity (R2 = 0.98) as critical for disease initiation. A significant positive correlation between FgSC DNA and DON (R = 0.63) and nivalenol (NIV; R = 0.82) were detected. Suggesting a greater risk of DON and NIV with greater FgSC DNA concentrations. Defining conducive periods associated with infection provides a platform to explore future risk indicators for disease intervention and mycotoxin mitigation.

P1.4-020

OCCURRENCE OF FUSARIUM SPP. IN GERMAN OAT FIELDS – RESULTS FROM A THREE YEAR MONITORING

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Text

In recent years the cultivation of oats in Germany as well as the interest in oat-based products has increased continuously. In particular, the nutritional excellence, as well as the position of oats as a "health crop" in cropping systems have increased the agronomic value of oats. In general, oats are considered a comparatively healthy crop regarding susceptibility to fungal pathogens. Nonetheless, elevated levels of mycotoxins in the harvested material have become an issue of concern in recent years. Such contamination is caused by the infestation with various Fusarium species. The present study was conducted in order to investigate the risk of Fusarium infection in German oat cultivation, to identify the involved Fusarium species and to analyze the range of associated mycotoxins. To this end, a monitoring was performed in the years 2020 to 2022. Samples from fungicide untreated plots were collected, and examined. In addition, agronomic and meteorological field data were recorded. Fusarium spp. were isolated from surface-sterilized oat grains on a nutrient medium. The obtained strains were identified both morphologically and by sequence analysis. Mycotoxins were determined by HPLC-MS/MS. The strains identified from 2020 to 2022 included the following species: F. poae, F. tricinctum, F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. langsethiae, F. cerealis. Major mycotoxins analyzed in grain samples were nivalenol, deoxynivalenol, T-2 and HT-2, and enniatins.

P1.4-021

THE PROTEOMES THAT FEED THE WORLD

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Text

Plants represent the nutritional basis of all life on earth and are essential for feeding an increasing human population while facing new challenges posed by climate change and plant pathogens.
While the genomes and transcriptomes of crops are increasingly elucidated, little is known about crop proteomes. To address this knowledge gap, we have launched a new initiative

with enormous socio-economic relevance at TUM with the Elitenetzwerk Bayern-funded International Doctoral Program “The Proteomes that Feed the World” at its core. One of the program’s overarching aims is to map the proteomes of all major tissues and organs of the 100 crop plants most important for human nutrition, thereby creating a Crop Proteome Atlas of extremely high value to academia as well as the agricultural industry. On top of the creation of the Crop Proteome Atlas, several sub-projects are investigating detailed aspects of plant proteomes in response to biotic stress. For instance, we aim to better understand the mode of action of the Fusarium mycotoxin deoxynivalenol (DON) and its protein biosynthesis inhibitor function in barley.
In preparation for the Crop Proteome Atlas project, a robust and reproducible protocol for the processing and analysis of a variety of plant tissues by liquid chromatography tandem mass-spectrometry (LC-MS/MS) was devised. This protocol constitutes a central component of the Crop Proteome Engine. All data will be made available on PRIDE and ProteomicsDB.

P1.4-022

COMBATING AFLATOXIN EXPOSURE RISKS THROUGH CONSERVATION AGRICULTURE

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Text
This study assessed the impact of Conservation Agricultural (CA) practices and planting soil fertility enhancing trees on toxigenic Aspergillus spp., peanut aflatoxin levels, and aflatoxin exposure risks in Zambia. Soil and peanut samples (270) were collected at farmgate of CA (peanuts grown in basins), AGF (peanuts grown in basins in farms containing Gliricidia sepium agroforestry trees), and NCA (peanuts grown on ridges). Aspergillus population density and aflatoxin level were quantified using the direct plate count and quantitative Neogen Lateral Flow techniques, respectively. Dietary exposure risk was computed using the aflatoxin dataset. Aspergillus spp. concentration [colony forming units (CFU)/g)] ranged from 10 to 7400 CFU/g. NCA supported higher propagules than CA or AGF. The S-morphotype (55%) was the most predominat Aspergillus species followed by A. parasiticus (34%), and A. flavus L strains (11%). Aflatoxin prevalence in peanuts averaged 64% but didn’t differ across farming techniques. Mean total aflatoxins were 110.4, 87.0 and 99.0 µg/kg for AGF, CA and NCA, respectively. Probable daily aflatoxin intake ranged from 12.7 to 767.1 ng/kg bw/d. The estimated liver cancer risk due to consumption of raw peanuts (0.334 - 20.128) was highest among children below 5 years. Therefore, CA has minimal impact on kernel aflatoxin level. Children are at higher risk of developing aflatoxin-related illnesses; thus, their exposure through diets should be controlled.

P1.4-023

MYCOTOXIN PRODUCING FUSARIUM SPECIES IN ESTONIAN GRASSLANDS AND SILAGE

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Text

Grass silage is an important part of animal feed in many European countries. Recent studies have shown mycotoxin contamination in silage, which can have a negative impact on animal health. The aim of this study was to identify mycotoxin producing *Fusarium* species from Estonian grasslands and to analyze the effect of fungicide applications on their presence. Two experiment plots were treated with two different commercially available fungicide products and one plot remained untreated as a negative control. The experiment was repeated on two consecutive years on different grassland plots. Samples were collected before and after fungicide applications and analyzed using species-specific PCR primers. The findings presented in this study did not confirm the effect of fungicides on *Fusarium* species on grassland plants. Evidence for potentially mycotoxin producing fungi was found. Three *Fusarium* species, *F. culmorum, F. cerealis and F. poae* were identified.

P1.4-024

FUSARIUM CULMORUM PRODUCES NX-2 TOXIN SIMULTANEOUSLY WITH DEOXYNIVALENOL AND 3-ACETYL-DEOXYNIVALENOL OR NIVALENOL

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Text

Fusarium culmorum is a major pathogen of grain crops. Infected plants accumulate deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ADON), or nivalenol (NIV), which are mycotoxins of the trichothecene B group. These toxins are also produced by the F. graminearum species complex. New trichothecenes structurally similar to trichothecenes B but lacking the carbonyl group on C-8, designated NX toxins, were recently discovered in atypical isolates of F. graminearum from North America. Only these isolates and a few strains of a yet to be characterized Fusarium species from South Africa are known to produce NX-2 and other NX toxins.

In this study, we report that among 20 F. culmorum strains isolated from maize, wheat, and oat in Europe and Asia over a period of 70 years, 18 strains produced NX-2 simultaneously with 3-ADON and DON or NIV. Rice cultures of strains producing 3-ADON accumulated NX-2 in amounts corresponding to 2–8% of 3-ADON (1.2–36 mg/kg). A strain producing NIV accumulated NX-2 and NIV at comparable amounts (13.6 and 10.3 mg/kg, respectively). In

F. graminearum, producers of NX-2 possess a special variant of cytochrome P450 monooxygenase encoded by TRI1 that is unable to oxidize C-8. In F. culmorum, producers and nonproducers of NX-2 possess identical TRI1; the reason for the production of NX-2 is unknown. Our results indicate that the production of NX-2 simultaneously with trichothecenes B is a common feature of F. culmorum.

P1.4-025

SEASONAL PREVALENCE OF AFLATOXIN AND STRATEGIES FOR MITIGATION IN THE GROUNDNUT, MAIZE AND SORGHUM VALUE CHAINS IN UGANDA

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Text

Aflatoxin contamination in food crops is a major public and policy concern in Uganda. However, there is limited consistent seasonal monitoring data on aflatoxin occurrence. Aflatoxin was monitored for three seasons in Northern, Eastern, Western, and Central regions of Uganda. Samples (993 maize, 169 groundnut, 92 sorghum) were randomly collected from farmer fields at crop physiological maturity. In groundnut, the mean aflatoxin level in 2021A and 2022A seasons was 12.1 ppb and 30.6 ppb, respectively. The Northern and Eastern regions had the highest aflatoxin level in 2021A (28.6 ppb) and 2022A (52.5 ppb), respectively. A similar trend was observed for maize, where average levels were 8.6 ppb in 2021A, 18.7 ppb in 2021B, and 46.7 ppb in the 2022A season. In 2021A only maize from Northern Uganda (18.0 ppb) had aflatoxin levels above 10 ppb, the EAC tolerance threshold. In 2021B, maize from the Northern region had the highest avg. aflatoxin levels (46.6 ppb) followed by Eastern (11.9 ppb). In 2022A all regions had aflatoxin levels above 10 ppb, and the Northern region had the highest (101.5 ppb), and Western region had the lowest (26.9 ppb). Aflatoxin levels in sorghum were below 10 ppb in all regions and seasons. The seasonal variations allude to the need to constantly monitor aflatoxin content in staple crops. This presentation highlights efforts to manage aflatoxin and points to gaps required to address the aflatoxin menace in staple crops in Uganda.

P1.4-026

MYCOTOXIN EXPOSURE IN FOODS EATING BY VEGANS AND VEGETARIANS: EVALUATING THE CURRENT RISK ASSESSMENT

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Text
Mycotoxins are secondary metabolites produced by filamentous fungi along the food chain. Despite the maximum legal limits legislated for in most countries, consumers are still exposed to these contaminants through their diets. Exposure to Ochratoxin A (OTA) is associated with liver failure and renal cancer and it is commonly detected in cereals, spices, nuts, dried fruit and beverages as coffee. A recent publication showed that serum OTA levels were two-fold higher in vegans than in omnivores. Another report calculated that the substitution of meat products with soy-based could lead to potential risk of renal cancer due to an increased intake of OTA, leading up to 1,208 extra cancer cases. Most of the publications assessed the contamination in raw material, but there is a lack of knowledge on role of organic plant-based products commonly consumed by vegans and vegetarians. Therefore, the main goal of this study was to quantify the OTA occurrence in plant-based milk and cereal processed foods. Samples were purchased in conventional supermarkets and OTA was quantified by ELISA kit. The results of this project will help to understand the OTA exposure through highly processed organic foods and provide foundations for a Risk Assessment based on a cross case analysis of the food chain, from farm to fork.

P1.4-027

MODELLING THE EFFECT OF WEATHER ON DEOXYNIVALENOL CONTAMINATION IN SWEDISH SPRING CEREALS USING MACHINE LEARNING ALGORITHMS

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Text
Fusarium head blight (FHB) is one of the most serious diseases of small-grain cereals, resulting in yield reduction and an accumulation of deoxynivalenol (DON) in grain. Weather conditions are known to have a significant effect on the production of toxins by fusaria. Since 2011, when about half of all oats grown in Sweden had a DON content too high for human consumption, almost all oats produced are tested for DON, generating a high cost to farmers and the grain industry. In this study, data from Swedish field trials on oats, spring barley, and spring wheat were analysed to identify the most crucial weather variables for DON contamination. Relationships between weather and DON content at harvest were analysed using Spearman's rank correlation coefficient. Based on the weather variables and the trial location, Machine Learning-based models were developed to classify the risk of grain DON contamination exceeding 200 µg/kg. Models based on Support Vector Machine performed overall best in predicting the risk of DON contamination with an accuracy between 70% and 81%. High relative humidity and precipitation around flowering, during grain development and ripening, were correlated with high DON levels. High temperatures during grain development and senescence reduced the risk of DON accumulation. In future studies, it might be of interest to determine whether the inclusion of pre-crop, agronomic factors, and crop resistance to FHB could further improve the performance of the models.

P1.4-028

ALTERNARIA SPECIES: A "FROM FARM TO FORK" PHYTOPATHOLOGICAL AND TOXICOLOGICAL GLOBAL CONCERN IN MEDITERRANEAN AREA

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Text
The fungal genus Alternaria represents a serious problem for agri-food crops, from tfield to post-harvest, for both ability to be pathogenic and produce several toxic metabolites. In particular, Alternaria species produce more than 70 metabolites, including alternariol, alternariol monomethyl ether, tenuazonic acid, altenuen mycotoxins. We report here, our last years studies on the occurrence of Alternaria species on different crops, in the Mediterranean area, their mycotoxin profile, and phylogeny. About 700 strains isolated from wheat, tomato, date palm, and the European sea-rocket, collected in Italy, Lebanon, and Tunisia, were studied. Phylogenetic analyses revealed that the most frequent species occurring on wheat kernels belonged to Alternaria and Infectoriae Sections and the majority of strains isolated from the other hosts belonged only to Alternaria Section. Few strains grouped in Ulocladiodes, Chalastospora and Pseudoalternaria Sections. More than 90% of strains included in Alternaria Section produced multiple mycotoxins, with variable amount. Our in-depth investigations demonstrate that geographical origin and host plants did not influence Alternaria species distribution and genetic diversity. Finally, the plasticity of this genus represents a great pathological and toxicological risk, not only in the Mediterranean Area, but also at worldwide level.

Necrotrophic plant pathogens

C9.2-1

MECHANISMS OF HOST SPECIFICITY IN THE GENUS BOTRYTIS

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Text
Fungi of the genus *Botrytis* are necrotrophic pathogens and *B. cinerea* is the best studied among them. With exception of *B. cinerea* and *B. pseudocinerea*, all other *Botrytis* species are considered to be restricted to a single host species or a group of closely related hosts. The genomes of 16 *Botrytis* species were sequenced, but features that provide clues about factors determining host specificity could not (easily) be identified from genome comparisons. In an effort to unravel mechanisms underlying host specificity, we focused on *B. elliptica*, a specialised pathogen of lily. Previous studies showed that quantitative differences in virulence of *B. elliptica* isolates are correlated with the cell death-inducing capacity of secreted proteins in liquid culture filtrates. We studied the proteins secreted by *B. elliptica* that induce cell death specifically on its host plant, and analysed the protein composition by mass spectrometry. Among the secreted proteins, one of the most striking was an enzyme

with chorismate mutase activity and a sequence motif that would target the protein to plant chloroplasts. I will discuss how this effector participates in virulence of *B. elliptica* on lily. I will also discuss features of minichromosomes (<300 kbp) that were detected in the genomes of several (but not all) *B. elliptica* isolates, and their occurrence in other *Botrytis* species.

C9.2-2

IS THE MECHANISTIC ACTION OF NLP-INDUCED PLANT MEMBRANE DAMAGE UNIVERSAL?

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Text

Necrosis- and ethylene-inducing 1-like proteins (NLPs) constitute a superfamily of proteins found in diverse phyla of plant-associated microorganisms. Numerous NLPs are cytotoxic and facilitate infections in a wide range of crops. The evidence of NLPs being toxic to both monocot and dicot plants is inconclusive. The target for interaction with the plant plasma membrane is a sphingolipid glycosyl inositol phosphoceramide (GIPC). Studies of NLP_{Pya} from *P. aphanidermatum* highlighted critical residues for interaction with the terminal hexose unit of GIPC and a unique mode of membrane damage was observed. Upon binding, the protein oligomerizes on the membrane, followed by the formation of transient heterogeneous pores that are permeable to small molecules. So far, other NLPs have not been studied for their plant plasma membrane binding and mechanism of damage and respective pore formation. We study the ability of MpNEP2 from *Moniliophthora perniciosa* to induce damage to the plant plasma membrane of mono- and dicot plants. We utilize the microfluidic system to study the binding and pore-forming activity of MpNEP2 to GIPC-containing vesicles. As shown previously in NLP_{Pya}, MpNEP2 also binds and exhibits pore-forming activity on GIPC-containing vesicles from dicots. In addition, the same action is observed in vesicles containing monocot GIPC. This is the first study of MpNEP2 to show GIPC as a receptor and explore putative pore-forming activity.

C9.2-3

PECTOBACTERIUM BRASILIENSE: WHAT HAVE WE LEARNED IN NEARLY TWO DECADES OF RESEARCH?

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Text

Pectobacterium brasiliense (Pbr) is a member of the Soft Rot Pectobacteriaceae (SRP) best known for causing blackleg and soft rot of potatoes. This species of Pectobacterium was first

reported nearly 20 years ago in Brazil. Since then, several Pbr isolates have been identified and shown to cause significant problems in different crop production systems globally. In addition, since the first report, a large body of research has been published leading to better understanding of the biology of the species. In our research, we use a combination of comparative and functional genomics, transcriptomic and metabolomic profiling to identify key virulence factors required for Pbr1692 colonisation of their main host, potato tubers. We found that there is a plethora of antibacterial systems upregulated in Pbr1692 during host colonisation. These include the type 6 secretion system (T6SS), pyocin and carbapenem. An interesting finding is the role of outer membrane vesicles (OMV) in ferrying some of these factors. We show that most of these factors are recruited to confer competitive advantage and enhance adaptation to different host niches rather than virulence. Finally, we show that there is a complex regulatory network of virulence factors and antibacterial agents by the different Pbr1692 transcription factors.

C9.2-4

IDENTIFICATION OF PTTNLS1 – A PUTATIVE PYRENOPHORA TERES F. TERES EFFECTOR INVOLVED WITH BARLEY RPT5-MEDIATED RESISTANCE

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Text

Net form net blotch of barley is a destructive foliar disease caused by the necrotrophic fungal pathogen *Pyrenophora teres* f. *teres* (*Ptt*). The strongest resistance to this pathogen is conferred by the resistance gene *Rpt5*, specifically the allele from Ethiopian landrace CI5791, although this resistance has recently been overcome by *Ptt* populations in Canada and Morocco. We have identified and began to characterize a putative effector protein that interacts with the CI5791 Rpt5 receptor-like protein and is broadly conserved across *P. teres* isolates. This protein, PttNLS1, is encoded by a gene underlying a virulence QTL on chromosome 8 that was found to be contributed by *Ptt* isolate 0-1 in a bi-parental mapping population between isolates 0-1 and Mor40-3. Interestingly, Mor40-3 is the only known isolate to possess a mutated copy of the gene encoding PttNLS1, resulting in a predicted truncation of the protein, possibly contributing to the increased virulence this isolate has on CI5791. Infiltration studies with the 0-1 isoform of PttNLS1 reveal a strong suppression of the induction of two pathogenesis-related (PR) genes, *PR-1b* and *PR4*. We propose that *PttNLS1* evolved to suppress host PTI and that the Rpt5 resistance receptor evolved to recognize this PttNLS1 and trigger early effector triggered immunity, providing the strong resistance observed in CI5791.

C9.2-5

SMALL MOLECULES, BIG IMPACT: MULTILEVEL REGULATION OF C-DI-GMP AND ITS EFFECTORS ON VIRULENCE FACTORS OF DICKEYA DADANTII

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Text
A small bacterial molecule, bis-(3?-5?)-cyclic dimeric guanosine monophosphate (c-di-GMP), acts as a second messenger that regulates various cellular processes in different bacterial species. The diguanylate cyclases (DGCs) synthesize c-di-GMP from two molecules of GTP, whereas the phosphodiesterases (PDEs) break down c-di-GMP to 5′-phosphoguanylyl-(3′-5′)-guanosine (pGpG) or guanosine monophosphate (GMP), together are responsible for controlling the intracellular levels of c-di-GMP. c-di-GMP effectors bind to c-di-GMP and are allosterically governed by this small molecule, further exerting diverse influences in the cell. Dickeya dadantii is an Enterobacterium that causes soft-rot disease in many economically important crops. In this study, we examined the molecular interactions between two PDEs, EGcpB and EcpC, and c-di-GMP effectors, BcsA and YcgR, in the regulation of the type III secretion system (T3SS) in D. dadantii. Furthermore, we explored the co-regulation between a novel putative transcriptional regulator, CdeR, and a DGC, GcpD, in regulating the T3SS and pectate lyase (Pel). We found that CdeR regulates the above two virulence factors of D. dadantii in a c-di-GMP-dependent manner. A multi-tiered regulatory mechanism that links the DGC, PDE, and c-di-GMP effector to the bacterial virulence factors was proposed.

C9.2-6

A COUNTER STAINING TECHNIQUE ELIMINATES THE NEED FOR TRANSFORMATION AND RESULTS IN MORE ACCURATE QUANTIFICATION IN MULTIPLE FUNGAL-PLANT INTERACTIONS

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Text
3D imaging using confocal microscopy is invaluable for visualizing plant-fungal interactions and the use of fluorescent proteins has been common, requiring transformation. We introduce a counter staining technique that uses propidium iodide and wheat germ agglutinin labeled with fluorescein isothiocyanate (WGA-FITC) to look at fungal colonization of plants. This method relies on the use of KOH to remove the cutin layer of the leaf, creating permeability of the staining solution, allowing the visualization of colonization. Subsequently, a quantitative volume analysis and program cell death (PCD) assessment was done. Volume analysis (fungal biomass) was performed using machine learning through pixel quantification to differentiate between fungal growth and plant cells using Imaris software. Because an early hallmark of PCD is the breakdown of organelles including the nucleus, PCD was quantified using Manders' overlap coefficient to assess the separation/overlap of red (plant DNA) and blue (plant autofluorescence) where color overlap indicates PCD. This technique worked effectively on both monocots and dicots, including the wheat-Parastagonospora nodorum, barley-Pyrenophora teres f. teres, and the sugarbeet-Cercospora beticola interactions. Since transformation is not required for this technique, it can be applied to any interaction, offering a higher quality cellular visualization, resulting in more accurate quantification of pathogen biomass and the analysis of PCD induction.

F9.2-1

PECTOBACTERIUM AND DICKEYA POTATO BLACKLEG PATHOGENS: EFFECT OF INTRA AND INTER-SPECIES STRAINS ASSOCIATIONS IN FIELD TRIAL

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Text

Blackleg causes high economic losses for the seed potato industry worldwide. The disease is caused by bacteria belonging to the genera Pectobacterium or Dickeya. Recent developments in sequencing technology led to refine their taxonomy. Since 2016, the number of described species has increased from 12 to 33, highlighting their great genetic diversity. To date, few data are available about their specific behavior on potato host.
In order to compare the aggressiveness of 5 different Pectobacterium and 2 Dickeya species, we inoculated the pathogens on tubers just before plantation in trial fields. Each inoculum consisted in a mix of 5 strains belonging to a same species. Then, different parameters reflecting the aggressiveness and fitness of the inoculated strains were observed, as blackleg expression or vertical transmission in harvested tubers.
The results showed differences between species for all the parameters studied, highlighting different colonization strategies on potato host. Moreover, the qPCR analysis of blackleg symptoms obtained after inoculation with a mix of strains belonging to two different species can reveal a possible antagonistic relation between pectinolytic bacteria species association. Finally, a metabarcoding sequencing approach performed to monitor each inoculated strain revealed the predominance of one strain in each analyzed symptom, not always the same, highlighting a pioneer effect during the symptom development.

F9.2-2

ROLE OF ANAEROBIC RESPIRATIONS OF CARBON SOURCES ON SURVIVAL AND ECOLOGICAL FITNESS OF DICKEYA GENUS

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Text

Bacteria are ubiquitous and occupy a very wide range of ecological niches where oxygen can be rapidly limited. In this case, bacteria develop flexible metabolic respiration. Therefore, demonstrating the possibility of using carbon sources as alternative terminal electron

acceptors (TEAs) and elucidating the underlying genetic pathways has great potential to help understand bacterial strategies to adapt and persist in the environment. This question is more relevant in the case of phytopathogenic bacteria which face many specific challenges to infect plants. It is the case for Dickeya, an emergent pathogen, found in various ecological niches, such as plant apoplast, and responsible for soft rot disease in a wide variety of plants. Understanding anaerobic respiratory pathways involved in Dickeya persistence and colonization will allow us to better understand its pathogenicity, and thus better counter it. In this study, we demonstrated that D. dadantii is able to use malate and asparagine as TEA. By constructing metabolic model of D. dadantii, we predicted pathways involved in anaerobic growth using asparagine and malate. Mutants affected in asparagine pathway were constructed and we evidenced the role of asparagine respiration in D. dadantii virulence. We also tested whether asparagine pathway is conserved among Dickeya and Pectobacterium genus.This study demonstrated that anaerobic respiration is an important trait involved in D. dadantii virulence.

F9.2-3

SMALL SECRETORY PROTEINS IN THE PATHOGENESIS OF SCLEROTINIA SCLEROTIORUM

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Text
Sclerotinia sclerotiorum is a typical necrotrophic fungal pathogen and has a wide host range including rape and soybean. Previous studies have shown that the cell wall degrading enzymes and oxalic acid play major roles in the pathogenesis of *S. sclerotiorum*. We found that small secretory proteins, such as SsSSVP1, SsCP1 and SsITL, also are important virulence factors of *S. sclerotiorum*. SsSSVP1 interacts with a plant mitochondrial protein QCR8 and hijacks it into cytoplasm, thereby disable its biological functions and induce cell death. SsCP1 interacts with PR1 in the apoplast and then maybe inhibiting the potential antifungal activity of PR1. SsITL interacts with a chloroplast-localized calcium-sensing receptor (CAS) in chloroplasts, and thereby reduces salicylic acid accumulation during the early stage of infection. These secretory proteins tend to attack the conserved proteins widely existing in host plants, which is consistent with the wide host range of *S. sclerotiorum*. Recently, we found that some new secretory proteins that induce host cell necrosis, such as SsNIP1 and SsNIP2, play important roles in the pathogenesis of *S. sclerotiorum*, and the mechanism of these proteins is still under further study. Together, our findings revealed a new mechanism of the pathogenesis of *S. sclerotiorum*, and could also help us to understand the pathogenic mechanism of necrotrophic fungal plant pathogen from a new perspective.

P9.2-001

INCIDENCE, DIVERSITY AND PATHOGENICITY OF DIAPORTHE SPECIES ASSOCIATED WITH SOYBEAN SEEDS IN SOUTH AFRICA

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Text

Seedborne Diaporthe species on soybean can cause seed decay, stem canker, and pod and stem blight, leading to economic losses. Despite their importance as soybean pathogens, information on Diaporthe spp. on soybean in South Africa (SA) is scarce. This study investigated the occurrence, diversity and pathogenicity of Diaporthe associated with soybean seed in SA. One hundred and sixty-two seed lots representing 11 cultivars were collected from 11 sites in SA from 2014 to 2015. Diaporthe isolates were recovered both years from all locations. Although the overall incidence was low (3.6% of seed infected), individual seed lots had up to 71% infected seeds. A subset of Diaporthe isolates were selected for species-level identification which was conducted through phylogenetic analyses of the ITS, EF1-α and TUB regions. Ten species were identified, of which D. ueckerae comprised the most isolates. Other species included some known soybean pathogens. Pathogenicity assays showed that D. longicolla contained the most virulent isolates, causing more than 88% damping-off, followed by D. ueckerae causing up to 70%. The D. ueckerae isolates caused a notable symptom which was confirmed to be associated with secondary metabolites experimentally in cell-free culture filtrate. This study showed that Diaporthe species pathogenic to soybean may be of major concern at specific sites in SA. This is the first report of D. foeniculina and D. ueckerae on soybean in South Africa.

P9.2-002

CHARACTERISTICS AND FUNGICIDES SENSITIVITY OF FUSARIUM INCARNATUM-EQUISETI SPECIES COMPLEX (FIESC) CAUSING MUSKMELON FRUIT ROT DISEASE IN TAIWAN

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Text

A newly emerging fruit rot disease of muskmelon caused by *Fusarium incarnatum-equiseti* species complex (FIESC) was reported in Taiwan in 2022. However, the species of FIESC causing melon fruit rot have been confirmed as pathogens during post-harvest. The aim of this study was to identify the FIESC species causing fruit rot in muskmelon in Taiwan and evaluate the sensitivity to fungicides. Fifteen FIESC isolates were collected from the diseased fruits located in different orchards, and the species were identified through phylogenetic analysis based on ITS rDNA, *tef1* and *rpb2* genes. Results indicated that three FIESC species were identified, including *F. irregular* (one isolate), *F. pernambucanum* (eight isolates), and *F. sulawesiense* (six isolates). The pathogenicity test confirmed that all the species could infect muskmelon (cultivar : Tainan No.13) and develop fruit rot symptoms. For fungicides test, the major species *F. pernambucanum* and *F. sulawesiense* were used to examine the reaction to carbendazim, chlorothalonil, fluopyram, fluxapyroxad, and trifloxystrobin. The results showed that carbendazim, chlorothalonil and trifloxystrobin have efficacy to inhibit mycelial growth and spore germination of most of *F. pernambucanum*, except FE02 isolate. In this study, *F. sulawesiense* were low sensitivity to five fungicides.

These results demonstrated that the sensitivities of two FIESC species to fungicides have high variation in Taiwan.

P9.2-003

MOLECULAR AND PHYSIOLOGICAL CHARACTERIZATION OF FUSARIUM OXYSPORUM CICERI ISOLATES FROM DIFFERENT CHICKPEA AREAS IN IKR, IRAQ

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Text
Thirty-two different F. oxysporum ciceri isolates were isolated from 54 chickpea fields in Sulaimani and Halabja governorates in 2021. Fourteen of the isolates were pathogenic and displayed various morphological traits. The ideal temperature range for Foc was 25-30°C. PDA was the best media for the fungal growth. The isolates showed a variety of growth patterns, including appressed, flat/velvet, fluffy to partial fluffy, and cottony. However, Foc-28 isolate in contrast to the others, displays mycelium growth that resemble nerves on PDA at 25ºC, making it stand out morphologically from the others. Microconidia (6.1-8.7x2.9-4.98µm), macroconidia (10.0-20.1x2.7-5.2µm), chlamydospores (7.2-15.0x 6.8-10.7µm), and colony diameter on PDA were all significantly differed between isolates. Significant differences in the pathogenicity and virulence of 14 Foc isolates were detected on 10 differentials. The isolates were classified in to two groups and nine physiological races accordingly. Each isolate's genomic DNA was amplified by the ITS primers to a maximum size of 400bp, producing a single band for each accession. The ITS sequences classified all the races into six clusters, which were all registered at NCBI Gen Bank under different accession numbers. Significant genetic diversity within populations and low genetic diversity between groups were discovered. The basic local alignment search tool (BLAST) analysis displayed a similarity to Foc genomic sequences of 95.1-100%.

P9.2-004

ROS IS ESSENTIAL FOR THE NORMAL MYCELIA GROWTH IN FUSARIUM GRAMINEARUM

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Text
Reactive oxygen species (ROS) have been proven to play an important role in cell differentiation and elongation. Studies on ROS have also been conducted in *Fusarium graminearum*, a filamentous fungus whose life cycle involves asexual sporulation and conidial germination. Here, we knocked out a transcription factor gene associated with conidial germination in *F. graminearum* and found that mutant exhibited simplified life cycle, in which the conidia that germinate directly generate further conidia without forming mycelia.

By detecting intracellular ROS, it was found that the intracellular ROS of mutant was significantly decreased compared with that of wild type. Moreover, when detected *NoxA* and *NoxB*, two genes related to ROS production, it was found that the gene expression in mutant was significantly down-regulated. In addition, the life cycle of the wild type was also simplified when Nox inhibitors was applied. Moreover, ROS was stained during conidial germination, it was found that ROS accumulated on the top of mycelia. However, under sporulation, ROS accumulated only in conidia and did not at the top of mycelium. Notably, in this study, we also found that induce intracellular ROS accumulation in mutant could restore normal conidial germination, but in wild type, the ROS accumulation induced sporulation.
Thus, since ROS accumulation leads to different results for wild type and mutant, we will further study how ROS regulate *F. graminearum* conidial germination and sporulation.

P9.2-005

DECIPHERING HOST-PATHOGEN AND FUNGAL-FUNGAL INTERACTIONS WITHIN THE LATENT FRUIT ROT OF WINTERBERRY PATHOSYSTEM: FROM THE FIELD TO THE BIOCHEMISTRY

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Text
Ilex verticillata (common winterberry) is a deciduous species of holly bearing colorful, persistent fruit, commonly used in landscape design and as woody cuts for winter seasonal decoration. *Diaporthe ilicicola* is a recently described fungal species that establishes latent infections in winterberry flowers at bloom, leading to necrosis when the fruit is fully mature. The formation of *D. ilicicola* pycnidia within the fruit epicarp was observed to create micro-wounds in the fruit cuticle facilitating co-infection by opportunistic necrotrophic fungi and leading to fast-progressing rot. *D. ilicicola* pycnidia formation was induced by increased intensity of white light *in vitro*, and by exposure to fruit ethanolic extracts *ex vivo*. Additionally, drops in field temperature were significantly correlated with disease incidence, suggesting that *D. ilicicola* infection may weaken the fruit cuticle and increase susceptibility to cold damage. Commercially available cultivars were screened for susceptibility to the disease and a quantitatively resistant phenotype was identified. Metabolomic analysis showed that the fruit of less susceptible cultivars display distinctly different profiles compared to more susceptible cultivars, with 89 unique metabolite features putatively associated with resistance. Future work will determine whether *D. ilicicola* asymptomatic infection can alter fruit cuticle integrity and identify compounds with bioactivity toward *D. ilicicola* or *I. verticillata* fruit.

P9.2-006

IN DICKEYA DADANTI, THE TYPE II SECRETION SYSTEM (T2SS) IS COVALENTLY ATTACHED TO THE BACTERIAL CELL WALL.

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Text

Dickeya dadantii is a broad-spectrum phytopathogenic γ-proteobacterium that provokes soft rot disease in various plants. *D. dadantii* pathogenicity is caused by an arsenal of plant cell-wall degrading enzymes secreted by the Type II Secretion System (T2SS) named Out. The Out system is a multiprotein complex spanning the cell envelope. Recently, we showed that OutB, an inner membrane T2SS component plays an important scaffolding role and that deletion of outB causes a striking reduction of pectinase secretion and virulence of *D. dadantii*. Here, we show that in *D. dadantii*, OutB is covalently attached to the PG. We investigated the molecular mechanisms of OutB attachment to peptidoglycan (PG) and the possible implication of this phenomenon in the virulence of *D. dadantii*. First, the covalent attachment of OutB was demonstrated after extraction, purification and analysis of the PG of *D. dadantii* by Western blot. Second, we characterized the enzymes implicated and identified the region and residues of OutB important for covalent attachment to PG: the two C-terminal lysines of OutB are involved in its attachment to PG and two L,D-transpeptidases, Ldt03 and Ldt84, catalyze this process. These results suggest that the covalent attachment of OutB to PG allows a better stabilization of the T2SS in the bacterial cell envelope. Moreover, it shows that the phenomenon of covalent attachment of proteins to PG in proteobacteria could be more widespread and remains largely unexplored.

P9.2-007

INTERACTIONS OF THE SPECIES OF THE ASCOCHYTA BLIGHT DISEASE COMPLEX AND HOST RESISTANCE

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Text

Ascochyta blight (AB) is an important seed-borne and foliar disease of field peas and is widely recognized as a major productivity barrier. The disease is caused by necrotrophic fungal pathogens, Ascochyta pisi Lib. (Didymella pisi sp. nov), Peyronellaea pinodes (Berk & A. Bloxam) Aveskamp, Gruyter & Verkley 2010 (Mycosphaerella pinodes (Berk & A. Bloxam) Vestergr. 1912, Pe. pinodella (Phoma medicaginis var. pinodella L.K Jones (Morgan-Jones & K.B. Burch), and Phoma koolunga. These pathogens can exist independently or together (known as the Ascochyta blight disease complex) within a pea field and even on single plants. We set up a study to monitor the three species of A. pisi, Pe. pinodes and Pe. pinodella in single-pea plants to reveal their interactions during pathogenesis with or without a biocontrol agent in a resistant or susceptible host. The results revealed dynamic changes in the complex over time with antagonistic/mutualistic interactions between species and host response. These findings are valuable to assist AB-resistance breeding and disease management with a biocontrol agent.

P9.2-008

GLOBAL DIVERSITY AND DISTRIBUTION OF NECROTROPHIC EFFECTORS IN A GLOBAL COLLECTION OF PYRENOPHORA TRITICI REPENTIS, CAUSAL AGENT OF TAN SPOT OF WHEAT

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Text

Pyrenophora tritici-repentis (Ptr), is a necrotrophic fungal pathogen that causes tan spot, a foliar disease of wheat. The effector repertoire of Ptr includes distinctive necrotrophic effectors ToxA, ToxB, and ToxC associated with disease development. The unexplained correlation between Ptr's geographical origin and its ability to produce distinct effector combinations suggests a divergent evolution of Ptr and Tox effectors. This study analyzed 52 Ptr isolates from diverse regions to determine Ptr's race structure, host range, and Tox gene diversity. Genomic DNA of these isolates was extracted and a subset of 25 isolates were sequenced using Illumina Mi-Seq platform. Sequencing data analysis reveals the presence of several SNP variations in Tox coding genes, and we predicted that these isolates would have differential response on wheat. PCR with ToxA, ToxB, and ToxC primers on additional isolates was performed to expand our ongoing Tox diversity analysis.Disease phenotyping of these isolates on wheat differentials characterize these isolates as race1, but races 2, 3, 4, and 5 were also observed, thus confirming our hypothesis of diversity in studied pool. These results, along with available functional genomic tools, will help breeders and pathologists to efficiently breed for tan spot-resistant wheat varieties, increasing revenue for wheat growers.

P9.2-009

DIVERSITY AND PATHOGENICITY OF GLOBISPORANGIUM AND PYTHIUM SPP. ASSOCIATED WITH PYRETHRUM IN AUSTRALIA

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Text

Pyrethrum (*Tanacetum cinerariifolium*) cultivation in Australia suffers from a persistent yield decline which in part is caused by a complex of soilborne pathogens. There has been no research on the relationship between oomycetes and pyrethrum yield decline. During surveys between 2018 to 2021, ten known *Globisporangium* species, two new *Globisporangium* species and three *Pythium* species were recovered from crown and root tissues of infected pyrethrum plants and from soils from 16 sites in Tasmania and Victoria, Australia. Identification of *Globisporangium* and *Pythium* spp. was based on morphological characters and multigene phylogenetic analyses using ITS, *Cox1* and *Cox2* sequences. *Globisporangium ultimum* var. *ultimum* was the most abundant in soils, while *G. sylvaticum* and *G. commune* sp. nov. were most abundant in pyrethrum plants. Seven *Globisporangium* species were pathogenic on both pyrethrum seeds (*in vitro* assays) and seedlings (glasshouse bioassays) causing pyrethrum seed rot, seedling damping-off and significant plant biomass reduction, while two *Globisporangium* species and three *Pythium* species only

caused significant symptoms on pyrethrum seeds. The results suggest that *Globisporangium* and *Pythium* species could be contributing to yield decline in pyrethrum in Australia. This is the first report of *Globisporangium* and *Pythium* species as pathogens of pyrethrum globally.

P9.2-010

NOVEL HARZIA IXTARENSIS FUNGUS ON ANNONA CHERIMOLA FRUIT IN MEXICO

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Text
Since 2005 in Íxtaro, Michoacán, symptoms of *Harzia* infection have been observed on immature *Annona cherimola* fruit with *Colletotrichum fragariae*-induced anthracnose lesions and mummified fruit. This study aimed to identify the *Harzia* sp. Four isolates were obtained from fruit exhibiting symptoms, cultured in four types of agar under various conditions, and characterized based on concatenated internal transcribed spacer ITS + large subunit and ITS + small subunit sequences. Additionally, the isolates were compared with two CBS species (two-type strains and two isolates) of *Harzia patula* and *H*. *tenella* under the same conditions as the *Harzia* isolates, and all known *Harzia* spp. in culture were included in two phylogenetic analyses. *H. ixtarensis* **sp. nov.** was proposed. Compared with *H. patula* CBS isolate 121524, which was the most closely phylogenetically related species, *H. ixtarensis* was characterized by slower colony growth (white to salmonish-beige), different percentages of two forms of conidia (elongated and globose; unicellular and hyaline to subhyaline), and smaller conidia. The conidia mainly germinated with two hyaline tubes without an appressorium. This study proposes the existence of a novel species, *H. ixtarensis*. It contributes to the evidence of the biodiversity of the genus *Harzia*, and its host range. Pathogenecity behavior is showed in a second abstract.

P9.2-011

ASSESSMENT OF APPLE AND PEAR CULTIVAR TOLERANCE AND AGGRESSIVENESS OF FUNGI ISOLATED FROM CANKERS AND FRUIT ROTS ON TREES

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Text
Several pathogenic fungi cause fruit rots and damage in the orchards and storages (e.g. *Neofabraea* spp., *Monilinia* spp., *Venturia* spp.) or both - cankers on trees and fruit rots in the orchards and storages (e.g. *Neofabraea* spp., *Monilinia* spp.). This study aimed to

assess apple and pear cultivar tolerance and aggressiveness of fungi isolated from fruit rots and cankers of apple and pear on trees. Four apple and four pear cultivars were used for the studies. In addition, apple rootstock B396 and pear seedling rootstock of local landrace 'Kazraušu pear' were included in the tests. The aggressiveness of nine fungal isolates belonging to *Neofabraea* and *Fusarium* was characterised by inoculating potted trees in the greenhouse. The differences in the aggressiveness among *Neofabraea* species and strains within species were found. *Fusarium* sp. strain isolated from apple canker was capable of causing cankers on both hosts. Several *Neofabraea* strains isolated from cankers were more aggressive than those obtained from fruits. The isolates from apples were also causing disease on pears and vice versa. Noticeable preference among the strains in the host species was noted in tree inoculation tests with rootstocks B396 and 'Kazraušu pear' seedlings. Further would be necessary to investigate the mechanisms determining the disease development for each pathogen and host on fruits (fruit rot) and trees (canker), and evaluate the role of rootstock in the host tolerance to canker.

P9.2-012

PR1-LIKE PROTEINS FROM CYTOSPORA CHRYSOPERMA SHARE COMMON AND DISTINCT ROLES IN FUNGAL VIRULENCE AND PLANT SUSCEPTIBILITY

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Text

Canker diseases caused by necrotrophic fungus Cytospora chrysosperma damages up to 80 species of woody plants. Accumulating evidences show that necrotrophy has evolved clever means to compromise host recognition and establish disease?possibly by employing the pathogenic PR1-like proteins which are emerging virulence-related immune suppressors. Here we identified three PR1-like proteins, CcCAP1-3. CcCAP1 and CcCAP2 were required for fungal virulence and their overexpression in Nicotiana benthamiana promoted Botrytis cinerea colonization. In addition, CcCAP1 inhibited plant immune responses, which was dependent on its PR1-like domain and nuclear localization; Yeast two-hybrid analysis identified plant RNA polymerase II subunit 11 (RPB11), eukaryotic initiation factor 3 (eIF3), splicing factor 3A subunit 2 (SF3A2) and plastocyanin (PCN) as CcCAP1-interactors. Overexpression of poplar RPB11 promoted fungal infection. While CcCAP2 but not CcCAP3 interacted with eIF3 and PCN. CcCAP1 and CcCAP2 were also involved in the regulation of genes associated with carbohydrate metabolism and candidate effector proteins, and CcCAP1 affected the expression of pathogenesis-related secondary metabolic gene clusters. Intriguingly they exhibited sterol- export and binding activity. Taken together, this study identified three PR1-like proteins from C. chrysosperma and characterized their roles in pathogen-plant interactions from the perspective of phytopathogen.

P9.2-013

PROTEOMIC ANALYSIS OF PENICILLIUM EXPANSUM INFECTING POSTHARVEST APPLES BASED ON LABEL-FREE AND PARALLEL REACTION MONITORING (PRM) TECHNIQUES

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Text

Blue mold caused by *Penicillium expansum* is the leading cause of apple decay during postharvest storage. It is of great significance to study the molecular mechanism of *P. expansum* infecting apples. In this study, label-free technology was used to study the proteomic changes of *P. expansum* infecting the apples at crucial time points, and the results were verified by PRM technology. The expression of target proteins measured by PRM was consistent with the expression trend of the proteomics results, indicating that the results were stable and reliable. Through bioinformatics analysis of the proteomics results, 267 differentially expressed proteins (DEPs) were screened, 136 up-regulated, and 131 down-regulated. Bioinformatics analysis of all DEPs showed that the DEPs at the early stage of *P. expansum* infection were associated with growth promotion and spore development, which improved the pathogenicity. Up-regulated expression of MAGE protein, heat shock protein, FKS2 and other pathogenic-related proteins positively regulated the infection. During the early infection, activation of metabolic pathways related to oxidative stress eliminated reactive oxygen species through catalase and cytochrome P450.These findings may help to further study the molecular mechanism of *P. expansum* infecting apple.

P9.2-014

THE BIOLOGICAL FUNCTION OF DNA METHYLTRANSFERASES IN THE NECROTROPHIC FUNGUS BOTRYTIS CINEREA

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Text

Botrytis cinerea is one of the most destructive phytopathogens. B. cinerea is difficult to control because of its strong stress resistance and flexible infection modes. The pathogenic mechanisms of B. cinerea have been studied at multiple levels, but little is known at epigenetic level. In this study, we focused on the function of DNA methyltransferases (MTases) in regulating the development and pathogenicity of B. cinerea. The expressions of the cytosine MTase genes were significantly inhibited during the infection process of B. cinerea. Treating B. cinerea conidia with the DNA methylation inhibitor 5-azacytidine led to compromised virulence. These results implied that DNA methylation is involved in the pathogenesis of B. cinerea. Among the four conserved DNA MTases, BcDIM2 and BcRID2 showed a strong synergistic effect. Double knockout mutant ?Bcdim2rid2 showed slower growth, attenuated oxidative tolerance, and complete non-pathogenicity, which is related to the reduced expression of virulence-related genes in ?Bcdim2rid2 and the induced resistance of the host. Although B. cinerea has multiple DNA MTases, the global methylation level is very low, and few 5mC sites can be detected through whole genome bisulfite sequencing. We speculate that DNA MTases may be mainly involved in the repeat-induced

point mutation (RIP) of B. cinerea, in which cytosine methylation is an intermediate state that is difficult to monitor.

P9.2-015

BIODIVERSITY OF BACTERIAL PLANT PATHOGENS FROM THE PECTOBACTERIACEAE FAMILY IN POLISH WATEWAYS

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Text

Pectinolytic bacteria from the *Pectobacteriaceae* family have been detected in waterways globally, including Europe, Asia, Northen America and Australia. Due to the ongoing climate change, it is anticipated that the number of crops subjected globally to irrigation during the vegetation period will constantly rise. Therefore the aim of this project was to describe the biodiversity and potential pathogenicity of *Pectobacteriacea*e isolates originating from Polish waterways.

During long-term monitoring of the presence of pectinolytic bacteria in natural water reservoirs in Poland, 57 isolates were detected and identified with the use of multiplex PCR and/or species-specific PCR as Dickeya or Pectobacterium spp. Based on the species-specific PCR, genotypic profiling (ERIC- and BOX-PCR) and phylogenetic analysis of recA and dnaX genes sequence, the presence of the strains from species: *D. chrysanthemi, D. aquatica, D. zeae, P. versatile* and *P. quasiaquaticum* was confirmed. Analysis of the ability to macerate potato tuber tissue and activities of pectinolytic, cellulolytic and proteolytic enzymes indicated that *D. chrysanthemi* isolates are the most homogeneous and have the highest level of pathogenicity in comparison to other analyzed species.

Preseted data indicated that the use of natural water reservoirs in Poland for irrigation of agricultural fields can lead to the spread of the soft rot *Pectobacteriaceae* in farmlands and as a results cause crops losses.

P9.2-016

IDENTIFICATION AND CHARACTERIZATION OF ALTERNARIA SPP. ASSOCIATED WITH CHERRY FRUIT BLACK ROT DURING PREHARVEST IN THE MAULE REGION, CHILE

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Text

In Chile, cherry trees are considered an important fruit crop, with an area of 48,960 ha

planted under Mediterranean conditions in the Maule Region, Central Chile. In the last five years, the occurrence of black rot during preharvest has increased in commercial orchards in the Maule Region. The goal of this work was to identify the causal agent associated with the black rot of cherries during pre-harvest in the Maule Region, and to evaluate the nutrients´ distribution in cherry fruits infected with black rot using X-ray microfluorescence spectroscopy (u-XRF). For this purpose, pre-harvest fruits with black rot were collected, and Alternaria spp. were isolated using Agar-Potato-Dextrose (APD, 2%) medium incubated for seven days at 20°C. From pure colonies, isolates of Alternaria sp. were identified at level species by morphology and phylogenetic analysis using the RPB2, ATPase, and calmodulin genes. Conidial suspensions of four Alternaria spp. were used for pathogenicity tests on ripe fruit. Based on the morphological and genetic characteristics, the species Alternaria alternata and A. arborescens were identified. The distribution of nutrients revealed a high concentration of potassium in the infected region. Otherwise, calcium was observed in the periphery of the symptomatic region, coinciding with the area of progress of the infection

P9.2-017

SECRETED PROTEIN FOCUBL IS ESSENTIAL FOR FULL VIRULENCE OF FUSARIUM F. SP. CUCUMERINUM ON CUCUMBER

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Text

Cucumber Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cucumerinum* (*Foc*) seriously threatens the yield and quality of cucumber. During infection, *Foc* delivers a large number of different secreted proteins into host plant tissues. So far, only a few proteins secreted by this fungus have been proved to be toxic effectors. We found a gene *FoCubl* was extremely up-regulated in the wild type (WT) during the early stage of infection and would be a virulence effector. The *FoCubl* has a single copy in the genome with a full length of 696 bp, containing an intron and a CDS length of 645bp, and encodes a 214-amino-acid protein. FoCubl has an N-terminal secretory signal peptide and a Cu_bind_like domain. *FoCubl* knockout mutants significantly reduced the aggressiveness, indicating its essential role in the full virulence on cucumber. Compared with the WT, the knockout mutants produced significantly less conidia, but did not exhibit differences in germination, suggesting an important role for FoCubl in conidiation. The deletion mutants did not show distinct changes in mycelial growth on PDA, however, accumulated significantly more mycelia in PDB. In addition, the deletion mutants were more sensitive to osmotic stress such as 1 M glycerol, glucose, NaCl, and KCl, respectively. In summary, these results suggest that FoCubl is a novel virulence effector of *F. oxysporum*. To our knowledge, this is first time to report an effector with a Cu_bind_like domain in *Fusarium* species.

P9.2-018

CHARACTERIZATION OF FUSARIUM SPP. ASSOCIATED WITH STRAWBERRY ROOT ROT IN CHINA

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Text

Strawberry is a popular and economically valuable fruit planted worldwide. Strawberry root rot can be caused by Fusarium, resulting in root death and reduced plant vigor and productivity. However, there is no detailed report on the identification of Fusarium spp. associated with strawberry root rot in China. In this study, strawberry roots with symptoms of root rot were collected from twenty-four provinces, autonomous regions, or municipalities across China. 159 isolates of Fusarium were isolated from these diseased roots. Based on morphological traits of fungal colonies on potato dextrose agar and carnation leaf agar, colony growth rate, morphological characteristics and size of conidium, conidiophore and chlamydospore and sequence analyses of internal transcribed spacer region of ribosomal DNA and translation elongation factor 1-α gene region, these isolates were identified as eight species of Fusarium, namely Fusarium oxysporum, F. acuminatum, F. incarnatum, F. equiseti, F. fujikuroi, F. asiaticum, F. solani, and F. falciforme, with F. oxysporum being predominate. The eight Fusarium species were all pathogenic on strawberry, and average disease incidence and average disease index of strawberry caused by F. oxysporum were 98.68% and 79.97, respectively, which were higher than those of strawberry incited by the other seven species of Fusarium. This is the first report that F. incarnatum, F. fujikuroi, F. asiaticum, and F. falciforme cause root rot on strawberry in China.

P9.2-019

OCCURRENCE OF SOME PHOMA, ASCOCHYTA AND DIDYMELLA SPECIES ON FORAGE AND FOOD FABACEAE IN ALGERIA: EVALUATION OF THEIR PATHOGENICITY AND HOST RANGE.

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Text

Food and fodder *Fabaceae* are of great importance in agricultural systems. In Algeria, they are used as a major protein supply for human and animal nutrition, also in crop rotation with cereals for enriching the soil with nitrogen. These *Fabaceae* are susceptible to many diseases, mainly caused by fungal pathogens. Surveys conducted during 2015-2019 have shown the presence of different types of symptoms on pea, chickpea, faba bean, alfalfa and berseem crops. These symptoms presented as necrotic brown spots, bordered by a clear margin on the different organs (leaves, stems, roots and pods), often with the presence of pycnidia. With the combination of morphological identification and molecular characterisation by sequencing the genes ITS, LSU, *tub2*, several species, belonging to 3 genera, were identified: *Phoma* (*P. herbarum*), *Ascochyta* (*A. rabiei*, *A. medicaginicola*, *A. nigripycnidia*), *Didymella* (*D. pisi*, *D. fabae*, *D. pinodes*, *D. pinodella*). Among these species *A. nigripycnidia* is reported for the first

time on berseem (*Trifolium alexandrinum*). The host specificity test showed that the host range is wider for forage pathogens than food ones, with varying degrees of speciation to their host of origin. However, the host range is wider for forage pathogens than for pathogens of food legumes species. These results will be used to establish a control strategy through a sustainable rotation of these crops.

P9.2-020

COLLETOTRICHUM SPP. IN HUNGARY: SIGNIFICANCE OF NEW SPECIES AND NEW HOSTS

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Text
Colletotrichum species are significant pre- and postharvest pathogens worldwide. Symptoms of anthracnose occur not only on fruits (e.g. sour cherry, strawberry, avocado, mango, blueberry, fig, banana) but also on ornamentals. The aim of the study was to identify the *Colletotrchium* species causing anthracnose on numerous hosts. For morphological studies, pathogens were isolated from infected tissues on potato dextrose agar (PDA) medium. The general characteristics of the colonies and the size and shape of the conidia (n=50) were examined. Reliable identification of *Colletotrichum* species on the basis of morphological features is not possible, especially within a given species complex. To identify the pathogen at the species level total DNA was extracted, and partial sequences of the internal transcribed spacer (ITS) region and calmodulin (CAL), histone3 (HIS3) genes were amplified by polymerase chain reaction and sequenced. Pathogenicity of the isolates was verified on their original host species. We identified several species within the *C. gloeosporioides* complex: *C. fructicola, C. musae, C. kahawae, C. siamense, C. asianum. C. godetiae, and C. fioriniae* from the *C. acutatum* species complex. To our knowledge, this is the first report of *C. fructicola, C. kahawae, C. asianum* species in Hungary.
Supported by the UNKP-22-3-I New National Excellence Program and the research of the ELKH TKI (project number: 3200107)

P9.2-021

ALTERNARIA SPECIES ASSOCIATED WITH LEAF SPOT DISEASE OF DRACAENA SPP. IN OMAN

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Text
Desert plant restoration programs in Oman are hindered by emerging diseases at Oman

Botanic Garden (OBG), which is planned to be one of the largest botanic gardens in the world. A study was conducted to characterize disease incidence, severity and causal agents of leaf spot diseases affecting two Draceana species, Draceana serrulata (endemic) and D. hanningtonii. A survey of more than 150 plants of D. serrulata and D. hanningtonii grown at OBG showed that the disease incidence is 100% in both plants. Disease severity, which was assessed based on the percentage of leaf coverage with spots indicated an average severity of 2.8 % for D. serrulata and 2.2 % for D. hanningtonii. Isolation of fungi from 10 randomly selected samples of each Dracaena species yielded 21 morphologically different fungi for D. serrulata and 34 fungal isolates for D. hanningtonii. Pathogenicity tests showed that two fungal isolates from D. serrulata, (DS18, DS20) and three isolates from D. hanningtonii (DH3, DH4 and DH6) were pathogenic on their respective host pants. Molecular identification of the pathogenic fungal isolates based on internal transcribed spacer (ITS- ITS5/4) region indicated that the leaf spot disease in both Dracaena spp is caused by Altenaria spp. This appears to be the first report of Alternaria leaf spot disease among D. serrulata and D. hanningtonii.

P9.2-022

ROLE OF NON-PROGRAMMED CELL DEATH INDUCING EFFECTORS OF PARASTAGONOSPORA NODORUM IN INFECTION PROCESS

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Text

Effectors are small, secreted proteins that play an important role in virulence of plant pathogenic fungi. Parastagonospora nodorum, which causes septoria nodorum blotch of wheat, secrets multiple necrotrophic effectors that induce programmed cell death (PCD) in susceptible wheat genotypes. To date, five necrotrophic effectors including SnToxA, SnTox1,SnTox267,SnTox3 and SnTox5 have been characterized. However, less is known about effectors that are involved in the necrotrophic lifestyle that do not induce PCD. Therefore, in this study we screened the proteome of P. nodorum strain Sn2000 using effectorP v3.0 and identified 556 predicted effectors. RNA-Seq data generated for samples collected at 0, 4, 12, 24, 48, 72, and 96 hours post inoculation of Sn2000 on the wheat line LP29 supported the in planta expression of 332/556 effectors. Differential expression analysis of effector genes expressed in time points relative to the expression at 0 hpi revealed that 77 effectors were differentially expressed. In addition, we identified 69 non-differentially expressed effector genes that consisted of at least 100 reads to be used in further analysis. InterProScan screening of these effectors revealed, 23 cell wall degrading enzymes, six chitin binding proteins, five proteases, four ROS-protection proteins, and eleven nutrient break-down enzymes, suggesting these non-PCD inducing effectors play a critical role in adhesion, penetration, and colonization stages of the infection process.

P9.2-023

EXPLORING A NOVEL PUTATIVE FUNGAL RIBOSOMALLY SYNTHESISED AND POST-TRANSLATIONALLY MODIFIED PEPTIDE FROM THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI.

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Text

When we consider the compounds produced by fungi, our first thoughts take us to their medical uses, while their more sinister employment in plant pathology and as mycotoxins often comes second. Intriguingly, a family of peptides exists which spans both areas, known as ribosomally synthesised and post-translationally modified peptides (RiPPs). RiPPs from bacteria and animals have anthropogenically useful functions; nisin as an antibiotic and ziconotide as a painkiller. However, the more recently identified fungal RiPPs, of those with known functions, are all to some extent cytotoxic varying in specificity regarding the organisms to which the toxins are targeted. Pertinently, the dikaritin group of RiPPs includes the known mycotoxins phomopsin and ustiloxin, with genome mining identifying a putative novel dikaritin RiPP cluster within the genome of the wheat pathogen *Zymoseptoria tritici*. The IPO323 strain of the fungus has 9 copies of a repeated YVIPVD sequence within its RiPP precursor peptide. YVIPVD copy number varies from 6 to 11 repeats between different *Z. tritici* strains indicating some level of selection on the peptide, the direction of which is currently unknown. Ongoing studies aim to discover the structure of this RiPP and its full biosynthetic pathway by utilising knockout strains of genes within the cluster. The role of the peptide in this important wheat pathogenic fungus has been assessed in terms of pathogenicity and resource competition but remains elusive.

P9.2-024

VIRULENCE REGULATORY NETWORK OF DICKEYA DADANTII: WHAT IS THE ROLE OF POST-TRANSCRIPTIONAL REGULATION?

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Text

The genus *Dickeya* are enterobacterial plant pathogens responsible for soft rot disease in a wide range of plant species, including economically important crops e.g. potato and rice. The infection process is divided into two main phases: the asymptomatic phase where *Dickeya spp.* produce early virulence factors to colonize the apoplastic spaces between plant cells; and the symptomatic phase, which is associated with the secretion of late virulence factors, i.e. plant cell wall degrading enzymes that macerate the plant tissue. Therefore, the spatial and temporal production of the virulence factors must be precisely controlled to ensure the efficient colonization and degradation of the host. While many transcriptional regulators are involved in controlling *Dickeya*'s virulence factors, knowledge of post-transcriptional regulation is still in infancy. Our first results on *Dickeya dadantii* RNA chaperons suggest a post-transcriptional regulation of virulence. Additionally, the obtention of *D. dadantii* transcriptional landscape

allowed us to identify RNAs predicted to interact with the mRNAs of virulence factors regulators that play a role in response to oxidative stress and changing metabolic content in the apoplast. Ongoing work aims to establish a link between regulatory RNAs, virulence factors, and environmental changes encountered by bacteria during infection, which will lead to a better comprehension of the complex virulence regulatory network of *D. dadantii*.

P9.2-025

HOST AND PATHOGEN GENETICS REVEAL AN INVERSE GENE-FOR-GENE ASSOCIATION IN THE P. TERES F. MACULATA - BARLEY PATHOSYSTEM

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Text
Spot-form net blotch (SFNB) of barley is a devastating foliar disease caused by the fungal pathogen *Pyrenophora teres* f. *maculata*. To deepen our understanding of the barley/*P. teres* f. *maculata* interaction, we screened a host population consisting of a cross between the resistant barley line PI 67381 and the susceptible line Hockett with a diverse set of *P. teres* f. *maculata* isolates, resulting in the identification of two major quantitative trait loci (QTL) associated with resistance/susceptibility on barley chromosomes 2H and 7H. We then screened a *P. teres* f. *maculata* pathogen population on PI 67381 and Hockett as well as other barley lines currently used in breeding programs and identified two major pathogen loci associated with virulence/avirulence on *P. teres* chromosomes 1 and 2. By screening the host population as well as host F_2 lines with pathogen progeny harboring the virulent allele at only one of the two identified loci (i.e. Chr1 or Chr2), we were able to determine that individual dominant susceptibility genes in the host were being targeted by individual virulence genes in the pathogen to facilitate colonization, providing evidence for an inverse gene-for-gene interaction, a hallmark of necrotrophic host-pathogen interactions. Finally, the genomic regions underlying the pathogen QTL were screened for candidate effector genes, which were then systematically evaluated in terms of effector function by performing CRISPR-Cas9 mediated gene disruptions.

P9.2-026

THE VIABILITY OF SCLEROTINIA SCLEROTIORUM SCLEROTIA EXPOSED TO DRY HEAT TEMPERATURES AND THE RUMEN OF CATTLE.

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Text
Sclerotinia sclerotiorum sclerotia can survive in soil and plant residues for several years thus, reducing inoculum prevalence and persistence is critical to disease management. We address two producer-posed questions regarding sclerotia viability within production systems. The first aim simulated crop stubble burning by assessing the effect of dry heat temperatures on sclerotia viability. The germination ability of sclerotia, collected from fields affected by soybean stem rot, grouped into four weight classes and exposed to temperatures between 125 and 200 °C for 5-, 10- or 15-min durations either buried at 5 cm in soil or on the surface (n = 384) was conducted. Larger sclerotia survived increasing temperatures and durations more readily than smaller structures, and temperatures exceeding 185 °C yielded all sclerotia non-viable. Sclerotia on the soil surface were less sensitive to increasing temperatures than buried sclerotia, likely due to rapid heat dissipation. Although viable sclerotia were reduced by dry heat temperatures, the elucidation of narrow windrow burning is required. The second aim determined the ability of sclerotia to survive in the rumen of cattle. Faecal samples of cattle which grazed on white mold-infected sunflowers were collected and enumerated (n = 104). Less than 4% of sclerotia in cattle manure germinated, suggesting cattle grazing on infested stubble poses a reduced risk of unintentional pathogen introductions to disease-free fields.

P9.2-027

SEARCHING FOR PUTATIVE VIRULENCE FACTORS IN THE PYRENOPHORA TERES F. TERES GENOME

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Text
Net form net blotch (NFNB) of barley, caused by the fungus Pyrenophora teres f. teres (Ptt) is an economically important foliar pathogen in many barley growing regions of the world, including Australia. This fungus is a heterothallic ascomycete where sexual recombination can lead to changes in disease expression in the host. Knowledge of the genetic architecture and genes involved in virulence is vital to increase the durability of NFNB resistance in barley cultivars. Through bi-parental and genome-wide association mapping we have identified and confirmed genomic regions associated with virulence in Ptt, the most significant of which were located on Ptt chromosomes 3 and 5. We have further defined fungal genomic regions by fine mapping and proteomics analysis using single QTL isolates to facilitate virulence gene identification and isolation and are correspondingly co-locating their interactions within the genomic regions in the barley host.

P9.2-028

HIGH HUMIDITY OR ABA COMPENSATES FOR T3SS AND DSPE MUTATIONS IN PECTOBACTERIUM CAROTOVORUM.

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Text
Pectobacterium carotovorum is a gram-negative phytopathogenic bacterium that causes soft rot on diverse plant species. It encodes and secretes a single type III secretion system (T3SS) effector protein, DspE. This effector is required for plant cell death when the pathogen is infiltrated into tobacco leaves, which is surprising since *P. carotovorum* also encodes multiple plant cell wall degrading enzymes (PCWDEs). The PCWDEs are up-regulated by AHL and secreted through the type II secretion system (T2SS). The AHL synthase, *expI*, was down-regulated in a *dspE*-mutant when the mutant was infiltrated into plant leaves, but not when the mutant was grown in culture medium. In addition, tobacco genes associated with abscisic acid (ABA) synthesis were up-regulated when wild type *P. carotovorum* was infiltrated into leaves, compared to infiltration of the *dspE* mutant. Co-infiltration of the *dspE* mutant and ABA resulted in plant cell death. The *dspE* mutant also caused plant cell death when tobacco plants were infiltrated with the *dspE* mutant and covered with plastic or when the infiltrated leaves were detached and placed in a moist chamber. When the plants were covered with plastic bags, expression of plant genes required for ABA synthesis was similar in leaves infiltrated with wild type *P. carotovorum* or the *dspE* mutant. These observations suggest that DspE function in plant cells indirectly upregulates AHL production and that DspE function can be compensated for by adding ABA.

P9.2-029

THE INFECTION PROCESS OF PHELLINUS NOXIUS IN WOODY PLANTS

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Text
While most wood decay fungi are saprophytic, a few are highly pathogenic to living trees. Brown root rot, caused by *Phellinus noxius*, mainly infects the stem base and roots of trees, causing root rot and wilting of the entire plant. Previous studies have only investigated the colonization of *P. noxius* on wood blocks, but how *P. noxius* infects living woody plants remains unknown. In this study, the infection process of *P. noxius* in woody plants was observed in artificially inoculated seedlings using improved microscopy techniques. To observe the initial stage of infection, stem inoculation was conducted on the seedlings of *Populus trichocarpa*, *Ficus benjamina*, and *Eriobotrya japonica*. *P. noxius* hyphae penetrated the epidermis through the opening or wounds and rapidly destroyed parenchyma cells, causing a cavity. The hyphae then extended through the ray parenchyma cells into the vessel and the pith of the xylem. To understand the late stage of infection, naturally infected root tissues were collected from the field. Numerous *P. noxius* hyphae were found in the cortex, and the hyphae in the xylem were only observed near bundles of reticulated mycelial cords or in the vessel. Understanding the pathogenesis of brown root rot has provided the basis for developing effective control strategies.

P9.2-030

A NOVEL PARASTAGONOSPORA NODORUM NECROTROPHIC EFFECTOR SNTOX8 INTERACTS WITH WHEAT CORRESPONDING RECEPTOR GENE SNN8

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Text

Septoria nodorum blotch (SNB) is an economically important disease of wheat caused by a fungal necrotrophic pathogen, Parastagonospora nodorum (P. nodorum). The majority components of SNB can be explained by interactions between effectors secreted by the pathogen and their matching susceptibility genes in the host. The necrotrophic interactions were found to induce programmed cell death (PCD) to promote the pathogen's proliferation leading to disease. In this study, we cloned and functionally validated SnTox8 and characterised its role in pathogenesis of this pathosystem.

SnTox8 has the typical characters of an effector including being a small Cysteine rich protein, having signal peptide and is light and heat sensitive. Based on protein modelling, SnTox8 is structurally dissimilar to most known fungal effectors. Quantitative trait loci (QTL) mapping identified the effector is targeting Snn8 located on wheat 2A chromosome to produce PCD. Ninety-nine percent of Australian P. nodorum isolates and eighty-four percent of commercial wheat cultivars carry SnTox8 and Snn8, respectively, making this interaction an important target for SNB resistance breeding program in Australia. In this talk, I will present the finding and its significant role in SNB as well as suggest a strategy to improve SNB resistance breeding.

P9.2-031

GENETIC AND REGULATORY MECHANISMS OF LIPASE ACTIVITY IN THE PLANT PATHOGENIC FUNGUS FUSARIUM GRAMINEARUM

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Text
Lipases, which catalyze the hydrolysis of long-chain tri-, di-, and monoglycerides into free fatty acids and glycerol, participate in various biological pathways in fungi. In this study, we examined the biological function and regulatory mechanisms of fungal lipases via two approaches. First, we performed a systemic functional characterization of 86 putative lipase-encoding genes in the plant pathogenic fungus Fusarium graminearum. The phenotypes were assayed for vegetative growth, asexual and sexual reproduction, stress responses, pathogenicity, mycotoxin production, and lipase activity. Most mutants were normal in the assessed phenotypes, implying overlapping roles for lipases in *F. graminearum*. In particular, Lip1 and Fgl1 were revealed as core extracellular lipases in *F. graminearum*. Second, we examined the lipase activity of previously constructed transcription factor (TF) mutants of *F. graminearum* and identified three TFs and one histone acetyltransferase that significantly affect lipase activity. The relative transcript levels of *LIP1* and *FGL1* were markedly reduced or enhanced in these TF mutants. Among them, Gzzc258 was identified as a key lipase regulator which is also involved in the induction of lipase activity during sexual reproduction. To our knowledge, this study is the first comprehensive functional analysis of fungal lipases and provides significant insights into the genetic and regulatory mechanisms underlying lipases in fungi.

P9.2-032

CONSORTIUM OF ENDOPHYTIC BACTERIA AND ARBUSCULAR MYCORRHIZA FUNGI PROTECT TOMATO PLANT AGAINST BOTRYTIS CINEREA

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Text
Plants have evolved to cope with environmental threats. Some beneficial microorganisms (BM) can improve plant defensive responses and helping the plant to survive. Among these BM, endophytic bacteria (EB) and arbuscular mycorrhiza fungi (AMF) are of the highest interest. By one side, some EB are known to have a protective effect in plant response against pathogens. They can penetrate the plant from the rhizosphere and colonize different tissues without producing any damage, also they help plants to adapt to stress. On the other side, mycorrhiza plants through mycorrhiza-induced resistance (MIR) mechanisms, which has been largely proved to be efficient for biocontrol. In our group, the *Bacillus mycoides*, isolate O2, was identified by sequencing the 16S rRNA gene. We showed that this isolate O2 in bioassays, can protect tomato plants and prime callose against *B. cinerea*, as the AMF *Rhizophagus irregularis* does. In nature, plants are in contact with different BM at the same time, thus we wonder how the consortium EB+AMF works against the pathogen. To study the protecting effect in plants of the consortium AMF + EB, preliminary phenotypic studies have been performed to see whether AMF and *B. mycoides*, isolate O2 synergize to protect the plant against a fungal infection. It has been shown that there is a clear protection exerted by the consortium AMF+EB in tomato plants Moneymaker variety, against *B. cinerea*. The

mechanisms underpinning this phenotype are going to be studied.

P9.2-033

PECTINOLYTIC BACILLUS PUMILUS NEW PATHOGEN OF POTATO IN TUNISIA

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Text

Potato soft rot leads to considerable damage in the fields and warehouses all over the world. Although it is well-known that bacteria from the genera Pectobacterium and Dickeya are the main causative agents of this disease, several recent studies indicated an involvement of pectinolytic Bacillus pumilus in the soft rot disease outbreaks in diverse geographical regions. In this view, the aim of this work was to identify bacteria responsible for soft rot disease in high potato production areas of Tunisia.
1001 samples of the affected potato tubers were collected in the growing seasons of 2018, 2019 and 2020 from eight governates of Tunisia. From this material 270 bacterial isolates were acquired. 20 of isolated bacterial strains indicted pectinolytic activity by forming deep cavities on Crystal Violet Pectate medium. All pectinolytic isolates were able to macerate potato tuber tissue under laboratory conditions. Phenotypic characterization showed that these isolates were Gram-positive bacilli, exhibiting pectinolytic, cellulolytic, proteolytic and amylolytic activities. Majority of the isolates indicated swimming and swarming motility. Application of MALDI-TOF MS, sequencing of 16S rDNA and API commercial tests allowed for assignment of 19 of the tested isolates to the species B. pumilus and 1 to the species Paenibacillus amylolyticus.
To the best of our knowledge, this is the first report of soft rot on potato caused by pectinolytic Bacillus and Paenibacillus spp. in Tunisia.

P9.2-034

HISTOPATHOLOGY OF RHIZOCTONIA ROOT AND CROWN ROT OF SUGAR BEET

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Text
Rhizoctonia root and crown rot (RRCR), caused by *Rhizoctonia solani* AG 2-2, is one of the most important soil-borne diseases of sugar beet (*Beta vulgaris*) throughout the world. Necrosis usually takes the form of dark, shallow lesions that often develop in a ladder-like pattern and spread laterally. While infection cushions on the root surface are commonly reported as the primary route of infection, our observations indicate that lesions associated with lateral roots may involve an alternate route of entry. In the current study, we utilized advanced microscopy to examine the physiological traits of sugar beet roots associated with the development of RRCR symptoms. Hyphae that entered through infection cushions on smooth root tissue were largely restricted to the outermost cortical layer between the outer cambium and the periderm. However, in areas where the outer cambium was discontinuous, hyphae were observed to penetrate deeper into the root tissue, suggesting the outer cambium acts as a barrier to fungal penetration. In contrast, no infection cushions were observed in tissue of the lateral roots and it appears the hyphae were able to invade relatively unimpeded due to the lack of secondary cambium in this area. A region of autoflurescence caused by an unknown factor was observed in advance of the hyphae. Our observations provide an updated histological study of RRCR and offers some physiological explanations for the characteristic symptoms associated with this disease.

P9.2-035

THE ROLE OF OXALIC ACID IN CLARIREEDIA JACKSONII PATHOGENESIS ON AMENITY TURFGRASS

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Text
Dollar spot is a destructive foliar disease of amenity turfgrass caused by the fungus *Clarireedia jacksonii*. Oxalic acid (OA) is an important pathogenicity factor in related fungal plant pathogens such as *Sclerotinia sclerotiorum*, however, direct evidence supporting the role of OA in the pathogenic development of *C. jacksonii* is lacking due to its recalcitrance to genetic manipulation. Herein, we developed a CRISPR/Cas9-mediated homologous recombination approach to overcome recalcitrant transformation and delete an oxaloacetate acetylhydrolase (Oah) gene that is required for OA's biosynthesis. We hypothesize that OA is an important pathogenicity factor for *C. jacksonii* and oah-deficient *C. jacksonii* mutants will result in reduced dollar spot disease. Two independent CRISPR-Cas9 mutants *ΔCjoah-1* and *ΔCjoah-2* were generated. The mutants, WT, and a *C. jacksonii* positive control strain (LWC) were inoculated on potted creeping bentgrass in a controlled environment chamber to measure dollar spot symptom development. Results showed that after 12 days, bentgrass inoculated with *ΔCjoah-1* and *ΔCjoah-2* exhibited 59.41% lower dollar spot severity than WT and LWC isolates. Moreover, OA production was significantly reduced by 71.51% at pH 5 and 78.07% at pH 8 in *ΔCjoah-1* and *ΔCjoah-2* compared to the WT and LWC. These results clearly demonstrate that OA plays a significant role in *C. jacksonii* pathogenesis on creeping bentgrass, and may offer novel directions for dollar spot suppression.

P9.2-036

ROLES OF ANAEROBIC RESPIRATIONS OF CARBON SOURCES FOR THE ADAPTATION AND SURVIVAL OF DICKEYA DADANTII

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Text

A.M. and C.B. contribute equally

Plant pathogenic bacteria face many specific challenges colonizing and/or infecting plants. Thus, for the bacterium Dickeya dadantii capable of colonizing different plants and living in anoxic environments of the rhizosphere and in plant tissues, the ability to grow in anoxic conditions represents an adaptive trait favouring its development and dissemination. We hypothesise that the capacity to anaerobically respire carbon compounds (C) constitutes a physiological advantage for phytopathogenic bacteria. Our objective is to highlight the ability of D.dadantii to anaerobically breathe C compounds, present in the apoplast. Apoplast's C compounds able to be used as terminal electron acceptors by D.dadantii were identified, and the conservation of their metabolic pathways in Dickeya and Pectobacterium genera is studied. The mutants of the identified metabolic pathways' genes are being built.

P9.2-037

FIRST REPORT OF CHERELLE WILT CAUSED BY NEW SPECIES OF COLLETOTRICHUM IN THE PHILIPPINES

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Text

Cherelle wilt (CW) and Cacao pod rot (CPR) have been causing a decline in cacao production (*Theobroma cacao*) in the cultivation. Several fungal species are reported as causal agents of both CW and CPR, however, there is not enough information indicating whether all of those pathogens are related. Thus, it is not clear if the causal agent of CW is also responsible for the occurrence of CPR. In this study, we collected a cherelle which turned black and covered with slightly white mycelia in June 2022 in the Philippines, and isolated a novel *Colletotrichum* species from the symptomatic CW. The pathogenicity test using the monoculture strain showed that the isolate is pathogenic to young cacao pods (with/without wound) but not to mature pods and leaves. This may indicate that the isolate is only the causal pathogen of CW and not of CPR and anthracnose of leaves. In molecular phylogenic tree based on ITS, *GAPDH*, *CHS1*, *ACT*, and *TUB2*, the isolate is an independent which belongs to the *Colletotrichum gigasporum* species complex (CGSC) consisting of nine known species. The size and number of septa of the ascospores were different from those of *C. gigasporum* and *C. taiwanense*. Moreover, all of the characteristic features of the appressoria, conidia and the colony do not much with any CGSC species.

Based on the aforementioned results, it is therefore concluded that the isolate is a new species of *Colletotrichum* and is being proposed to be added as a causal pathogen of CW.

P9.2-038

DEFENCE OF BRASSICA NAPUS TO RHIZOCTONIA SOLANI AG2-1 AND HOST-PATHOGEN INTERACTIONS IN THE SOIL ENVIRONMENT

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Text

Rhizoctonia solani Anastomosis Group (AG) 2-1 causes damping-off and root/hypocotyl rot of Brassica napus(Oilseed Rape; OSR) resulting in significant crop losses in field. Resistance variation and the regulation of defence of OSR to AG2-1 or the effects of the soil environment on host-pathogen interactions have not yet been fully elucidated. Presently, cultural practices and/or seed treatment remain as the main methods of disease control. We carried out a series of experiments to determine the varietal responses of B. napus to AG2-1 and used confocal microscopy and computed micro-tomography to visualise infection and to quantify the temporal effects of soil type, moisture and seed treatment on pathogen growth and development. Molecular approaches and functional analysis with Arabidopsis thaliana mutants were employed to define the defence response to AG2-1. Auxin, abscisic acid, and the MYC2 branch of jasmonate signalling contributed to plant susceptibility, whilst induced systemic resistance was enhanced by NAPDH RBOHD, ethylene signalling and the ERF/PDF branch of jasmonate signalling. AG2-1 development and infection were favoured by low soil moisture irrespective of soil type, with excessive moisture causing preferential pathogen growth on the soil surface in the absence of seed treatment. The findings from these studies identify defence pathways, sources of resistance and soil environmental factors that can lead to improved OSR management of soil-borne R. solani.

P9.2-039

COLLETOTRICHUM SPECIES ASSOCIATED WITH PRE-HARVEST CITRUS ANTHRACNOSE IN SOUTH AFRICA

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Text

Colletotrichum is the causal agent of pre- and post-harvest anthracnose on many important crops such as citrus. Although *C. gloeosporioides* is known to occur on South African citrus, a European survey identified *C. karsti* from imported fruit from South Africa for the first time in 2017. The aim of this study was therefore to determine which *Colletotrichum* species are associated with pre-harvest citrus anthracnose symptoms in different regions in South Africa.

This was done by sampling different plant parts, namely leaves, twigs, green and mature fruit, fruit stems and flowers, in 20 citrus-growing areas in four provinces in South Africa. A total of 1141 *Colletotrichum* isolates were obtained. Representative isolates were identified by amplifying and sequencing GAPDH, TUB2 and ITS gene regions. The main *Colletotrichum* species associated with citrus in all the growing regions were *C. gloeosporioides* and *C. karstii*. The two species were identified in an even ratio from all citrus varieties surveyed and were isolated from all plant parts sampled. Navel oranges had the highest incidence. *Colletotrichum gloeosporioides* was obtained from more fruit than *C. karstii* and more *C. karstii* isolates were obtained from leaves. This is the first large-scale study to investigate the distribution of *Colletotrichum* species associated with anthracnose symptoms, in South African citrus orchards.

P9.2-040

THE ETIOLOGY OF ALTERNARIA BROWN SPOT AND ALTERNARIA CORE ROT OF CITRUS IN SOUTH AFRICA

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Text

Many small-spored *Alternaria* species cause serious damage to fruit, such as Alternaria brown spot and Alternaria core rot in citrus orchards worldwide. The aim of this study was to clarify which small-spored *Alternaria* species are associated with these diseases in South African citrus orchards. Fungal isolations were made from leaves, twigs, young and mature fruit, fruit stems and flowers from 33 citrus cultivars in four main citrus production areas in South Africa. *Alternaria* isolates were identified by amplifying and sequencing the rpb2, endoPG and OPA10-2 gene regions and by fungal morphology. Most of the isolates were identified as *A. alternata* and divided into two clades. *Alternaria alternata* was mostly isolated from soft citrus types, and specifically from fruit. *Alternaria alternata* was furthermore associated with brown spot and core rot fruit lesions. This study showed that small-spored *Alternaria* species were found from different plant parts including leaves, twigs, young and mature fruit, fruit stems and flowers. Results of this will lay the foundation for decision making in future disease management strategies of Alternaria brown spot and Alternaria core rot of citrus.

P9.2-041

DEVELOPMENT OF MICROSATELLITE MARKERS FOR POPULATION STUDIES OF ROSELLINIA NECATRIX.

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Text

Rosellinia necatrix is a soil-borne pathogen, the causal agent of white root rot (WRR), affecting several economically important ornamental plants and fruit trees. This fungus was first reported in South Africa in 1974, on apple and pear trees, and grapevines in the Western Cape. In 2016, it was reported on avocado orchards in Limpopo for the first time. Since then, WRR has also been detected in KwaZulu-Natal and Mpumalanga. Despite these reports, limited knowledge is available on the origin, spread and population biology of this fungus. Therefore, the aim of this study was to develop a set of polymorphic microsatellite markers to study the genetic diversity and population structure of *R. necatrix* in South Africa. Using genome sequences of three isolates from *R. necatrix*, 29 primer pairs were designed to amplify microsatellite regions consisting of tri-, tetra-, penta- and hexa-nucleotide repeats. Polymorphism at each microsatellite locus was determined through the amplification of six isolates from Israel, Spain and South Africa. Thirty isolates from Limpopo were used to plot a genotype accumulation curve. The efficacy of the microsatellite markers for population genetic analyses studies was demonstrated on two populations of *R. necatrix* from South Africa. The markers developed in this study are a useful tool to study the population structure and genetic diversity of *R. necatrix* populations in countries where it is an important pathogen.

P9.2-042

EFFICIENT ISOLATION AND TRANSFORMATION PROTOCOL OF ZYMOSEPTORIA TRITICI PROTOPLAST FROM THE GLOBAL REFERENCE STRAIN IPO323

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Text

Zymoseptoria tritici is a devastating filamentous fungus and the causal agent of Septoria tritici blotch (STB) in wheat. It has been documented STB could cause up to 50% yield loss in severe conditions while it is also responsible for up to 70% of the total fungicide usage on wheat in Europe. *Z. tritici* has one of the most expansive genome resources for any plant pathogen, with over 1000 publicly available genomes along with over 20 high-quality long-read assemblies. However, our ability to take full advantage of this rich dataset is limited by the lack of a high-throughput method for genetic manipulation of this species.

This is especially more complicated in *Z. tritici* as the novel global reference strain IPO323 has anecdotally been reported to be difficult to protoplast. Adding to this difficulty was the only published enzyme (glucanex, Sigma) used to generate *Z. tritici* protoplasts has now been discontinued. Here we developed a protocol for protoplast isolation and transformation of the *Z. tritici* reference IPO323 and other modern *Z. tritici* isolates using the cheap and commercially available winemaking enzyme ‘extralyse’ (Laffort). We will present an overview of our

optimised protoplast isolation method, and transformation efficiency of IPO323 using PEG-mediated transformation.

P9.2-043

GENOMIC AND PHENOTYPIC CHARACTERIZATION OF A NEW GENUS OF THE PECTOBACTERIACEAE FAMILY AND RECTIFICATION OF THE OUTLINE OF THIS FAMILY

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Text

The bacterial order *Enterobacterales* is currently divided into eight recognized families: *Budviciaceae*, *Enterobacteriaceae*, *Erwiniaceae*, *Hafniaceae*, *Morganellaceae*, *Pectobacteriaceae*, *Thorselliaceae*, *Yersiniaceae*, and the not yet recognized genus *Bruguierivoracaceae*. The *Pectobacteriaceae* family comprises plant pathogens able to provoke diverse diseases, including plant maceration due to the production of pectinases disrupting the plant cell wall. To better understand their natural diversity, a survey of pectinolytic bacteria was performed in lakes of the French region La Camargue near the Mediterranean Sea. Sixteen atypical pectinolytic isolates were obtained from brackish water of three lakes. In phylogenetic trees, the novel strains formed a new clade of *Pectobacteriaceae*, separate from the previously described genera of this family: *Affinibrenneria*, *Brenneria*, *Dickeya*, *Lonsdalea*, *Musicola*, *Pectobacterium*, and *Samsonia*. Phylogenomic study of representative members of the order *Enterobacterales* clearly indicated that *Acerihabitans* does not belong to *Pectobacteriaceae* and should be reclassified in the *Bruguierivoracaceae* family. In contrast, the relative position of *Symbiopectobacterium* in the *Enterobacterales* tree supports its appurtenance to *Pectobacteriaceae*. Finaly, based on phenotypic, genomic and phylogenetic characteristics, we propose the creation of a new genus including the sixteen pectinolytic isolates from Camargue brackish lakes

P9.2-044

EXOSOME TRAFICKING DURING THE ASYMPTOMATIC PHASE IN BOTRYTIS CINEREA

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Text

Botrytis cinerea is known as a necrotrophic phytopathogenic fungus: the pathogen kills the

plant cells before feeding on them. However, an asymptomatic growth is also reported in *B. cinerea* that could last over several weeks under certain conditions. Our group started a study with the assumption that various biotic and abiotic parameters might favor the switching of *B. cinerea* from the asymptomatic growth to the necrotrophic lifestyle. Another highlight is the ability of the model plant *Arabidopsis thaliana* to secrete exosomes in response to *B. cinerea*. The plant exosomes internalize the fungal cell and deliver signaling molecules that downregulate the fungal virulence. Our working hypothesis is that plant exosome trafficking contributes to lower the fungal virulence and maintain the asymptomatic phase of the fungus during its infection. Using a combination of fluorescent reporter systems in *Arabidopsis* transgenic lines, various developmental and physiological stages in addition to the way of inoculation of *B. cinerea* were assessed to (1) determine the best conditions necessary to analyze the asymptomatic phase ; (2) observe by fluorescence microscopy the exosome trafficking between the host and its pathogen. In an integrative biology perspective, this study will contribute to better understand the complex molecular dialogue between *B. cinerea* and its host; and at term the behavior of this pathogen in the field.

P9.2-045

TAN SPOT SEVERITY IN THE FIELD IS EXPLAINED ONLY PARTIALLY BY KNOWN NECROTROPHIC EFFECTORS OF PYRENOPHORA TRITICI-REPENTIS IN NORDIC SPRING WHEAT

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Text

Tan spot caused by Pyrenophora tritici-repentis (Ptr), a necrotrophic fungal pathogen, is one of the most prevalent diseases of spring wheat (Triticum aestivum L.) in Nordics and especially in Finland. Three Ptr necrotrophic effectors (NEs) are currently known; ToxA and ToxB, which are proteins, and ToxC, a small polar molecule. Here we tested the sensitivity of 181 spring wheat genotypes, mostly Nordic cultivars and breeding lines, to purified ToxA and ToxB effector proteins to examine the importance of these virulence factors behind tan spot epidemics in Nordics. Both ToxA and ToxB sensitivities were present in the tested wheat material, ToxA sensitivity being more common than sensitivity to ToxB. We compared the sensitivity data with observations from artificially inoculated field experiment in Finland in 2022 and found that both ToxA and ToxB sensitivity explained only little of phenotypic variation in tan spot susceptibility. ToxA and ToxB have previously been described as important facilitators of tan spot susceptibility in NE-sensitive lines, but our results indicate other factors being more important in Nordic wheat production area. We will continue the study by collecting second year of field data to better capture year-by-year variance. We will also explore the genetic diversity of Ptr populations in Finland to better understand the factors driving the epidemic development from the fungus perspective.

P9.2-046

RHYTHMIC PATTERNS: EVIDENCES TOWARDS A BI-SINUSOIDAL DYNAMIC OF APICAL SECRETION IN BOTRYTIS CINEREA

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Text

The development and morphology of filamentous fungi rely on the polar delivery of secretory vesicles to growing hyphal apexes. Those vesicles bring lipids required for the extension of the plasma membrane and parietal enzymes involved in the synthesis of the cell wall. While extensively studied in plant pollen tubes that also exhibit polar growth, the temporal dynamic of vesicles at fungal apexes has been understudied. Here, the chitin synthase CHSIIIa of the phytopathogenic fungus *Botrytis cinerea*, known to be transported by secretory vesicles, has been fused with eGFP. Confocal microscopy has been used to study the temporal dynamics of labelled vesicles. First, pulses of vesicles were observed in actively growing hyphae that were neither observed in non-growing hyphae nor in differentiated penetrative structures. Second, the kinetic measurement and analysis of the fluorescence signals collected from growing hyphae highlighted a rhythmic behaviour characterized by a bi-sinusoidal pattern. This pattern has been characterized using sinusoidal regression and Discrete Fourier Transform. To our knowledge, this is the first description of a bi-sinusoidal dynamic of vesicle secretion in filamentous fungi. Together, these results shed new light on the current models of fungal cell elongation.

P9.2-047

IN VITRO GERMINATION OF PHAEOCYTOSTROMA SACCHARI CONIDIA, THE CAUSAL AGENT OF STALK ROT DISEASE OF SUGARCANE (SACCHARUM SPP.)

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Text

The stalk rot disease of sugarcane, caused by *Phaeocytostroma sacchari*, is considered a secondary disease. However, it is frequency and importance have increased in Brazil. The objective of this work was to evaluate the in vitro conditions for the germination of *P. sacchari* conidia. The colonies were grown in PDA medium for 30 days, under photoperiod 12/12 h at 25ºC. Suspension of 10^5 conidia mL^{-1} was obtained through serial dilution and counting in a hemocytometer. For the evaluation of germination, 20 µL of conidial suspension was deposited in water agar (WA) culture medium, on a microscope slide. Different methodologies were tested, being added to the WA medium, in the ration 0,5:1 (extract:suspension): (i) extract of stalk bark, (ii) extract of midrib, (iii) sugarcane juice, and (iv) sterile water, as control. Each treatment was constituted by three repetitions. Incubation took place in BOD at temperatures of 22ºC, 25ºC and 28ºC. The germination rate was evaluated 12, 18 and 24 hours after

incubation. To interrupt germination, a drop of lactophenol blue was deposited. A conidium whose germ tube was longer than it is width in it is median region was considered germinated. The beginning of conidial germination was observed 12 hours after incubation for treatments containing extracts. Germination rates greater than 50% were observed 24 hours after incubation at 28ºC, for treatments containing sugarcane extracts. No conidium germinated in the control treatments.

P9.2-048

SUGARCANE STALKS ROT: RESURGENCE DISEASE

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Text

In the last decades, the main diseases associated with the crop have been well managed through the use of varietal resistance. However, lately there has been a resurgence of diseases previously considered of secondary importance, and others, still little known. Such diseases occur separately, or sometimes simultaneously, apparently originating from infections and co-infections caused by necrotrophic pathogens. The objective of this work is to highlight the presence of a disease associated with a complex of symptoms, which summarize stem rot, concomitantly due to fermentation and characteristic odor, and the presence of coincident wilted stems, which lead to a large reduction in production. Symptoms are observed from the beginning of maturation, with gradual evolution in subsequent phases. Such symptoms have been observed in several varieties, and in different bioecological conditions. The damage resulting from such diseases are very high, with losses of up to 32% of stalks with symptoms of the disease. Reductions in productivity of the order of 22.9 tons of stalks per hectare were also reported. Phaeocytostroma sacchari, Colletotrichum falcatum and Fusarium spp., the most frequent fungi, have often been isolated from diseased stalks. Associated with the absence of spatial and space-time discontinuity, it is speculated that the high frequency and population of such pathogens is associated with a high source of inoculum resulting from the absence of cycle break of these fungi.

New Developments in Fungicide Resistance

C5.5-1

FUNGAL CLONE WARS: HOW HYBRIDISATION AND CLONAL EXPANSION LED TO A NEW TYPE OF DMI RESISTANCE IN BARLEY SPOT FORM NET BLOTCH

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Text

Barley spot form net blotch and net form net blotch diseases are caused by *Pyrenophora teres* f. *maculata* (Ptm) and *P. teres* f. *teres* (Ptt), respectively. Ptt and Ptm show high genetic diversity in the field due to intraspecific sexual recombination and interspecific hybridisation of the two species, although the latter is considered rare. Here we describe the detection of Ptt × Ptm hybrids with demethylase inhibitor (DMI) fungicide resistance (HR Ptm) and discuss the implications for barley disease management. The genomes of one putative hybrid, three Ptm and ten Ptt isolates were sequenced, and recombination analyses performed at the intergenic and whole genome levels. Of the 12 chromosomes, 11 showed significant recombination events in the intergenic regions while variable recombination rate showed significant recombination across all the chromosomes. Further genotyping of fourteen Ptt, fifteen Ptm, 48 HR Ptm, and two P. teres isolates from barley grass, showed that all HR Ptm isolates were clonal and not clustered with Ptt or Ptm. Locus specific analyses of the DMI target *Cyp51A* gene showed four recombination breakpoints, including the F489L point mutation that is correlated with DMI resistance. These results confirm the occurrence of natural recombination between Ptt and Ptm and indicates that the HR Ptm likely acquired DMI fungicide resistance through interspecific recombination, followed by clonal expansion of this genotype in barley-growing areas of Western Australia.

C5.5-2

STATUS OF RESISTANCE EVOLUTION OF BARLEY DISEASES AND CURRENT CONTROL STRATEGIES IN EUROPE

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Text

Barley is affected by different diseases, whereas Ramularia leaf spot (Ramularia collo-cygni), scald (Rhynchosporium secalis) and net blotch (Pyrenophora teres) are of high importance in most European barley growing regions. These diseases have been efficiently controlled for many years by various modes of action especially by QoIs, DMIs and SDHIs. For R. collo-cygni, QoI resistance mediated by the G143A mutation in the cyt b, is widespread and SDHIs and DMIs are also affected by mutations in their respective target genes. However, both modes of action, especially the DMIs, still contribute to effective Ramularia control. R. secalis is efficiently controlled by QoIs and SDHIs, there are only single reports on adapted isolates, which have no practical relevance so far. For P. teres, SDHI resistance affects SDHI field efficacy with regional differences and is mediated by various mutations in the SDH subunits B, C and D. QoI adaptation in P. teres is conferred by the mutation F129L in the cyt b and widespread in various European regions, however some QoIs such as pyraclostrobin show high field efficacy on F129L-adapted populations. A single detection of a G143A-mutated isolate of P. teres from Denmark in 2019 was intensively investigated and studies showed that this event was based on a partial cyt b gene transfer by P. tritici-repentis. In last seasons

monitoring studies, based on sensitivity assays and molecular analysis, such isolates have not shown up again.

C5.5-3

FUNGICIDE RESISTANCE IN MOST RELEVANT BRAZILIAN CROPS

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Text

The first cases of Asian Soybean Rust (ASR) in Brazil were reported in season 2001, causing high level of defoliation and yield reduction in brazilian's soybean fields. The use of fungicides to control this disease has been essential among the years. Initially this disease was controlled with triazoles solo, but in 2005 the first reports were of reduced performance of this fungicide group was observed in the field, being later detected some mutations in the fungus associated with resistance to triazoles. After this event, to control this disease was adopted the strategy of fungicides mixes. The combination of strobilurins and triazoles began to be applied and keep good level of performance until season 2012/2013 when was identified the mutation F129L that impact partially the performance of strobilurins in all regions in Brazil. In 2014 was introduced into the market the carboxamides fungicides group to control ASR. Considering the area cultivated and disease pressure level in different years in 2017 was reported first case of mutation that effect carboxamides (I86F). This last mutation associated to mutations for triazoles and strobilurins generates a new challenge for fungicides to control ASR. The strategy adopted to reduce the impact of this mutation in control of ASR was associated to use multi-site fungicides in tank mixtures, select short cycle cultivars, anticipated the planting time to avoid or reduce the better weather condition for disease evolution.

C5.5-4

FIELD PROFILING OF FUNGICIDE RESISTANCE FREQUENCIES

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Text

Fungicide resistance is an increasing risk to crop production and knowledge around the frequencies of relevant resistant genotypes is critical for informed management strategies. Using droplet digital PCR of leaf lesion DNA and the net blotch pathosystem in barley, we demonstrate the ability to quantify both *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*, and 23 genotypes associated with different levels of sensitivity to DMI (demethylation inhibitor) or SDHI (succinate dehydrogenase inhibitor) fungicides. Across 20 fields sampled in Western Australia in 2021, genotypes associated with reduced sensitivity or resistance to

DMIs were detected in every field (3 to 92% of the population). Genotypes associated with reduced sensitivity or resistance to SDHIs were detected in 15 fields (1 to 89% of the population). The most frequently detected pathogen was *P. teres* f. *maculata*, while the most frequently detected genotypes were the PtTi insert (*Cyp51A* promoter) and N75S (*SdhC* subunit). Across the combined field data, the frequency of reduced sensitivity or resistance genotypes was 31% for DMIs and 18% for SDHIs, likely reflecting the historical use of each fungicide. Fungicide applications varied between fields, however no strategy resulted in populations with less than 10% of both DMI and SDHI reduced sensitivity or resistant genotypes. This workflow will improve investigations into fungicide management impacts in the field for both researchers and growers.

C5.5-5

FUNGICIDE RESISTANCE MECHANISMS AND MANAGEMENT IN FUSARIUM GRAMINEARUM SPECIES COMPLEX

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Text

Fusarium head blight (FHB) caused by *F. graminearum* (Fg) and several other Fusarium spp. is a devastating disease of cereal crops worldwide. In addition to yield losses, mycotoxins deoxynivalenol (DON) and its derivatives produced by Fg complex in infested grains represent a serious threat to human and animal health. Currently, the application of fungicides during wheat anthesis is a primary method for management of FHB. In this presentation, we will review recent advances in 1) chemical control and fungicide resistance situations in FHB; 2) molecular mechanisms of Fg complex to various fungicides, including benzimidazoles, cyanoacrylates, sterol demethylation inhibitors, and succinic dehydrogenase inhibitors; 3) management strategy against fungicide resistance in FHB. In addition, DON has been the most frequently detected mycotoxin in cereal grains worldwide, we will also summary effects of fungicide applications on DON management.

C5.5-6

PREVALENCE AND CO-OCCURRENCE OF TWO FUNGICIDE RESISTANCE MARKERS IN ERYSIPHE NECATOR

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Text

Emergence of fungicide resistance in plant pathogenic fungi impairs chemical control. *Erysiphe necator*, the pathogen causing grapevine powdery mildew has been effective in developing fungicide resistance. This includes resistance against demethylation inhibitors, and boscalid, a succinate dehydrogenase inhibitor. DNA markers of the resistance are nucleotide changes in the coding genes of the proteins targeted by those fungicides, *CYP51* and *sdhB*. In *E. necator*, these markers are known as A495T and A794G, respectively. By combining datasets on the prevalence of A495T (in 1415 samples) and A794G (in 505 samples) obtained by simple diagnostic methods, we aimed at investigating (i) if the prevalence of the markers is influenced by fungicide treatments, and/or the wine region of sample origin; and (ii) the co-occurrence of A495T and A794G in individual samples. The prevalence of A495T was not significantly different between treated and untreated fields. Presence of A794G was strongly correlated with fungicide treatments. The prevalence of both markers was dependent on the geographic origin of the samples, i. e., wine-region. Moreover, the presence of the two markers in the same sample was found not to be independent, that is, one marker occurred in the same sample more frequently if the other marker was also present. Our results, beside others, imply that fungicide use should be adjusted regionally, and should ideally be based on monitoring of fungicide resistance.

F5.4-1

FROM MITOCHONDRIAL GENOME TO STROBILURIN RESISTANCE: IN SILICO ANALYSIS OF GROUP I INTRON EVOLUTION IN FUNGAL PLANT PATHOGENS

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Text

The QoIs, also known as strobilurins, represent a group of fungicides with arguably the greatest resistance risk. In 2023, the FRAC reported more than 50 species with a resistant status to QoIs. Resistance is mainly caused by the replacement of Glycine into Alanine (G143A) in the target cytochrome b protein. This substitution is conferred by a single nucleotide mutation in the cytochrome b gene. In fungi, a group I intron, situated directly after codon 143, prevents QoI resistance by blocking the G143A mutation. However, group I introns have the ability to move from a donor gene into an intronless acceptor gene. The mobility of group I introns has been speculated to be a compensation mechanism to restore the potential for QoI resistance mutation. On this basis, an extensive in silico analysis of whole mitochondrial fungal genomes was performed to characterize the distribution of the group I introns among CFR fungal species. Our results showed that one subtype of group I is associated with QoI resistance and that CFR fungi can be classified according to the presence/absence of this subtype in their genome, resulting in their capacity to acquire the G143A mutation, and thus become resistant. Accordingly, this classification allows to predict the potential of QoI resistance among fungi. At a time when the use of synthetic fungicides can lead to resistance and environmental problems, this predictive model could be a valuable tool in managing the use of QoIs for disease control.

P5.4-001

RESISTANCE OF BLUMERIA GRAMINIS F. SP. TRITICI TO AZOLE FUNGICIDES IN CHINA

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Text

Azoles are commonly used control Wheat powdery mildew caused by Blumeria graminis f. sp. tritici (Bgt). Tebuconazole and epoxiconazole were used for more than 10 years, while prothioconazole was registered for the control of wheat powdery mildew in 2019 in China. To investigate the current status of azole resistance in Bgt. Totally 106 isolates collected in 9 provinces in China were determined by leaf segment method. The EC50 values of Bgt isolates in 2019 was significantly right shift in comparison with the isolates before 2000 to all three azoles. In addition, there was positive cross resistance among the three azoles. According to amino acid subsitutues of BgtCYP51, 7 types were classified. Type I (79S136Y175K442F496R), type II (79S136W175K442F496R), and type III (79T136F175N442F496R) were dominantly genotypes including 89.34% isolates, while type I as the wild type were only 10.66%. Currently, type II, and type III as the main resistance type were dominantly genotypes in China. The EC50 of Type II and type III isolates to three azoles were significantly higher than that of wild Type I. The expression levels of Bgtcyp51 gene were increased in the isolates reduced sensitivity to the three azoles. The findings in this study suggest that azole resistant is very common in Bgt in China. Molecular basis of azole resistance were caused by Y136F, Y136W, S79T/Y136F/K175N mutations, over-expression, and increased gene copy numbers of Bgtcyp51 gene, individually or synergetically.

P5.4-002

MOLECULAR CHARACTERIZATION OF PROPICONAZOLE RESISTANT TILLETIA HORRIDA ISOLATES IN THE UNITED STATES

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Text

Rice kernel smut, caused by Tilletia horrida, is a new emerging disease threatening rice production in the US. Heavy use of propiconazole fungicides of the demethylation inhibitor (DMI) class for midseason preventive applications has resulted in reduced efficacy or even failures to control kernel smut in recent years. Propiconazole resistance might be involved in observed control efficacy reductions. In this study, 63 isolates of T. horrida were collected from across the US. Three of the isolates from organic rice, where no fungicides were applied ever, were used to establish the propiconazole baseline sensitivity of 0.2 ml/ml. While most isolates shown no inhibition at the concentration of 10 ml/ml, only 14 isolates tolerated at 25 ml/ml, with a single isolate that was not inhibited even at 50 ml/ml. Variations in CYP51 gene, the target gene for propiconazole resistance, in the resistant and sensitive isolates were further characterized, and mutations were confirmed to be present in CYP51 gene of the resistant isolates. The results show, for the first time, that propiconazole

resistance has developed in the US T. horrida populations. There is an urgent need for further research on searching for effective fungicides alternative to propiconazole for managing kernel smut in rice.

P5.4-003

QOI FUNGICIDE RESISTANCE IN SUNFLOWER PHOMOPSIS

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Text
Phomopsis stem canker of sunflower (*Helianthus annuus*), primarily caused by *Diaporthe helianthi* (syn. *Phomopsis helianthi*), can cause yield losses up to 40% worldwide. For managing Phomopsis stem canker, fungicides containing Quinone outside Inhibitor (QoI) are recommended, which have a high risk to select resistant fungal strains. The objective of this study was to assess sensitivity of *D. helianthi* to azoxystrobin (QoI) fungicide in the greenhouse. The experiment was established in a factorial completely randomized design [isolates (10) x fungicide concentration (3)] and repeated once. For each factorial combination, three seeds of a *Diaporthe*-susceptible hybrid were sown in two pots each containing potting mix. At four true leaves growth stage, fungicide was sprayed using a backpack sprayer. Plants with no fungicide served as control. After 24-h, all plants were inoculated using mycelial-contact inoculation method and maintained at 25°C. Disease severity was rated 10-d post-inoculation, and analyzed using relative treatment effects (RTE). A significant isolate-fungicide concentration interaction (P<0.0001) was observed. For isolates with QoI-reduced sensitivity, significant differences in RTE were not observed between control and fungicide-treated plants using 95% confidence intervals. The molecular basis of QoI resistance was identified as G143A mutation, and farmers must consider alternating fungicides with different modes of actions for Phomopsis stem canker management.

P5.4-005

DMI RESISTANCE IN CERCOSPORA BETICOLA IS MODULATED BY CYP51 CODON BIAS

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Text
Cercospora leaf spot (CLS) is the most damaging foliar disease of sugar beet globally. To combat CLS, multifaceted efforts are widely employed, including breeding for resistance,

cultural practices, and the application of fungicides. However, populations of Cercospora beticola have become resistant to most fungicides used for CLS management, including those in the sterol demethylation inhibitor (DMI) class of fungicides. In this study, we sampled nearly 600 isolates of Cercospora beticola from MN and ND during the 2021 sugar beet growing season. For each isolate, EC50 values were determined for DMIs tetraconazole (Eminent), prothioconazole (Proline), difenoconazole (Inspire), and mefentrifluconazole (Revysol). Using the CYP51 gene sequence for each isolate, we determined that the synonymous E170 mutation and the synonymous/nonsynonymous L144(F) can be used to predict resistance to these four DMIs. The prevalence and accuracy of the six mutation combinations were calculated and specific combinations can predict resistance with greater than 90% accuracy. Interestingly, one prevalent mutation combination resulted identified cross-resistance to difenoconazole and mefentrifluconazole, but sensitivity to tetraconazole and prothioconazole. This data reveals the importance of codon bias in fungicide resistance and is the first demonstration of the use of synonymous mutations to predict fungicide resistance.

P5.4-006

LOCATION, LOCATION, LOCATION: THE FIRST RULE OF POSTHARVEST EPIDEMIOLOGY FOR LOCATING FUNGICIDE RESISTANT BLUE MOLD FUNGI IN COMMERCIAL PACKINGHOUSES

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Text

Apple blue mold (*Penicillium* spp.) impacts fruit quality and increases the chances of mycotoxin contamination in processed fruit products. Blue mold is the primary postharvest apple disease in the USA. Management relies on synthetic fungicides; consequently, fungicide resistance has been reported. Therefore, the role of inoculum sources (IS) was evaluated as reservoirs of *Penicillium* spp. having fungicide resistance phenotypes. Ten orchards and packinghouses were sampled in the Mid-Atlantic, USA, between 2020 and 2022. The surfaces of harvesting equipment, packinghouse facilities and cold room air, dump tank water, and apple fruit surfaces were sampled. *Penicillium* spp. were isolated from each IS using Petri dishes containing PDA amended with discriminatory doses of pyrimethanil, fludioxonil, and thiabendazole. The frequency of *Penicillium* spp. per IS was determined, and representative isolates were identified to species using DNA sequencing. *Penicillium* spp. from apples with blue mold were assessed on discriminatory doses as above. The packinghouse and cold room air, and dump tank water had the highest frequency of fungicide resistant isolates (10%-65%), while the frequency was 5%-34% on rotten apple fruit. *P. expansum* was the most frequently isolated species in both, IS and apples with blue mold. The differences in the IS indicate that targeted management practices are needed and can now be executed, based on these findings, to prevent fungicide failures on stored fruit.

P5.4-008

THE IN VITRO AND IN VIVO PHOSPHITE SENSITIVITY OF PHYTOPHTHORA CINNAMOMI ISOLATES FROM AVOCADO ORCHARDS IN TAIWAN

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Text

Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi* is one of the most important diseases of avocado in Taiwan. The application of phosphonate fungicides has been used to manage this disease for decades in many countries, and it had been observed that the prolonged use of phosphite had led to a reduced sensitivity to phosphite in *P. cinnamomi* isolates. In this study, the phosphite sensitivity of 50 *P. cinnamomi* isolates from 18 Taiwanese avocado orchards was tested with phosphite-amended Ribeiro's modified medium. The results showed that most isolates were sensitive or intermediate sensitive to phosphite, while only 8 isolates were tolerant to phosphite, found in only 4 orchards. Interestingly, there was no extensive phosphonate application history in these 4 orchards, and the genotypes of the tolerant isolates were all different among these orchards. Three isolates representing sensitive, intermediate sensitive and tolerant isolates were inoculated to avocado treated with different rates of phosphite. The detached root bioassay showed that the tolerant isolate had higher colonization rates than the other two isolates when the root phosphite concentrations were over 1000 µg/g-fresh weight. The information obtained in this study will be helpful for establishing the critical root phosphite concentration required for suppressing *P. cinnamomi* in avocado, and managing the development of phosphite resistance.

P5.4-009

DETECTION AND CHARACTERIZATION OF FUNGICIDE RESISTANT NET BLOTCH PATHOGEN PYRENOPHORA TERES F. TERES ISOLATES FROM ESTONIA

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Text

Reliance on fungicides in disease control creates a selection pressure for the evolution of resistance in fungal pathogens. One of the major pathogens of barley in Northern Europe is *Pyrenophora teres f. teres* (*Ptt*). FRAC has classified *Ptt* as medium-risk pathogen for developing resistance to fungicides. We isolated a total of 206 *Ptt* single-spore isolates from nine different counties across Estonia in 2021 and 2022. The baseline sensitivity of Estonian population to demethylase inhibitor (DMI; Cyp51), succinate dehydrogenase inhibitor (SDHI; SdhC and SdhD) and strobilurin (QoI; CytB) fungicides was established. Generally, fungicide sensitivity in the Estonian *Ptt* population is high or moderately declined. Among the samples, we found single isolates showing resistance to tested fungicides. The prevalence and contribution of different resistance mechanisms involved in fungicide sensitivity were investigated in *Ptt* population. A high proportion of *Ptt* isolates carry a DMI resistance-

associated substitution F489L in Cyp51. Further investigation revealed a 134 bp insert in Cyp51 promoter in few isolates only from year 2022. Differences in sensitivity to the SDHI fungicides were observed among the isolates with mutations C-S135R and D-H134R in SDH subunits. The sensitivity to azoxystrobin decreased in CytB F129L mutated isolates. The data presented confirm the ongoing evolution of fungicide sensitivity in the *Ptt* population.

P5.4-010

PROTEOMIC ANALYSIS REVEALED THAT THE OOMYCETICIDE PHOSPHITE MAY POSSESS A MULTI-MODAL FUNCTION IN AN OOMYCETE PATHOSYSTEM

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Text
Phytophthora cinnamomi is a phytopathogenic oomycete that causes dieback disease in a wild variety of dicots of significant conservational and horticultural importance. The oomyceticide phosphite is widely used to minimise the impact of dieback however, the mode of action of the chemical has not been fully deciphered. It is unclear whether it only works directly on the pathogen or also through the host. Additionally, resistance to phosphite is emerging in *P. cinnamomi* isolates and other oomycete phytopathogens. In this study, we used a label-free quantitative proteomics approach to investigate possible modes of action of phosphite on the pathogen as well as a model host. Phosphite was applied to sensitive and resistant *P. cinnamomi* isolates. Significant changes in protein abundances associated with general metabolism, stress, signalling and regulation of gene expression were observed in the sensitive isolate only. When the model host *Lupinus angustifolius* was treated with phosphite, enrichment of proteins that are associated with photosynthesis, carbon fixation and lipid metabolism in the host was observed. An increase in the production of a range of defence-related proteins was also observed. Based on our findings, we proposed possible models of the multi-modal action of phosphite that directly targets the pathogen as well as alters plant metabolism and immune response. This will be discussed.

P5.4-011

MEFENOXAM SENSITIVITY TYPING OF SOUTH AFRICAN PHYTOPHTHORA ISOLATES

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Text
Citrus nursery plants infected with *Phytophthora citrophthora* and *Phytophthora nicotianae* are treated with mefenoxam, a systemic fungicide, effective for the control of oomycetes. Resistance to mefenoxam in oomycetes has recently been reported in several countries

including South Africa. However, the genetic mutations responsible for the resistance are unknown. Furthermore, the full genome for *P. citrophthora* has not yet been published which leads to further complications in reliable mutation detection. In this study, 11 *P. citrophthora* and *39 P. nicotianae* isolates with unknown sensitivity to mefenoxam were subjected to fungicide sensitivity evaluation. The isolates were exposed to eight mefenoxam concentrations ranging from 0 to 100 ppm to classify isolates as sensitive, intermediate, or resistant to mefenoxam. Six isolates were sensitive to mefenoxam (relative growth (RG) at 100 pm < 10%), 36 intermediate (RG 10 - 30%) and eight resistant (RG > 30%). Three isolates from each category were selected per species for whole genome sequencing. The full genome sequences from isolates with differing mefenoxam sensitivity levels can be compared to identify the genetic markers responsible for resistance development. Once the mutations are identified, a diagnostic quantitative PCR can be developed to detect these and predict an isolate's sensitivity level accurately. The monitoring of resistance to mefenoxam is vital to fungicide efficacy and its responsible use in citrus production.

P5.4-012

CROSS-RESISTANCE OF CLARIREEDIA JACKSONII TO DMI FUNGICIDES

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Text

Dollar spot is the most common turfgrass disease of the Great Lakes region in North America, and is controlled by repeat fungicide applications every season. There are reports of decreased sensitivity to propiconazole, a demethylation inhibiting (DMI) fungicide, in populations of *Clarireedia jacksonii*, the causal agent. Cross-resistance occurs when an organism shows resistance to more than one fungicide with the same mode of action, and had been reported for *C. jacksonii* and DMI fungicides in earlier studies, but not for more recently introduced DMI fungicides. The purpose of this study was to compare resistance values among 20 isolates of *C. jacksonii* with varying sensitivity to propiconazole (very sensitive to highly resistant to propiconazole) and compare them using correlation analysis. EC_{50} values (effective concentration for 50% inhibition) were generated for each isolate for each fungicide, and these values were subjected to pairwise correlation analysis. Among the 11 fungicides examined in amended agar tests, pairwise correlation values (R) ranged from 0.56 to 0.97 ($p<0.007$). The results suggest that there is significant cross-resistance among these DMI fungicides examined in *C. jacksonii*.

P5.4-013

FUNGICIDE RESISTANCE ACTION COMMITTEE (FRAC) MOA POSTER

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Text
Fungicides have become an integral part of efficient food production. The loss of a fungicide to agriculture through resistance is a problem that affects us all. FRAC works to prolong the effectiveness of fungicides liable to encounter resistance problems and to limit crop losses should resistance occur. Fungicide resistance management strategies must combine the long-term conservation of fungicide effectiveness with relevant use patterns that are sufficient to satisfy the needs of the farmer. Thus to have a chance of success, any strategy must be reached by agreement and depend upon a commitment to implementation from all supply companies involved. Also, it must be understandable and acceptable to the farmer. FRAC provides background information as well as annually updated Fungicide Resistance Management Recommendations for fungicides of the major modes of action. The FRAC Mode of Action (MoA) classification provides growers, advisors, extension staff, consultants and crop protection professionals with a guide to the selection of fungicides for use in an effective and sustainable fungicide resistance management strategy. Here we present usefull information about Fungicide Classification and Resistance Management.

P5.4-014

DEVELOPMENT OF A MICROPLATE ABSORBANCE ASSAY FOR ASSESSING FUNGICIDE SENSITIVITY OF FILAMENTOUS FUNGI AND COMPARISON TO AN AMENDED AGAR ASSAY

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Text
An absorbance assay using 96-well microplates to assess the sensitivity of *Clarireedia jacksonii*, a non-sporulating fungus, to the fungicide propiconazole was developed as an alternative to the labor intensive amended-agar assays. This microplate assay allowed for the assessment of multiple isolates at different fungicide concentrations with many technical replications in a single plate. Among methods tested for inoculating microplate wells, our “microplug” method was the simplest to perform, requiring only a micropipette with 1 ml tips to punch plugs from agar plates. The EC_{50} values for 30 isolates of *C. jacksonii* with varying sensitivities to propiconazole were calculated for the microplate absorbance assay and for a traditional amended-agar assay. A correlation of $\log_{10}$ transformed EC_{50} values of both assays revealed a significant relationship ($R = 0.75$, $p < 0.001$). Additionally, the microplate assay was more sensitive in detecting resistance ($EC_{50} > 0.1$ µg/ml) and revealed five isolates to be resistant to propiconazole that were not found as such with the amended agar assay. Only one of the assessed isolates was observed to have a significantly different EC_{50} value between the two assays that resulted in a different sensitivity classification. These results imply that the microplate absorbance assay as implemented in this study is not exactly equivalent to the traditional amended agar assay for estimating EC_{50} values, but further developments may bring the values closer.

P5.4-015

FUNGICIDE RESISTANCE OCCURRENCE IN THE CUCURBIT POWDERY MILDEW FUNGUS IN THE UNITED STATES

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Text
Fungicides at risk for resistance are critical for effective cucurbit powdery mildew (CPM) control. Podosphaera xanthii isolates were collected from research and commercial plantings each year since 1990 mostly in New York. Their fungicide sensitivity was determined with a leaf disk bioassay. Resistance to FRAC code 11 fungicides, boscalid (FRAC 7), quinoxyfen (13) and cyflufenamid (U6) were detected 3, 6, 8 and 5 years, respectively, after products with these became commercially available for CPM in the USA. Cyflufenamid resistance developed quickly despite label restrictions compelling implementation of a good resistance management program (no consecutive applications allowed and maximum of two applications per crop) and availability of up to three other effective chemistries (FRAC 3, 13, 50) to use in rotation. Resistance to FRAC code 1 and 11 fungicides continues to be very common although these have not been recommended for years. Boscalid resistance is somewhat common. Sensitivity to FRAC 3 chemistry has exhibited little change since 1990s, thus while the pathogen is fully resistant to the first fungicide in this group (triadimefon), fungicides developed subsequently have provided good to excellent control in field efficacy trials. Isolates have been detected with resistance to FRAC 1, 7 (boscalid), 11, 13, and U6 chemistry and with reduced sensitivity to fluopyram (7) and metrafenone (50). Multi-fungicide resistance is a challenge to effectively managing CPM.

P5.4-016

DOES RESISTANCE OF WINTER WHEAT VARIETIES AFFECT THE INFECTION AND SPREAD OF FUNGICIDE-RESISTANT ZYMOSEPTORIA TRITICI MUTANTS?

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Text
Septoria leaf blotch (SLB) caused by *Zymoseptoria tritici* is one of the most important diseases in wheat. Resistant varieties and application of DMI and SDHI fungicides are used to controll SLB. In order to avoid resistance and maintain the effectiveness of fungicides for as long as possible, different groups of active substances should be used alternately and attention should be paid to a diverse cultivation of resistant wheat varieties. However, the extent to which the resistance of wheat varieties influences the infection and spread of fungicide-resistant *Z. tritici* mutants in the pathogen population is still unclear. Here we tested different wheat varieties and estimated their susceptibilities to *Z. tritici* under field conditions. Furthermore, we isolated *Z. tritici* from field samples and re-inoculated isolates with different virulences to a range of wheat varieties growing under glasshouse conditions. Additionally, the effect of different fungicidal agents on *Z. tritici* infection depending on variety resistance was investigated. For this purpose, infestation development was visually recorded over a period of 35 dpi and microtiterassays with DMI (Mefentrifluconazole, Epoxiconazole) and SDHI (Bixafen, Fluopyram) fungicides were performed. Results indicated a high effectivness of Mefentrifluconazole against *Z. tritici*, while Epoxiconazole, Bixafen and Fluopyram showed high variances. Relations between wheat varities and the virulence of the pathogen were rather weak.

P5.4-017

FUNGICIDE SENSITIVITY AND FITNESS PROFILES OF VENTURIA INAEQUALIS: AN INTEGRATED APPROACH TO EFFECTIVE APPLE SCAB CONTROL

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Text

Nowadays chemical control, based on repeated fungicide application, represents the principal means to reduce the severe damages caused by *Venturia inaequalis*, the ascomycete responsible of apple scab. The onset of resistance is a consequence of pathogen adaptation under field conditions. Fungicide resistance is a widespread phenomenon that involves single site classes and should be monitored to predict the shift in pathogen populations sensitivity and prolong the active life of fungicides. The aim of this work was the phenotypic characterization of *V. inaequalis* strains collected at different altimetric areas in Northern Italy orchards for the sensitivity profile to five active substances (dodine, trifloxystrobin, cyprodinil, myclobutanil, boscalid). Fitness traits, like mycelial growth and conidial production (concentration and size), were assessed on each strain. The overall results shown an altitude-dependent spore production and an equal competitivity between resistant and sensitive strains. Some advantages in terms of mycelial growth and conidia production were found for cyprodinil and trifloxystrobin resistant strains, suggesting that the latter could spread and replace the sensitive ones in filed populations. Moreover, the occurrence of multidrug resistant strains along with the lack of fitness penalty underlines the relevance of antiresistance strategy application as a mean to achieve an effective disease control in an ever-changing resistance scenario.

P5.4-018

DOSE RESPONSES OF REDUCED SENSITIVE AND RESISTANT ISOLATES OF PYRENOPHORA TERES F. MACULATA AND P. TERES F. TERES TO DEMETHYLASE INHIBITOR AND SUCCINATE DEHYDROGENASE INHIBITOR FUNGICIDES

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Text

Net- and spot-type net blotches of barley, caused by the related fungal pathogens

Pyrenophora teres f. teres (Ptt) and P. teres f. maculata (Ptm), respectively, are major diseases of barley in many parts of the world. As host resistance to both diseases is moderate at best, fungicides such as demethylase inhibitors (DMI) and succinate dehydrogenase inhibitors (SDHI) are often used to control these diseases. In Australia, in recent years there have been increasing detections of cases of resistance (R) and reduced sensitivity (RS) to both DMI and SDHI fungicides in both pathogens. Practices that promote frequencies of the RS and/or R types in the pathogen populations reduce efficacies of fungicide products. However, frequencies of the R or RS strains at which products become ineffective in the field is unclear. In this presentation we will report on results from field and glasshouse studies on dose-responses of Ptm and Ptt to DMI and SDHI fungicides. Results such as these are key inputs in (economic) models for determination of threshold frequencies, of the R or RS or mixtures of both in the pathogens populations, at which it would be beneficial to adjust treatment programmes when resistance is increasing.

P5.4-019

RESISTANCE OF PHYTOPHTHORA COLOCASIAE TO AZOXYSTROBIN IN FUJIAN, CHINA

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Text

Taro leaf blight caused by Phytophthora colocasiae is the mainly disease in taro production. The quinone outside inhibitor (QoI) azoxystrobin has been uesd to manage this disease. Despite the application of azoxystrobin, the disease still occurs frequently in Fujian, China. In order to explore the reason, the sensitivity of 40 Phytophthora colocasiae strains collected from different cities in Fujian to azoxystrobin were evaluated by mycelial growth inhibition assay. The result showed that 92.5% strains possessed relative growth greater than 80% at discriminatory dose of 10 μg/mL supplemented with salicylhydroxamic acid at 50 μg/mL, while 7.5% strains grew less than 80%. The further analysis of mitochondrial cytochrome b gene showed that two types of strains had only one nucleotide variant, which leading to codon change at 425(GGT to GCT) in the cyt b gene and an amino acid substitution at position 142 (glycine to alanine). The above data indicated high resistance frequency of P. colocasiae to azoxystrobin in Fujian, China. The taro grower should stop use of QoIs in management of taro leaf blight in these fields.

P5.4-020

GEOGRAPY IS THE MAIN FACTOR BEHIND THE DISTRIBUTION OF A DMI-FUNGICIDE RESISTANCE MARKER IN ERYSIPHE NECATOR

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HUNGARY; (3) Food and Wine Research Institute, Eszterházy Károly Catholic University, Eger, HUNGARY

Text

Erysiphe necator, the causal agent of grapevine powdery mildew (GPM) is controlled mostly by sterol demethylation inhibitor (DMI) fungicides, which act by inhibiting eburicol 14α-demethylase (CYP51), a key enzyme in the biosynthesis of ergosterol. The occurrence and spread of the fungicide resistance in GPM populations have been reported; a common marker of DMI resistance is an A to T nucleotide substitution at position 495 (A495T) in the *CYP51* gene. We analysed the effects of (i) wine region, cultivar and DMI fungicide treatment, (ii) season and (iii) year of collection on the probability of the presence of A495T in Hungarian vineyards. More than 2000 field samples were collected from GPM populations in six wine regions, and the presence of A495T was assayed with sequencing of a fragment of the *CYP51* gene or qPCR. Overall, A495T was present in approx. 17 percent of the samples. A495T was detected in all wine regions; its prevalence ranged between 3 and 35%. The occurrence of A495T differed significantly among wine regions and grape cultivars, and sampling years but did not show seasonality. The treatment with DMI-fungicides did not have a significant effect on the presence of the A495T marker. These results suggest that local differences are among the main factors influencing the prevalence of a DMI-resistance marker and this should be considered during fungicide treatments.

This research was supported by the Hungarian Scientific Research Fund (NKFIH OTKA FK142735).

P5.4-021

EFFECT OF CYP51 GENE EXPRESSION LEVELS AND HAPLOTYPE DIVERSITY ON RESISTANCE PHENOTYPES OF FIELD ISOLATES OF CERCOSPORA BETICOLA

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Text

Cercospora leaf spot, caused by *Cercospora beticola*, is one of the most widespread foliar diseases of sugar beet. Over the last few decades there has been a decrease in the sensitivity of *C. beticola* to triazoles. Several mechanisms involved in this sensitivity diminution have been identified such as: i-mutations in the *Cyp51* gene leading to a change in the *α*-14-demethylase structure of the triazole target protein; ii- the overexpression of *Cyp51* gene; iii- the increase in membrane efflux through transporters. The present study has focused on the involvement of the overexpression of the *Cyp51* gene in the different haplotypes of *C. beticola* isolated in France. Quantitative PCR was optimised to study *Cyp51* gene expression across all haplotypes know as our knowledge. *Cyp51* expression was studied in 29 strains (10 haplotypes). Compared wild-type strains, this study showed that strains carrying one or more mutations in the *Cyp51* gene constitutively overexpressed this gene. However, no clear relationship was found between the level of *Cyp51* expression and the level of resistance to triazoles in strains carrying the same substitutions on the *α*-14-demethylase. *Cyp51* expression induced by triazole application was also studied in strains sensitive and resistant to triazoles fungicides. This study provides new insights into the relative importance of constitutive and induced overexpression of the *Cyp51* gene, which

encodes the *α*-14-demethylase, in different haplotypes of *C. beticola*.

P5.4-022

NATURALLY OCCURRING PROPICONAZOLE-TOLERANT FUNGAL ISOLATES IN THE PHYLLOSPHERE OF AGROSTIS STOLONIFERA

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Text
Agrostis stolonifera is commonly used amenity turfgrass and can experience frequent fungicide applications because of intensive management. The frequent use of fungicides has given rise to problems with fungicide resistance in several major turfgrass pathogens. However, there are other organisms in this environment, which may be naturally tolerant of particular fungicides and of which little is known. The purpose of this work was to examine the identity and diversity of these organisms during two growing seasons by sampling asymptomatic leaf blades of *A. stolonifera* from research plots which were only established one or two years before the sampling and had no or very little exposure to fungicides. Leaf samples were obtained from March through October in two years, and were plated on media amended with 1 ug/ml of propiconazole and antibiotics. From among the 21 morphotypes, we were able to identify 19 different species, three of which were yeasts. The five species that showed the greatest representation among the ~2200 isolates were as follows: *Microdochium bolleyii* (23%), *Rhizoctonia solani* (11%), *Papiliotrema flavescens* (9%), *Cryptococcus aspenensis* (9%), and *Mucor nidicola* (8%). Why these species are naturally tolerant of propiconazole remains to be explored, as well as whether the types of genes and mutations that confer this tolerance are the same as the ones that produce acquired resistance in other species.

P5.4-023

DIFFERENTIAL SENSITIVITY TO SINGLE-SITE FUNGICIDES MAY EXPLAIN WHY RAMULARIOPSIS PSEUDOGLYCINES IS THE PREVALENT RAMULARIOPSIS SPECIES ASSOCIATED WITH RAMULARIA LEAF SPOT (RLS) IN COMMERCIAL UPLAND COTTON FIELDS IN BRAZIL

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Text
Ramulariopsis gossypii (Rg) and *R. pseudoglycines* (Rpg) are the etiological agents of Ramularia Leaf Spot (RLS) in Brazil, with Rpg being the prevalent species in commercial

fields. Resistant varieties and chemical control are the most important strategies used to manage RLS. However, the unsatisfactory response to some single-site fungicides suggested adaptation to fungicides currently used. Differential fungicide resistance could also explain the prevalence of Rpg. To address these hypotheses, we used a combination of classical and cutting-edge genomic tools. ITS and RPB2 sequence analysis from 110 *Ramulariopsis* sp. isolates revealed the occurrence of Rpg in 104 samples, and Rg in six samples. Rg was only found in samples from unsprayed breeding plots. Whole genome resequencing (WGS) of 71 isolates indicated that a combination of SNPs and expansion of the number of copies of the *Cyp51* gene (CNV) are associated with increased resistance to triazoles only in Rpg. *β-tubulin* mutations associated with benzimidazole resistance were observed for both Rpg and Rg. The G143A substitution of CytB associated with strobilurin resistance was also only observed in Rpg. The resistance genes currently been used in breeding programs aiming develop RLS resistant cultivars are not effective against Rg. The findings reported here highlight the importance of the integrated used of chemical control and resistant varieties to maintain the durability of the main sources of RLS resistance.

One health: impact of resistance to antibiotics and fungicides in plant pathogens

C7.4-1

MEDICAL IMPLICATIONS OF AZOLE FUNGICIDE USE ON ASPERGILLUS FUMIGATUS: A ONE HEALTH CHALLENGE REQUIRING A MULTIDISCIPLINARY APPROACH

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Text

Aspergillus fumigatus is a saprobic fungus that causes a range of diseases in humans, including invasive aspergillosis. Triazoles represent that main treatment option for *Aspergillus* diseases, with very limited alternative treatment options. Resistance to azoles has emerged globally, which was shown to be mainly driven by environmental exposure of *A. fumigatus* to azole fungicides. Azole resistance causes important challenges in the management of invasive aspergillosis, including resistance diagnosis and treatment. Clinical studies have shown a 25% higher mortality rate in patients with azole-resistant invasive aspergillosis compared to azole-susceptible infection. Studies in the (agricultural) environment have shown that stockpiling of plant waste material and the presence of azole fungicide residues drives azole resistance selection in *A. fumigatus*. Such hotspots have been identified in flower bulb waste, green waste and wood chippings. Current studies are directed towards monitoring interventions aimed at reducing the acculumlation of (resistant) spores in the environment and tracking transmission routes from the environment to the patient. Dual use of antifungal drugs represents a risk to human health and may require

authorization procedures to include activity against non-target human fungal pathogens as part of the risk assessment. This is relevant given the development of new fungicides and antifungals drugs with the same mode of action, such as the DHODH-inhibitors.

C7.4-2

SENSITIVITY TO AZOLE FUNGICIDES IN NORDIC POPULATIONS OF PARASTAGONOSPORA NODORUM CAUSING STAGONOSPORA NODORUM BLOTCH OF WHEAT AND ASPERGILLUS FUMIGATUS CAUSING INVASIVE ASPERGILLOSIS IN HUMANS

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Text

Fungicide resistance is an increasing challenge to global food production and human health. *Zymoseptoria tritici*, a major leaf pathogen on wheat, has developed resistance to azoles through mutations in the cyp51 gene and is increasingly difficult to manage. Development of resistance to antifungal azoles in *Aspergillus fumigatus* leaves health professionals with few alternatives for effective treatment. In the national funded project 'Navazol- navigating the threats of azole resistance development in human, plant and animal pathogens in Norway', we isolated *Parastagonospora nodorum*, the dominating leaf pathogen in Norwegian wheat and *Aspergillus fumigatus* from agricultural and landscape samples. The fungal isolates were tested on 6-8 different concentrations of Proline, a prothioconazole containing fungicide, to determine the concentration that reduced fungal growth by 50% (EC_{50}). An R script was developed to facilitate computation of the EC_{50} value based on the log-logistic function. Testing 50 *P. nodorum* and *A. fumigatus* isolates showed low prevalence of fungicide resistance (EC_{50} >10ppm) under Norwegian conditions. These results suggested that disease management in Norwegian field crops have not increased the risk of fungicide resistance in either plant, nor human pathogens. However, as crop production adapts to global changes, monitoring of azole resistance and optimizing demand-guided fungicide input are essential to maintain effective plant and human health management.

C7.4-3

THE HUNT FOR KILLER ASPERGILLUS FUMIGATUS IN THE ENVIRONMENT - SURVEILLANCE OF TOMATO AND CORN FIELDS IN OHIO

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Text

Azole resistance in clinical isolates of *Aspergillus fumigatus* has been linked to the intensive use of azole fungicides in the environment. Although *A. fumigatus* is not a phytopathogen,

agricultural azole fungicides have activity against it and resistance can develop through off-target contact with A. fumigatus. Soil and leaves from 16 tomato fields and residue from 15 corn fields in Ohio were collected across four 6 m transects. Most (68%) of the fields received at least one application of a triazole fungicide during the growing season. *Aspergillus* spp. were isolated and screened for resistance to three medical triazoles–itraconazole, posaconazole, and voriconazole in accordance with European Commission on Antimicrobial Susceptibility Testing (EUCAST) guidelines. A total of 49, 46, and 58 putative *A. fumigatus* isolates were recovered from soil, tomato leaves and corn residue, respectively. No resistant isolates were recovered from the tomato fields. Two isolates from corn, one from a field that was not treated with a triazole fungicide (MLI996-21) and one from a field treated with a triazole fungicide (MLI1004-21), exhibited growth on RPMI-agar amended with clinical breakpoint concentrations of itraconazole. On broth microdilution MIC testing, MLI1004-21 revealed reduced sensitivity to itraconazole. These results suggest that corn and tomato crops as produced in Ohio are not hotspots for medical triazole-resistant A. fumigatus, although further investigation is warranted.

C7.4-4

ELUCIDATING THE IMPACTS OF ANTHROPOGENIC ACTIVITIES ON BENEFICIAL AND PATHOGENIC PLANT MICROBE INTERACTIONS

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Text

Plant microbiomes are increasingly exposed to anthropogenic activities. The growth of multi-resistant bacteria and emerging plant and human pathogens are acknowledged as a global concern. We suggest that different human activities are commonly linked to decrease of plant microbial diversity that likely disturb the plant microbe interaction and lead to the disease outbreak. By combining a multi-omics approach, we extensively investigated the impact of anthropogenic activities i.e., management practices and use of antibiotics on plant associated bacterial communities. We also used a lichen, a model of native microbiota, to obtain mechanistic insights into the effects of antimicrobial exposure on natural microbiota. We observed that putative beneficial bacteria are among the most sensitive responders to the anthropogenic activities. The overall bacterial community structures were altered by the anthropogenic activities including the use of antimicrobial compounds, which resulted in a relative increase of low abundant taxa with r-strategy and potential traits as emerging pathogen. Moreover, using shotgun metagenome and RNA sequencing, we revealed different strategies of these bacterial groups to overcome antimicrobial exposure i.e., regulating influx and efflux systems to limit uptake of antimicrobial substances. Our study provides indications that beneficial and pathogenic plant microbial interaction are compromised when the plant host is exposed to anthropogenic activities.

C7.4-5

USE OF ANTIBIOTICS TO CONTROL PLANT PATHOGENIC BACTERIA: GENETIC AND GENOMIC CONSIDERATIONS

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Text

Plant pathogenic bacteria (PPB) cause devastating losses of crops, e.g. in the wine industry (*Xylella fastidiosa*) or pear orchards (*Erwinia amylovora*). On a worldwide scale, farmers resort to antibiotics to control these diseases, despite the threatening impact of antibiotic resistance genes (ARGs) on human and animal health. There is an urgent need to evaluate the current use of antibiotics in plant agriculture as ARGs have already emerged in PPB. There is a need to investigate the contribution of mobile genetic elements to the spread of antibiotic resistance among PPB and other bacteria.
Streptomycin is the most widely used antibiotic on plants. Tn*5393* and its variants are the main streptomycin resistance vectors in PPB. They are also found in *Salmonella enterica* and *Klebsiella pneumoniae*, two major human pathogens. Comparing the genetic organization of the Tn*5393* variants shows that the insertion of IS elements and other transposons have resulted in more complex entities, potentially carrying other ARGs. In PPB, Tn*5393* structures are relatively simple, but in human or animal pathogens, variants of the transposon show the potential complexity that could be attained in PPB within a few years, with the formation of expanded transposons carrying several ARGs.
Our study highlights that, besides understanding the potential transfers between bacteria, there is a crucial need for more detailed information on the type and quantities of antibiotics used as plant protection products.

P7.4-001

DMI SENSITIVITY OF BASELINE AND FUNGICIDES EXPOSED ISOLATES OF ALTERNARIA ALTERNATA FROM TANGERINES

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Text

Alternaria brown spot, caused by Alternaria alternata, is the main fungal disease that affects tangerines and their hybrids. In Brazil, the disease is mainly controlled by the use of fungicides from the group of Quinone outside inhibitor (QoI) in mixture with 14α-Demethylation inhibitor (DMI), in addition to copper fungicides. The inefficiency of QoI fungicides in controlling the disease in tangerine orchards at the State of São Paulo was reported in 2017 and in 2021, the resistance of A. alternata isolates to QoI has been proven. Given the scenario of loss of sensitivity to QoI fungicides this study aimed to evaluate the sensitivity of A. alternata to fungicides from DMI group. To achieve this goal, the evaluation of A. alternata sensitivity to DMI fungicides was performed by determining the effective concentration of tebuconazole and difenoconazole to inhibit 50% (EC_{50}) of mycelia development. The EC_{50} of tebuconazole and difenoconale were determined, respectively, for 170 and 103 isolates of A. alternata, by the Spiral Plater technique. A. alternata isolates were collected from tangerine commercial fields in 2003 (baseline), 2017, 2018 and 2021. All

baseline isolates were sensitive to both fungicides (EC_{50}<1.0 µg.ml^{-1}). From 2017 to 2021 only 17 % and 1% of A. alternata isolates showed reduced sensitivity to tebuconazole and difenoconazole, respectively. A shift in sensitivity of A. Alternaria collected from 2003 to 2021 was noticed only for tebuconazole.

P7.4-002

TEMPERATURE AND HOST-DEPENDENT FUNGICIDE EFFICACY

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Text

Fungicides are one of the most important strategies for the controlling plant diseases. Research on fungicides has focused on their biochemical properties and strategies to mitigate pathogen resistance. The influence of ecological factors on the efficacy has been largely ignored. We investigated the effects of temperature, host diversity, and host resistance on the efficacy of fungicides. Fungicide efficacy was measured in vitro by calculating the mycelial growth rate of the pathogen in the presence of fungicides relative to that without fungicides or by calculating EC50 in three independent experiments each with 150-300 *Phytophthora infestans* genotypes. In Experiment 1, the pathogen populations from nine locations were exposed to five temperatures (13 ?, 15 ?, 19 ?, 22 ? and 25 ?). In Experiment 2 and 3, pathogen populations from potato populations differing in genetic diversity or quantitative resistance were tested for their tolerance to two fungicides of different action modes. Fungicide efficacy was found to respond quadratically to experimental temperatures, with the lowest efficacy at the optimal growth temperature of the pathogen. Fungicide efficacy was also found to be positively correlated to host diversity but negatively correlated to quantitative host resistance and these association patterns were independent of fungicide property. These results suggest that fungicide doses should be adjusted according to air temperature and host genetics during field practice.

P7.4-003

EFFECTS OF FUNGICIDE AND HOST RESISTANCE ON CONTROL OF LEPTOSPHAERIA MACULANS AND L. BIGLOBOSA

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Text

Phoma stem canker is a damaging disease of oilseed rape (Brassica napus), caused by two related fungal pathogens Leptosphaeria maculans (Lm) and L. biglobosa (Lb). Control of severe UK phoma stem canker epidemics relies on use of fungicides. To investigate effects

of different fungicides on control of Lm and Lb, field experiments with different cultivars and in vitro experiments with different isolates were done. Results from field experiments with six cultivars over three cropping seasons showed that fungicides Proline (prothioconazole) and Refinzar (penthiopyrad+picoxystrobin) reduced phoma stem canker severity caused by Lm and Lb measured by amounts of Lm and Lb DNA in stem tissues but there were variations between different cultivars or different seasons. Ten Lm and ten Lb isolates were tested for sensitivity to three fungicides (prothioconazole-desthio, picoxystrobin and penthiopyrad) in-vitro. All fungicides were effective in control of Lm and Lb growth, with prothioconazole-desthio being the most effective. Lb isolates were less sensitive to prothioconazole-desthio than Lm isolates. To investigate differences between Lm and Lb in fungicide sensitivity mechanisms, five Lm and five Lb isolates with different levels of fungicide sensitivity were inoculated onto cultivar Topas and leaves were sampled at 7 and 12 days post inoculation for gene expression analysis. Results showed different levels of expression of LmCYP51 and LbCYP51 genes among the Lm and Lb isolates.

P7.4-004

IN VITRO SENSITIVITY TO SOME FUNGICIDES OF MONILINIA LAXA

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Text

Brown rot caused by *Monilinia laxa*, *M. fructigena*, and *M. fructicola* causes severe losses in important commercial crops such as peach and nectarine, apricot, plum, sweet and sour cherry, almond, apple, and pear, with a significant economic impact. *Monilinia laxa* is the most common species in Türkiye. Fungicides are frequently used to control this pathogen. Resistance to fungicides, which provide effective and rapid pathogen control, is currently a major issue. In this study, the resistance of 35 isolates of *M. laxa* to captan, cyprodinil, and thiophanate methyl fungicides was evaluated under *in vitro* conditions. Significant differences were found in the EC_{50} values of fungicides among fungal isolates. According to the results of the petri dish assay, the isolates with the highest EC_{50} values were KOKI1 (32451,2 ppm) in captan, ISKA20 (1,980 ppm) in cyprodinil, and ISKA32 (4,142 ppm) in thiophanate methyl. It is required to develop integrated control programs that can be used for the pathogen's management.

The work was supported by the Ministry of Agriculture and Forestry, the General Directorate of Agricultural Research and Policies (TAGEM), Project No. TAGEM/BSAD/A/21/A2/P1/2716.

P7.4-005

DETERMINATION OF IN VITRO ANTIFUNGAL EFFICIENCIES OF SOME FUNGICIDES AGAINST THE DIPLODIA BULGARICA AS A NEW PATHOGEN OF APPLE IN TÜRKIYE

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Text

The apple (*Malus domestica*) is one of the most important commercially grown fruit crops in the world. In Türkiye, apples are an economically important horticultural crop more than 4,300,000 t of apples are produced every year, making the country the third largest producer worldwide. Members of the family Botryosphaeriaceae can cause canker, dieback, gummosis, fruit rot, and twig blight diseases in numerous woody hosts. Recently, a botryosphaeriaceous species (*Diplodia bulgarica*) associated with canker and fruit rot symptoms in apples was reported in the Egirdir district of Isparta province, Türkiye. The aim of this study is to determine the sensitivity of *D. bulgarica* to some fungicides. Nine fungicides (captan, chlorothalonil, dodine, fluazinam, fosetyl-al, mancozeb, tebuconazole, thiophonate methyl, and trifloxystrobin) were tested in vitro for their antifungal effects against the pathogen at different concentrations. Probit analysis was used to determine the mean effective concentration (EC_{50}) values. The most effective fungicides were fluazinam, tebuconazole, and thiophonate methyl, for which EC_{50} values were <1.0 mg/L.

P7.4-006

ANTIBIOTIC RESISTANCE OF KLEBSIELLA SPP. ISOLATED FROM AGRICULTURAL PRODUCTS AND AGRICULTURAL ENVIRONMENTS IN KOREA

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Text

A new concept called One Health, which unites the health of humans, animals and the environment, is being used to control the upcoming epidemic. Plant health is also important in terms of providing healthy food for humans. Although various agricultural antibiotics are used to control bacterial diseases in crops, antibiotic-resistant bacteria in agriculture have hardly been investigated. This study was conducted to provide basic data for antibiotic resistance research through the investigation of antibiotic resistant bacteria in agricultural products and agricultural environments in Korea. *Klebsiella* spp., including *Klebsiella pneumoniae*, were isolated from lettuce and pepper, agricultural water, soil and compost using selective media. A total of 56 *Klebsiella* spp. was isolated, especially these bacteria were isolated in high proportions from soil and agricultural water. As a result of the resistance test of the isolates against 16 antibiotics including ampicillin, 78% of the strains showed resistance to one or more antibiotics. Among the antibiotics, the ratio of resistant bacteria to cefoxitin and ampicillin was the highest at 52% and 27%, while showing sensitivity to all 9 antibiotics including gentamicin. Investigation of antibiotic resistance to various harmful microorganisms such as *Escherichia coli* is also in progress, and continuous data accumulation will provide the basis for various antibiotic resistance studies in the future.

P7.4-007

OCCURRENCE OF ANTIBIOTICS RESISTANT PSEUDOMONAS SPP. ISOLATED FROM VEGETABLES AND AGRICULTURAL ENVIRONMENTS IN SOUTH KOREA

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Text
The purpose of this study was to develop safe agricultural production technology by controlling agricultural environmental factors in relation to antibiotic-resistant bacteria in humans. In this study, prevalence and antibiotic resistance of *Pseudomonas* spp. were investigated in 137 different samples collected from lettuce (n = 12), garlic chives (n = 14), soil (n = 26), compost (n = 20), and irrigation water (n = 26) in 26 farms between 2021 and 2022 in the Republic of Korea. Species of Pseudomonas were prevalently isolated from samples as follows; *P. aeruginosa* 36.5% (n = 50), *P. putida* 14.6% (n = 20), *P. plecoglossicida* 11.7% (n = 16), *P. guariconensis* 9.5% (n = 13), *P. taiwanensis* 7.3% (n = 10), and Pseudomonas other species 20.4% (n = 28). The most isolates of *Pseudomonas* spp. showed high susceptibility to gentamicin (97.8%), amikacin (96.3%), and ceftazidime (89.7%), but some isolates showed resistance against to antibiotics such as imipenem (8.2%), ciprofloxacin (6.5%) and colistin (5.1%). Among them, 15 isolates of *P. aeruginosa* resistant to three antibiotics were from irrigation water 9, soil 2, garlic chives 2, and lettuce 2. In the future, more samples will be analyzed to track the source of antibiotic resistance in agricultural environments, and a systematic examination of how to acquire antibiotic resistance should be carried out.

Particle based delivery of biomolecules for crop protection

C6.4-1

BIOCLAY – DELIVERING RNA SPRAYS TO CROPS USING CLAY PARTICLES FOR SUSTAINABLE CROP PROTECTION

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Text
Innovation driving sustainable crop protection resonating with health of the planet and the

future consumer is key to food and nutritional security. The ongoing usefulness of chemical pesticides suffers from issues such as residual toxicity, run-off, pest specificity and resistance. RNA based biopesticides or 'RNA sprays' for plants as a next generation crop protection platform without the need for genetic modification is gaining momentum globally. Series of papers have shown that exogenous application of doble stranded RNA (dsRNA) can induce RNAi-mediated protection against pests and pathogens. However, the instability of topically applied naked dsRNA on the leaf surface is a major limitation. The BioClayTM technology to deliver dsRNA as biological active using clay particles (Layered double hydroxide) as carriers presents a non-GM, residue free, specific, and environmentally sustainable alternative to chemical pesticides. RNAi effectors delivered as BioClay are stable, do not get washed off and provide protection to the sprayed and unsprayed leaves of a wide range of host plants against the targeted pest or pathogen. BioClay platform is being progressed to target viruses, insect pests such as white flies and fungi including Botrytis, Verticillium, Fusarium and Phytophthora. Real world application of RNA based biopesticides with sustainable credentials will be governed by factors such as cost-effective production of dsRNA, the regulatory landscape and public licensing.

C6.4-2

USING NANOCARRIERS FOR TARGETED RNAI THERAPY IN CONTROLLING PLANT PATHOGENS

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Text

Plant diseases cause significant economic losses in agriculture worldwide. Traditional methods of disease control, such as chemical pesticides, have been found, in many cases, to be ineffective and harmful to the environment. Therefore, strategies to sustainability control plant diseases are much needed and nanotechnology is gaining attention as a potential solution for such problems. Nanocarriers are small particles that can be engineered to target specific organelles in plants and deliver biocargos, such as RNA interference (RNAi) precursors (siRNAs and dsRNAs), to plant cells. These particles offer a more targeted and effective way to control plant pathogens compared to traditional methods. Our research focuses on designing and optimizing these nanocarriers for efficient delivery of RNAi precursors to plant cells. We have synthesized and characterized different nanocarriers and evaluated their ability to protect and deliver nucleic acid to plant cells. Our results show that the protein nanocarriers have high stability, are able to protect the genetic material from degradation, and efficiently deliver it to the target cells. Moving forward, our studies will focus on optimizing the design of these nanocarriers for practical applications.

C6.4-3

CELLULOSE-BASED NANOPARTICLES TO DELIVER ACTIVE NATURAL COMPOUNDS: A SUCCESSFUL CASE IN THE MANAGEMENT OF THE TOMATO BACTERIAL SPECK DISEASE

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Text

Nanotechnology could represent an innovative way to control plant pathogens, providing alternative solutions to the pesticides traditionally employed to manage plant diseases. In this work a novel nanoparticles-based formulation (NPF) made of cellulose nanocrystals as carriers, high-amylose starch as excipient, and chitosan and gallic acid as active principles, was evaluated to control the worldwide distributed pathogen *Pseudomonas syringae* pv. *tomato* (Pst), the causal agent of the tomato bacterial speck disease. We firstly described the antimicrobial properties of cellulose nanocrystals alone, which displayed the capability of reducing bacterial flagellar motility, inducing cell aggregation and delaying biofilm production by preventing surface adhesion. In combination with chitosan and gallic acid, NPF at 2% was able to inhibit *in vitro* cell multiplication up to 80%. The application of the NPF on host plants reduced the disease severity compared to copper hydroxide as much as reduced the spread of Pst, which was evaluated by Real-Time qPCR. This work provides valuable characterizations of the several mode of actions of a novel bio-based NPF, which could be potentially useful to control Pst even in organic agriculture regime.

C6.4-4

THE USE OF ARTIFICIAL NANOVESICLES FOR DSRNA DELIVERY IN SPRAY-INDUCED GENE SILENCING (SIGS) FOR CROP PROTECTION

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Text

Fungal pathogens are a major threat to global food security, and current management strategies are limited to mainly chemical control, which is harmful to human health and the environment. Therefore, innovative, eco-friendly, strategies for combating fungal pathogens must be developed. Recent advances, in which our research group has been pioneer, have shown that Spray-Induced Gene Silencing (SIGS) via topical application of pathogen gene-

targeting RNAs can inhibit plant diseases caused by fungal pathogens that can effectively take up RNAs from the environment. These antifungal RNAs can be versatility designed to be species-specific and to target multiple genes simultaneously. Though promising, a major drawback to SIGS approaches is the instability of RNA in the environment, which can be rapidly degraded when exposed to rainfall, high humidity, and UV light. Previously, we discovered that plant hosts utilize extracellular vesicles to protect and deliver small RNAs into fungal cells to suppress fungal virulence, so inspired by this naturally occurring pathway, we examined three artificial nanovesicle formulations to shield pathogen gene targeting double-stranded RNAs from degradation for topical application to control the fungal pathogen, Botrytis cinerea. All three formulations are effective in both RNA delivery and protection, and greatly extend the length of RNA-mediated plant protection.

C6.4-5

SYNTHETIC LIPID NANOPARTICLES: FABRICATION AND USE FOR PLANT DISEASE PROTECTION

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Text
Many plant diseases and particularly those caused by bacteria lack effective treatment to fend off pathogens and minimize yield losses. We have previously shown that membrane vesicles of Gram-negative bacteria prime the plant immune system and protect it from subsequent infection. This knowledge was exploited to develop synthetic lipid nanoparticles (LNPs) as a delivery vehicle of biomolecules to induce disease resistance in plants. Fluorescently labelled LNPs with different lipid composition were synthesized and applied to plant to determine uptake, distribution and persistence. Plant uptake was more effective when LNPs were applied through the root system compared with leaf applications. From the roots, LNPs were transported to the stem and leaves preferentially via the vascular system, and were detectable at least 25 days post-application. To examine whether LNPs can activate plant immunity, we rationally designed LNPs coated with the plant immune elicitor flg22 (LNP-flg) and used them to treat Arabidopsis plants. LNP-flg induced a robust upregulation of immune gene markers in seedlings and elicited a reactive oxygen species burst in disc leaves. Further, plants pretreated with LNP-flg promote a significant reduction in bacterial growth in infected as well as in distant leaves. This priming effect was evident up to a week after LNP application. In summary, our results indicate that rationally designed LNPs may represent a promising alternative to mitigate plant diseases.

C6.4-6

POTENTIAL USE OF CHITOSAN-BASED NANOPARTICLES IN CROP PROTECTION

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Text

The last decades, increased food production demand along with climate change is expected to make crops more disease and stress vulnerable. In addition, pesticide use reduction in the frame of Green Deal necessitates the need for development of new generation plant protection products. In this challenging era, the role of nanotechnology in crop protection needs to be considered. In this study, chitosan nanoparticles (CNPs) and CNPs with enclosed Cu^{+2}, Gibberellic acid (GA3) or Salicylic acid (SA) were produced and tested for phytotoxicity and defense stimulating efficiency on *A. thaliana* Col-0 control plants and *npr1*, *hsp90.2* and *RNAiHsp90.1* mutant lines by examining ROS production through NBT and DAB staining. In the absence of disease and after artificial inoculation with *P. xanthii* conidia, it was shown that various SA-CNPs formulations induced *PR1* resistance marker gene at 5ppm and reduced disease severity on leaves of *PR1prom::GUS* transgenic plants. Additionally, CNPs were tested on a commercial courgette variety for efficacy against *P. xanthi*i in relation to their particle sizes and concentrations. However, confocal microscopy on leaves and protoplasts of *N. benthamiana* and *A. thaliana* after FITC-CNPs treatment, revealed a time- and size-dependent CNP movement in cell membranes. Therefore, CNP potential use should be balanced between effectiveness and safety.

P6.4-001

EFFECT OF DIFFERENT CONCENTRATION OF SIO2, TIO2 AND ZNO2 NANOPARTICLES ON GERMINATION PERCENTAGE, VIGOUR AND SEED HEALTH PARAMETERS OF CHICKPEA SEEDS

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Text

Explosive demand of higher agricultural productivity to feed the increasing population demands innovative technologies to improve the seed quality in order to harness the full potential of genetic resource and other agronomic inputs. The use of nano-based technology for seed treatment has a potential to upgrade the seed quality with minimalistic exposure to chemicals making it ecologically and economically sustainable. The present study is an attempt to assess the prospects of nanotechnology as an alternative approach to improve the seed quality. The seeds of chickpea variety Pusa-547 were treated using zinc, silicon, and titanium oxides in various combinations (viz., 50, 100, 250, 500 and 750 ppm). The observations for various seed quality parameters were recorded to find out the best treatment that could improve the physiological and biochemical attributes of seed. Amongst the various treatments given to the seeds of the chickpea variety Pusa-547, the treatment Dry Bulk ZnO@ 500 ppm recorded significantly the highest values for most of the quality parameters and thus it can be used to enhance the seed quality.

P6.4-002

THE EFFECT OF SODIUM CHLORIDE AQUEOUS SOLUTION ON THE GROWTH AND DEVELOPMENT OF SUNFLOWER PLANT (HELIANTHUS ANNUUS L.) AFTER THE SEED GERMINATION STAGE INSIDE THE GREENHOUSE.

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Text

The response of sunflower plant (Helianthus annuus L.) after early germination to sodium chloride was investigated at the stages that come after germination and early seedling growth. Sunflower plant were treated with a series of five different concentrations of sodium chloride aqueous solution 0, 50, 75, 100 and 200 mM and tap water for control and were allowed to grow under cultivation conditions in special pots at the rate of one plant per pot. The results showed that the highest concentration of salinity (200 mmol NaCl) led to a decrease in the growth rate and growth rate of sunflower plant. Fresh and dry masses of sunflower plant were significantly reduced at 0-200 mM NaCl aqueous solution. A decrease in stem growth and length, in addition to its diameter, was observed with increasing NaCl concentration. The results also show that up to 75 mM NaCl, all growth indicators and fresh and dry biomass were moderately salinity tolerant. Thus, the present study concluded that sunflower plant can be grown in brackish soil.
Key-words: Sunflower plant, NaCl, Aqueous solution, Soil, Greenhouse conditions

P6.4-003

COST-EFFECTIVE RNAI BIOPESTICIDE FOR POTATO VIRUS MANAGEMENT

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Text

RNA interference (RNAi) applications are one of the sustainable possibilities for crop health management that could be used to replace other chemically based alternatives. One of the most relevant to farming applications in the case of crop treatment is spray-induced gene silencing (SIGS). Potato Virus Y (PVY) is the most widespread and damaging virus in potato production across the world. We have produced dsRNA fragments of conserved regions that cover the whole viral genome to be sprayed in gene silencing experiments. The experiment was designed to generate the dsRNA under a newly designed bacterial expression system developed based on Golden Gate assembly. The dsRNA fragment was applied to Nicotiana tabacum plants to test its ability to inhibit viral infection by topical spraying application. By

using qPCR, frequent samples were taken and tested to calculate the silencing percentage. In addition, symptom development was recorded in three levels mild, moderate & severe.

P6.4-004

A NOVEL PHYTO-FUMIGANT VOLATILE FORMULATION UNVEILS THE SUPPRESSIVE NATURE OF DAMPING OFF AND FUSARIAL WILT PATHOGENS IN TOMATO

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Text

Soil-borne fungal diseases are a major threat to vegetable crops that severely affects plant growth and development. The present study aimed to evaluate Plant/microbial volatile organic compounds (p/mVOCs) against *P. aphanidermatum (Pa)* and *F. oxysporum f. sp. lycopersici* (FOL).
Among them, the volatilomes of *M. spicata (Ms)* and *C. citratus* (Cc) showed the maximum inhibitory effect on mycelial growth of the target pathogens. The volatilomes of *T. asperellum* (Ta) showed the maximum inhibitory effect on Pa and FOL. To identify the nature of VOCs involved in the suppression of pathogens, the volatilomes of *Ms* and *Cc* leaves were subjected to HS GCMS, and the volatilomes of *Ta* were subjected to GC-MS-ATD. The results revealed the production of carvone by *Ms*; citronellol by *Cc*; isopentyl alcohol and limonene by *Ta* with increased peak area percentage. To develop a new phytofumigant formulation, the effective volatilomes were immobilized separately in a vermiculite bound with castor oil as the vermiculite ball formulation. Studies on defense gene expression revealed that PR1 and LOX were highly expressed after 48h of exposure to the *Ms* vermiculite balls against Pa in tomato plants. Similarly, tomato plants inoculated with FOL also revealed increased expressions of PR1 after 72h and WRKY after 48h on exposure to the *M. spicata* balls. The research concluded that p/mVOCs at low concentrations positively affect plant growth promotion and induce systemic resistance against plant pathogens.

P6.4-005

DEVELOPMENT OF A REPRODUCIBLE SYSTEM FOR ASSESSMENT OF BOTRYTIS BUNCH ROT DISEASE IN GRAPEVINES AND THE IMPACT OF BIOCLAY™ RNAI TECHNOLOGY ON DISEASE INCIDENCE.

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Text
The fungal pathogen *Botrytis cinerea* causes the grapevine diseases botrytis bunch rot (BBR) and grey mould. BBR outbreaks in vineyards can cause substantial economic loss from a reduction in yield and a downgrade in fruit quality. We have developed a consensus

flower *B. cinerea* inoculation protocol for inducing and assessing infection throughout berry development and veraison. Further, we have used this methodology to assess impact of the novel botryticide BioClay™ on BBR. BioClay™ utilises double-stranded RNA (dsRNA) to trigger RNAi silencing of targeted virulence-related pathogen genes, with the stability and durability of the dsRNA improved through combination with clay particles. We report on the effectiveness of BioClay™ against *B. cinerea* infection in Riesling grapevines and discuss adaptability of the system for testing other crop protectants in grapevine.

P6.4-006

CHALCONES, A NEW ALTERNATIVE OF BIOCONTROL AGAINST THE ROOT ROT CAUSED BY PHYTOPHTHORA CINNAMOMI

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Text

The development of new ecofriendly methods to control Phytophthora cinnamomi (Pc) root rot disease that is threatening the survival of oak woodlands in the Mediterranean Basin is essential. An alternative to standard fungicides is chalcones (1,3-diphenyl1-2-proper-1-one), aromatic compounds naturally produced by plants and synthesizable. The main goal of this work was to determine the biocide activity against Pc of two chalcones, a dihydrochalcone (DC) and a thiolchalcone (TC). Their effect, individually and combined, were evaluated at three doses (10-6, 10-5 and 10-4 M), on the mycelial growth of the pathogen by in vitro experiments. DC and TC separately and in combination at the highest dose (10-4 M) reached a mycelial growth significantly lower than the untreated controls, reaching percentages of mycelial growth inhibition higher than 65% with both chalcones combined. Based on these results, the commercial product Nemanol (Telluris Biotech India) composed by DC and TC at 10-4 M was chosen to be tested against Pc survival and root disease development by in planta experiments. A significant reduction of the viable inoculum density was recorded in soil treated with Nemanol before and after soil infestation. In addition, Lupinus luteus seedlings growing in soil treated before soil infestation showed a severity of root symptoms significantly lower than the plants that grew in untreated and infested soil. Our results support the efficacy of chalcones to control Pc root disease.

P6.4-007

ALLEVIATION OF BIOTIC AND ABIOTIC STRESS ON PLANTS BY TREATMENT OF A CYCLIC DIPEPTIDE(L-PROLINE-L-GLYCINE) FROM BACILLUS VELEZENSIS BS07M

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Text

The strain, Bacillus velezensis BS07 has been reported to induce multiple spectrums of systemic resistance and growth promotion in various crops. the strain was compared with the representative strains of B. velezensis BS07M by comparing 16s rRNA sequence analysis and utilization of carbon sources such as L-arabinose, D-Xylose, Mannose, and Trehalose with B. amyloliquefaciens DSM7, B. siamensis KCTC13613, and B. velezensis LMG22478. Cyclic dipeptide, cyclo(L -Pro -L -Gly) was purified by various column chromatography from culture filtrates of BS07M. The compound was identified as cyclo(L -Pro-L -Gly) based on the analysis of NMR spectra. The compound induced systemic resistance in the cucumber plant against C. orbiculare. Infiltration or spray of the compound at a low concentration (0.1 ppm) induced systemic resistance against P. capsici, P. carotovorum, etc., and involved in defense gene activation. We investigated the BS07M combination with cyclic dipeptides on tobacco or red-pepper seedlings for priming; this caused a significant yield increase and soft rot disease suppression as well. The ISR mechanism was supported by enhanced expression of defense-related genes on chili-pepper after pathogen challenge by using the RT-PCR technique. These results suggest that a bacterial metabolite, cyclo dipeptides, and bacterial cell combination is a great potential for disease suppression and yield increase in various crops.

P6.4-009

TOPICAL APPLICATION OF RNA INTERFERENCE TO IMPROVE GRAIN CROP HEALTH AGAINST THE FUNGAL PATHOGEN FUSARIUM GRAMINEARUM

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Text

Fusarium Head Blight (FHB) is a fungal disease affecting grain crops caused by Fusarium graminearum. It can lead to yield loss and produce mycotoxins during infection, which is harmful to humans and animals. Chemicals such as Demethylation Inhibitors are used to reduce the incidence of FHB. However, its overuse can cause potential harm to soil-beneficial microorganisms and lead to fungicide resistance. RNA interference (RNAi) is a gene-silencing mechanism that has emerged as a promising tool to overcome pests and diseases. We are investigating its potential as an exogenous application for treating FHB in wheat and barley. Confocal microscopy confirmed environmental uptake of exogenous dsRNA within the germ tubes, with variable uptake efficiency. With these results, an in vitro method for screening potential target genes using target-specific dsRNA was developed to investigate fungal inhibition. In planta seedling assays using the Fusarium graminearum – wheat/barley pathosystems were also tested by spray application of dsRNA. The in vitro assays also showed some inhibition of the fungi, indicating the potential to upscale. Whereas the in planta assays showed significant inhibition in barley but surprisingly poor inhibitory effects in wheat, possibly due to low uptake efficiency. The findings suggest high potential in using target-specific dsRNA as a biocontrol against F. graminearum, but more research is required to test

its potential against FHB in mature plants.

P6.4-010

A NOVEL NANOPARTICLE-BASED FORMULATION FOR THE MANAGEMENT OF KIWIFRUIT BACTERIAL CANKER AND OLIVE KNOT WHILE BOOSTING THE HOSTS INNATE IMMUNITY

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Text

Pseudomonas syringae pv. *actinidiae* (Psa) and *P. savastanoi* pv. *savastanoi* (Psav) are impacting bacterial pathogens and are managed by following good agornomical practices and by the preventive application of cupric salts. There is an urgent need to find efficient and bio-based solutions to mitigate bacterial diseases. We formulated a novel nanoparticle-based formulation (NPF) composed of cellulose nanocrystals and high-amylose starch as carriers and excipient, while chitosan and gallic acid were included as active antibacterial. The NPF was tested *in vitro* and *in vivo* at 2% against Psa and Psav. Antibacterial assays demonstrated that the NPF inhibited the cell multiplication from 50% to 80%. In-broth assays demonstrated that the NPF drastically reduced the ability of the *Pseudomonas* spp. to produce biofilms and its adhesion to plastic surfaces. The application of the NPF on host plants reduced the disease severity compared to standard cupric salts, as much as reduced the spread of Psa, which was evaluated by Real-Time qPCR. Interestingly, the application of NPF boosted innate immunity in Olive and Actinidia plants, by up-regulating several salicylic acid responsive genes. This work provides novel and valuable information regarding the several mode of actions of a novel bio-based NPF, which could be potentially useful to control Psa and Psav even in organic agriculture.

P6.4-011

RNAI AS A GREEN BIO-FUNGICIDE TO MANAGE BOTRYTIS GREY MOULD IN CHICKPEA

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Text

Botrytis cinerea, the causal agent for Botrytis grey mould disease, is one of the devastating necrotrophic fungal pathogens responsible for significant yield losses in a wide range of crops including chickpea. Under favourable environmental conditions the pathogen infects all plant parts including the flowers and pods, making it difficult to manage this disease especially during the canopy closure stage. Breeding for host resistance has been elusive,

and repeated use of broad-spectrum fungicides may lead to loss of sensitivity through adaptation. Consequently, the use of double-stranded RNA (dsRNA) as RNAi-based bio-fungicides to target functional sequences triggering RNAi machinery are considered as an alternate management approach. In this study, we demonstrated that exogenous application of dsRNA targeting B. cinerea Dicer-like (DCL) genes protects against B. cinerea infection In Planta up to 14 days post inoculation (DPI) under controlled environment conditions. In vitro assessment on the direct impact of dsRNA, showed significant reduction in B. cinerea growth up to 120 hours post inoculation (HPI). Histopathology revealed significant reduction in spore germination, germ tube length and formation of appressoria, suggesting that topical application of dsRNA targeting B. cinerea specific genes may effectively control B. cinerea growth and disease development sustainably within natural agroecosystems.

P6.4-012

FORGOTTEN NUTRIENT THAT ENHANCES THE PASSIVE DEFENCE PATHWAY, SO REDUCING DISEASE LEVELS?

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Text
A characteristic of the passive defence pathway is that the thickness and strength of the leaf surface inhibits penetration by pathogens. Silicon is one of the most widespread elements on the planet and is found in the trichomes of plant. However much of the silicon is unavailable to plants as plants can only take up silicon if it is in a bio- available form such as silicilic acid. The literature on the use of silicon nutrient demonstrates a wide range of potential benefits to plants. In particular disease levels on a range of crop species are reduced when silicon nutrient is applied regularly. The work presented here shows disease reduction in strawberries, courgettes, dragon fruit, wax apple, cocoa, rice and rubber. The work presented demonstrates the up take of silicon and how the silicon is deposited on the surface of strawberry leaves and deposited under the walls of the epidermis and palisade cells thus strengthening the leaf and inhibiting penetration by pathogens (and pests). Epidemic development is significantly reduced on the crops tested. The use of bio available silicilic acid enables disease reduction and reduction in environmental impact due to the reduced need for fungicides, thus contributing to sustainable production.

Pathovars of Pseudomonas and Xanthomonas spp.: do they really exist?

C7.5-1

THE PATHOVAR CONCEPT MISREPRESENTS THE REALITY OF DISEASE AS A MULTIFACTOR PROCESS; IT'S TIME TO MODERNIZE

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Text

The pathovar concept intends to define groups of closely related strains with like pathogenic potential - in terms of the susceptible plant species and the type of symptoms. In turn, pathovar identity is supposed to be useful for diagnostics and disease management. We argue that the pathovar concept cannot meet its intended goals for two fundamental reasons. Firstly, expression of symptoms is the outcome of a process that involves a multitude of factors as represented in the disease triangle. Some of these factors have stochastic or chaotic behaviors. Hence, the outcome of the interaction of a bacterial strain with a plant is conditional and not absolute. This leads to the second obstacle: defining standard criteria by which pathovar identify is attributed objectively. These criteria should specify if pathovar is defined according to natural disease outbreaks or host range tests under controlled conditions. The former ignores differences in opportunity for infection and impedes anticipation of new disease emergences, especially by strains from environmental sources. The latter is faced with unlimited options for host species, cultivars, plant age and physiology and environmental conditions. Furthermore, for numerous plant pathogens, extensive host range tests do not reveal distinct pathovars. We propose that disease potential be estimated by situating bacterial genotypes in a map of risk likelihoods that account for all relevant factors contributing to disease outbreaks.

C7.5-2

PATHOVARS OF XANTHOMONAS CAMPESTRIS, DIAGNOSTICS AND DISEASE RESISTANCE

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Text

Plants from the *Brassicaceae* family can be attacked by different *Xanthomonas campestris* pathogens. *Xanthomonas campestris* pv. *campestris* (Xcc), the cause of black rot of brassicas, is one of the most important diseases of crops like cabbage and cauliflower worldwide. Ornamental *Brassicaceae* plants like wallflowers and garden stocks and some common weeds can also be infected by *X. campestris* pathogens including *X. campestris* pv. *incanae* (Xci). In contrast to Xcc and Xci, which cause vascular diseases, *X. campestris* pv. *raphani* (Xcr) causes leaf spots in a larger range of *Brassicaceae* and other hosts including

tomato. At least two pathovars, Xcc and Xcr, have race structures defined by the ability of causing disease in different brassica differential accessions. We are characterising the pathogenicity and sequencing a collection of *Xanthomonas* spp. isolates from different origins and spanning over 60 years, including different pathovars of *X. campestris*. The results of our work allowed us to reclassify some pathovars from *X. campestris* as other species, thus clarifying the taxonomy. We are designing diagnostic testing for important pathovars and races of *X. campestris*. Screening brassica collections has led to the identification of sources of resistance and we are developing chlorophyll imaging and whole plant imaging techniques to study *X. campestris* infections. We aim to characterise and map resistances and identify resistance-linked markers that can assist future breeding.

C7.5-3

BACTERIAL PATHOVAR DOES NOT ALIGN WITH CURRENT (META)GENOMICS-BASED PATHOGEN IDENTIFICATION

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Text

Rapid whole genome and metagenomics sequencing is redefining how we track bacterial pathogen outbreaks in real time. Diagnostic clinics, centers and front-line pathologists are increasingly implementing these technologies to support producers in identification of emerging, unknown and unculturable agricultural threats. Current bacterial pathogen typing and naming with pathovar designations limits our ability to properly identify the causal agent of infection especially in the context of the complexity of the microbiome. Pathovar designation is phenotypic and does not always follow the genetics of a species, which is critical for tracking the evolution of an outbreak. Pathovars are also impractical and rely on host range or phenotypic assays, which are slow and varies at each laboratory. Genome based approaches instead allow for standardized identification and precisely track organism evolution especially new variants of emerging importance. Metagenomics also captures genetic content of the whole biome, and pathovar testing is not possible for an organism detected only by metagenome sequencing. Moreover in light of the metagenome, our definitions may even shift of defining a community of organisms for disease development rather than a single one pathogen, one symptom concept. This presentation will describe our current work using genomics and metagenomics for real-time pathogen tracking to push past pathovar typing for pathogen characterization.

C7.5-4

PHYLOGENY-ASSISTED SUBSPECIFIC CLASSIFICATION OF XANTHOMONADS INFECTING TOMATO AND PEPPER

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Text

Bacterial spot of tomato and pepper is caused by multiple taxa of bacteria within *Xanthomonas*, including *X. euvesicatoria* and *X. perforans*. Recent studies have proposed consolidation of two species as pathovars of *X. euvesicatoria*, owing to >95% average nucleotide identity (ANI) between two species. However, *X. perforans* and *X. euvesicatoria* are both pathogenic on the same set of host plants, thus the use of pathovar as defined in the International Code of Nomenclature of Prokaryotes is not useful in discerning between the two. To infer the taxonomic position of the two species, we calculated whole genome ANI and inferred core genome phylogeny from whole-genome-sequenced pathovars of *X. euvesicatoria sensu lato*. We found that *X. euvesicatoria* (ANI > 99.7%) and *X. perforans* (ANI > 99.4%) are classified into separate (ANI < 99.1%) clusters, with monophyly supported by core genes, and thus are taxonomically distinct. We also identified a divergent group of *X. perforans* strains that form a distinct cluster only distantly related to typical *X. perforans* (< 99.2% ANI). Thus, we propose reclassification of *X. euvesicatoria sensu stricto* as *X. euvesicatoria* subsp. *euvesicatoria* and split *X. perforans* into subsp. *perforans* and a yet unnamed subsp. of *X. euvesicatoria*.

C7.5-5

TIME TO REVISIT THE PATHOVAR CONCEPT, TOWARDS A POLYPHASIC APPROACH ?

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Text

The pathovar concept was proposed as a provisional solution to avoid losing names of plant pathogenic species, that were not retained on the Approved Lists of Bacterial Names. Pathogenicity tests, to determine host range and symptomatology, are the cornerstone of this special purpose definition. However, they suffer from many drawbacks. Their outcome largely depends on inoculation conditions. Extended overlapping host range or gradient of virulence can sometime hinder pathovar circumscription, and large-scale assays to determine the extent of host range are seldom conducted.
During the last decades, our understanding of pathogenicity mechanisms, host specificity and pathogen evolution has benefited from genetics and genomics. Host adaptation of *Pseudomonas* and *Xanthomonas* strains, was shown to be driven by the evolution of the repertoire of type three effectors (T3E). Within *Xanthomonas arboricola*, it was shown that the most aggressive pathovars form three distinct clonal complexes, while less aggressive ones and non-pathogenic strains formed a recombinant network of strains with fewer or no T3E and sometime no type three secretion system. These differences of genetic structure and gene content are consubstantial with the ecological success of the strains and their ability to cause epidemics. Should we give more weight to these biological traits in the pathovar definition ?

Such a polyphasic approach could help to reconcile the pathovar concept with a natural classification.

P7.5-001

PELTIGERA LICHENS IN ICELAND: YET ANOTHER RESERVOIR OF THE PLANT PATHOGEN PSEUDOMONAS SYRINGAE?

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Text

The study of Pseudomonas syringae has recently shifted from an agrocentric context to an ecological perspective. The discovery of new niches inhabited by P. syringae improves our knowledge of the adaptive skills of this bacterial group and helps us realize the extent of its dispersion. The objective of this doctoral research project is to determine if lichens, the major vegetation of Iceland – an island where only 1% of its land is used for agriculture – harbor P. syringae with pathogenic potential for crops. Of the 16 different types of lichen analyzed, species of only one genera were consistently found to harbor P. syringae: Peltigera spp. Those strains isolated from Peltigera were later compared in terms of fitness and pathogenic potential with strains with well-known epidemiological importance on ten different plant species. For this question, population dynamics and virulence of lichen strains and reference epidemic strains were compared by inoculating species of crop plants of which some are grown in Iceland (barley, cucumber, kale...). Surprisingly, P. syringae isolated from Peltigera lichen has fitness and pathogenic potential similar to P. syringae from epidemics on crops in the plant species tested. Overall, the results of this work offer a unique opportunity to mark the starting point of observations on potential disease emergence as temperature, prevailing weather, and land use change in Iceland as consequences of climate and global change.

P7.5-002

EVALUATION OF PCR PRIMERS DERIVED FROM FOUR PROTEIN-CODING GENES AGAINST XANTHOMONAS SPECIES TO EXPAND MULTI-LOCUS SEQUENCING STUDIES

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Text

Recently, MLSA-based approaches are increasingly being applied for the re-evaluation of some plant pathogenic bacterial species including Xanthomonas species. Nucleotide primers, *dnaK*, *fyuA, gyr*B, and *rpoD*, have been used for PCR amplification and sequencing

from *Xanthomonas* species. But their specificity to extended species is not fully tested. This study was performed to evaluate their amplification ability with 32 *Xanthomona*s strains consisting of 28 species and 5 pathovars. All the four primers did not amplify the target genes from *X. campestri*s pv. *armoraciae*, *X. theicola*, and *X. hortorum* pv. *carotae*. The primers for dnaK amplified the target sequences from 26 *Xanthomonas* strains, but did not from *X. hyacinthi, X. translucens* pv. *translucens*, *X. sacchari*, and *X. theicola*. The primers for *fyu*A amplified the target sequences from 19 *Xanthomonas* strains, but did not from *X. hyacinthi, X. translucens* pv. *translucens, X. sacchari*, and *X. theicola, X. arboricola* pv. *corylina, X. codiaei, X. campestris* pv. *campestris, X. alfalfae* subsp. *alfalfa, X. gardneri, X. vasicola* pv. *vasculorum*, and *X. nasturtii*. The primers for *gyrB* amplified the target sequences from 28 *Xanthomonas* strains, but did not from *X. translucens* pv. *translucens*. The primers for *rpoD* amplified the target sequences from 28 *Xanthomonas* strains, but did not from *X. hyacinthi* and *X. sacchari.* Application of MLSA based analysis of the four gene sequences of 32 Xanthomonas strains was discussed.

P7.5-003

A NEW BACTERIAL DISEASE OF OLEANDER

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Text

Nerium oleander L. of the family *Apocynaceae*, is an evergreen shrub or small tree of the Mediterranean region. The abundant and long-lasting flowering makes it a popular ornamental plant. Only a few bacteria can infect oleander such as *Pseudomonas savastanoi* pv. *nerii, Agrobacterium tumefaciens* and *Xylella fastidiosa*. We have been investigating oleander canker disease for years, and during sample collection we observed atypical symptoms on the leaves and stems of oleander. In our work, we aimed to identify the pathogen. The collected samples were decontaminated, homogenized and streaked on King B agar. The isolates formed yellow-coloured, smooth-edged and convex bacterial colonies on the medium. The isolates were Gram negative, oxidase negative and induced a hypersensitive reaction in tobacco leaves. The biochemical properties were determined by API20E tests. Pathogenicity was performed on young oleander plants with bacterial suspension. Two weeks after the artificial infestation, brown necrosis was observed on the leaves of the oleander. The pathogen was successfully reisolated Koch's postulates were fulfilled. For molecular identification of the pathogen, the 16S rDNA region were amplified using a universal bacterial primer pair. According to symptoms, colony morphology, biochemical features, pathogenicity and molecular methods, the pathogen was identified as *Xanthomonas campestris*.

The research was supported by the ELKH TKI (project number: 3200107).

P7.5-004

CHARACTERIZATION OF XANTHOMONAS ARBORICOLA PV. JUGLANDIS ISOLATES IN HUNGARY

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Text

*Xanthomonas arboricol*a pv. *juglandis* is the causal agent of walnut blight, the most important bacterial disease of *Juglans* species, which affects a high percentage of pistillate flowers and fruits, but does not kill bearing trees. Symptoms of the disease consist of dark brown to black spots on new leaves, stems and nuts. Many nuts fall prematurely; others reach full size, but their kernel become blackened, dried and wrinkled.
In Hungary, walnut blight disease occur and cause important damage in gardens and orchards. Since 2020, we have been constantly monitoring the symptoms. The collected leaf and fruits samples were decontaminated, homogenized and streaked on King B agar. After 24 hours of incubation at 26 °C the Gram property of isolates was determined by KOH test. We examined all isolates to induce a hypersensitive reaction on tobacco leaves. Biochemical API test was also used for identification. Finally, the pathogenicity of the isolates was checked. In the case of walnut blight, immature walnut fruits and leaves were artificial infected with a bacterial suspension. For molecular identification of the isolates, the 16S rDNA region were amplified using a universal primer pair (63F, 1389R). *Xanthomonas arboricola* pv. *juglandis* isolates that cause walnut blight disease have been identified by classical and molecular methods in Hungary.

This project was supported by the János Bolyai Research Scholarship (bo_671_20) of the Hungarian Academy of Sciences”.

P7.5-005

FIRST REPORT OF PSEUDOMONAS SYRINGAE PV. SYRINGAE CAUSING A LEAF SPOT DISEASE ON WATERMELON PLANTS IN GREECE

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Text

In May 2022, a bacterial infection was found during microscopic examination, in watermelon seedlings, with symptoms differing from known watermelon bacterial diseases. The disease destroyed more than 3.000 plants at the nursery. On the leaves lamina, small oily, watery round spots were observed which grow in concentric rings and may merge. The spots acquire a brown necrotic center with a watery margin and/or a weak chlorotic halo. Similar elongated spots/cankers were observed on the stems, which may damage part or all the leaf lamina. From the infected tissues, on King's B medium, bacterial colonies were constantly

growing in pure culture with diffuse blue fluorescent pigment. Isolates in LOPAT tests showed the phenotype [+ - - - +]. Based on the morphological and biochemical profile, the isolates were characterized as members of Pseudomonas syringae species complex, a fact that also confirmed by the analysis of 16S rRNA. In addition, phylogenetic analyses based on the sequencing of multiple loci (MLSA) utilizing four housekeeping genes (gapA, gltA, gyrB, rpoD), showed that isolates from watermelon seedlings were grouped with corresponding sequences of other strains of Pseudomonas syringae pv. syringae. Koch's postulates were fulfilled on watermelon plants. This is the first report of Pseudomonas syringae pv. syringae, causing a new watermelon disease in Greece.

P7.5-006

THE GENOMIC CHANGES THAT MAY HAVE CONTRIBUTED TO THE ADAPTATION OF XANTHOMONAS VASICOLA PV. VASCULORUM TO EUCALYPTUS GRANDIS.

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Text
Xanthomonas vasicola is one of the 27 described *Xanthomonas* phytopathogens, and members of this genus form characteristic yellow colonies due to the production of xanthomonadins. These phytopathogens cause diseases in 124 monocotyledonous and 368 dicotyledonous plants. In the mid-2000s, an outbreak of bacterial blight and dieback caused by *X. vasicola* pv. *vasculorum* occurred in a newly established *E. grandis* plantation with a single clonal origin, and it was hypothesised that this phytopathogen jumped from the adjacent sugarcane fields onto *Eucalyptus*. A comparative genomics study was undertaken to understand the molecular changes that may have contributed to this host jump and the adaptation of this bacterial pathogen to *E. grandis*. The phylogenetic relationship of five *E. grandis* strains and eighteen *X. vasicola* strains of other hosts was determined. Genomic islands and insertion sequences were identified, and some insertion sequences predicted were present within these islands. In addition, orthologous clusters of *X. vasicola* strains from different hosts were predicted, and the phylogeny of clusters unique to the *E. grandis* strains was inferred. Together these analyses identified unique genes from the *E. grandis* strains involved in chemotaxis, plant cell wall degradation, the suppression of reactive oxygen species, and the T4SS. Therefore, it is possible that these genes may have contributed to the adaptation of *X. vasicola* pv. *vasculorum* to *E. grandis* from sugarcane.

P7.5-007

INOCULATION TECHNIQUES OF THE AGENTS OF COMMON BACTERIAL BLIGHT IN PHASEOLUS VULGARIS

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Text

Common bacterial blight (CBB) is an endemic disease of common bean (Phaseolus vulgaris) caused by Xanthomonas phaseoli pv. phaseoli and X. citri pv. fuscans. In favourable conditions, it reduces yield of beans up to 40 %. Despite the numerous efforts to improve the genetic resistance of common bean to CBB, a complete resistance is lacking and the efficacy of known resistance varies depending on the strains tested. Methods for disease assessment and resistance scoring differ by their efficacy in differentiating the virulence of strains and the resistant genotypes. Effective disease phenotyping methods is essential for searching novel resistant genotypes, therefore we compared 4 different inoculation methods with two Xanthomonas spp. strains (USB 749, USB 771) on 2 bean varieties. The methods were ranked according to the time required to complete the procedure and according to their ability to discriminate the virulence of the two strains. This comparative study allows to select the appropriate method of infection depending on the research objective considered and will be essential for our future breeding activity.

Plant pathogens interactions in multi stress conditions (abiotic and biotic stresses): viruses and other pathogens?

C5.7-1

PLANT-VIRUS-VECTOR INTERACTIONS UNDER ABIOTIC STRESSES

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Text

Biotic and abiotic stresses shape plants communities and distribution in ecosystems. Among biotic stresses, plant viruses can cause significant losses in agriculture. Moreover, the majority of plant viruses rely on a third-party for host-host spread, insects being by far the most important vectors of these plant pathogens. Therefore, environmental conditions that influence the vector biology will as a result influence virus transmission efficiency, spread

and persistence in ecosystems. In this context, climate change disturbs multiple processes that may impact the epidemiology of plant viruses interactions under multi stress conditions, adding another layer of uncertainty in crop protection, as well as current and future food security. The impact of different abiotic stresses, especially related to climate change (temperature, water availability, CO2 etc..), on plant-virus-vector interactions will be presented and discussed.

C5.7-2

SEARCHING FOR SUSTAINABLE AND EFFICIENT SOURCES OF DISEASE RESISTANCE UPON FLUCTUATING CLIMATE: CHARACTERIZATION OF THE WHEAT-SPECIFIC RESPONSES TO FUSARIUM HEAD BLIGHT UNDER CONTRASTING IRRIGATION

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Text

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, is a prominent disease of small grain cereals impacting yield and grain quality. In wheat, many efforts are still produced to find alternative sources of resistances, especially focusing on susceptibility factors. Despite the important role of the abiotic environment in the establishment of the disease, only few studies have included the impact of future climate scenarios on the expression of the plant susceptibility or resistance. Focusing on fluctuating water regimes, this work aims to initiate the phenotypic and molecular characterization of the FHB-induced responses in wheat facing soil water deficit preceding infection. A setup of mild drought of different durations during spike development demonstrated a specific and non-additive response to the constraint combination. A strong decrease of symptoms in plants subjected to previous drought during at least 6 days have been observed compared to well-watered. A relationship between symptom reduction and water stress duration was observed. Metabolite profiling and dual-transcriptomics of spike samples is used to identify molecular mechanisms specifically set up upon combined stress as compared to well-watered plants and plants subjected to respective individual stresses. These results will provide new candidates for the selection of sustainable and efficient sources of FHB resistance with promising potential in the context of climate change.

C5.7-3

DROUGHT EXACERBATE VIRUS EFFECT ON CANOLA PLANTS

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Text

Canola (Brassica napus L.) is one of the most important crops worldwide. It is affected by several pathogens which can cause significant losses and increase production costs. Aphid-borne, turnip yellows virus (TuYV, Polerovirus, Family: Luteroviridae) is one of the most damaging and difficult to control. Under future climate, for many parts of the world, more intense and prolonged drought events are predicted, adding another factor that can significantly impact the epidemiology of plant diseases. In this study, we aimed to understand the impact of drought on canola plants previously infected with TuYV. We conducted glasshouse trials, where TuYV infected and noninfected canola plants were exposed to different watering regimes as well as terminal drought. Canola plants were inoculated with TuYV at three leaf stage then at four leaf stage, water treatments were initiated. Several parameters including: height, number of leaves, growth stage and leaf area were recorded as well as chlorophyll content, water use efficiency, symptom expression and biomass, to assess the impact of virus infection and drought on canola growth. TuYV exacerbated the effects of water stress and terminal drought on biomass and other plant growth parameters when compared to non-infected plants. These results suggest that drought could aggravate the negative impact of TuYV on canola crops as a consequence of climate change.

C5.7-4

THE BOTRYTIS CINEREA PECTIN LYASE BCPNL1 IS INVOLVED IN PATHOGENICITY AND ITS PECTINOLYTIC ACTIVITY CONTRIBUTES TO THE IMPACT OF HOST NITROGEN NUTRITION ON DISEASE SEVERITY

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Text

Host nitrogen nutrition is known to impact the expression of plant defense genes but also pathogen virulence factors. In this study we found that pectinolytic enzymes of *Botrytis cinerea* are strongly affected by plant nitrate nutrition during infection of *Arabidopsis thaliana* at the transcriptional level leading to changes in the final oligogalacturonide (OG) production. We report here the importance of *BcPNL1*, the first pectin lyases (PNLs) of *B. cinerea* described as involved in pathogenesis and OG production during the infectious process. Indeed, CRISPR-Cas9 *ΔBcpnl1* deletion mutants were hypovirulent on several hosts plants and the absence of PNL products found by LC-MS/MS analysis of OGs produced during the interaction confirmed that BcPNL1 is the major PNL of *B. cinerea*. Furthermore, during infection with the *ΔBcpnl1* mutants, negative regulators of the jasmonate (JA) defence pathway such as *AtJOX3* and *AtJAZs* were down regulated whereas the JA marker gene *AtPDF1.2* was strongly induced. These results confirmed a previously mentioned role of OGs produced by PNL in repression of the JA pathway. We also revealed that varying nitrogen nutrition status of the

plant have a different impact on defense gene expression during infection with the *ΔBcpnl1* mutant. Altogether, we provide evidences that plant cell wall degradation and particularly BcPNL1 activity are important factors contributing to the complex interaction between host nitrogen nutrition and pathogenesis.

C5.7-5

CAN TEMPERATURE ADAPTATION DRIVE RALSTONIA SOLANACEARUM RANGE EXPANSION IN THE FUTURE?

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Text

The *Ralstonia solanacearum* species complex (RSSC) consists of a group of phytopathogenic β-proteobacteria with extensive genetic diversity responsible for lethal wilts or rots in a variety of plants worldwide. RSSC strains have adapted to survive across different environments and to infect a wide range of plant hosts. While human agricultural activity has played an important role in the distribution of the pathogen, it is less clear how RSSC strains will adapt and spread in light of global warming. In this work, I will use experimental evolution to expose the cold-adapted strain UW551 to increasing environment temperatures in the laboratory and quantify the effect on its virulence and fitness. Secondly, I will evaluate how the warm-adapted strain GMI1000 evolves in response to exposure to a cold environment. These experiments will help to assess whether RSSC strains currently inhabiting cold regions will become a more severe threat due to global warming, and if strains inhabiting warmer regions can potentially adapt to colder climates, leading to pathogen migration and range expansion in the future. These studies will also provide insights into the genes and mechanisms used by RSSC strains to adapt and survive under changing environmental temperatures.

C5.7-6

BARLEY SHOWS REDUCED FUSARIUM HEAD BLIGHT UNDER DROUGHT AND MODULAR EXPRESSION OF DIFFERENTIAL EXPRESSED GENES UNDER COMBINED STRESS

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Text

Little is known about the interplay of biotic and abiotic stress responses in crop plants. Spring barley is susceptible to Fusarium Head Blight (FHB), which is highly affected by weather conditions. We combined barley ear infection with Fusarium culmorum with or without

previously applied drought stress in the greenhouse and studied the influence of drought on FHB severity in three differently susceptible spring barley varieties. We found strongly reduced FHB severity in susceptible varieties under drought stress. We conducted a global transcriptome analysis of barley spikes and correlated physiological stress markers such as abscisic acid with co-expressed gene networks using weighted gene correlation network analysis. These networks may help to explain common or genotype-specific responses to complex stress situations. An infection-related module contained co-expressed genes for defence, programmed cell death and mycotoxin-detoxification indicating that diverse genotypes use a similar defence strategy towards FHB albeit with different success. Further networks are highly associated with drought or genotypes, but no network is correlated with the combination of drought with infection, indicating a modular composition of single stress responses. Our analysis further (re-)discovered both established and new players in response to stress and fungal toxins and provides a route for diverse analyses of combined physiological and global gene expression data.

F5.7-1

PHENOTYPING BIOTIC-ABIOTIC INTERACTIONS AFFECTING RICE GRAIN YIELD TO DISCOVER TOLERANT GENOTYPES

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Text

High temperatures are known to exacerbate rice panicle blight caused by Burkholderia glumae. Our long term goal is to develop rice with increased tolerance to combined heat and disease stresses. Our first steps have been to develop a robust experimental system to test how heat stress tolerance interacts with B. glumae infection. A set of rice genotypes with contrasting tolerance to heat was inoculated with B. glumae at anthesis stage. Genotype response to bacterial infection was measured by quantifying the proportion of empty spikelets as well as the total number of grains obtained from inoculated panicles. Preliminary results identified promising rice genotypes that can tolerate heat stress and the infection by B. glumae separately. These rice genotypes will be valuable for studying the molecular mechanisms responsible for tolerance and enable development of new rice varieties that withstand combined stresses under field conditions.

P5.7-001

IT'S COMPLICATED! LINKING CA2+ SIGNALLING WITH DOWN-STREAM RESPONSES TO OSMOTIC STRESS AND PAMPS IN ARABIDOPSIS THALIANA ROOTS.

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Text

Signal transduction enables plants to detect and respond to environmental stress. Calcium (Ca^{2+}), considered a key signalling molecule in plants, regulates gene expression, hormone release and cell death. However, it remains unclear how specificity of Ca^{2+} signalling responses is achieved. Firstly, we established a bi-directional dual-flow-RootChip microfluidic device to expose G-CaMP3 *A. thaliana* primary roots to asymmetric solute gradients. Fluorescent live-cell imaging detected and quantified cytosolic Ca^{2+} and root growth. We observed the Ca^{2+} signal induced by different osmolytes (NaCl, PEG and Mannitol) and pathogen associated molecular patterns (flg22, PEP-13, chitin). The signal dispersed differently depending on treatment condition and orientation (shoot-to-root, root-to-shoot, transverse), exhibiting a directional response. Secondly, a label-free quantitative proteomic approach was used to link early signalling with changes in protein profiles and phosphorylation events. For this, we compared roots treated for 5 minutes with 20% PEG, 500 µM flg22 and ½ MS medium (control). Up to 300 phosphoproteins and 6000 proteins were detected in each sample, showing distinctive treatment groups with supervised clustering methods. Proteins of interest with functions in osmotic stress adaptation and immunity were identified and further analysed. We will discuss their potential role in signalling to distinguish between different stressors to fine tune root adaptation processes.

P5.7-002

SUFFICIENT COUMARIN ACCUMULATION IMPROVES APPLE RESISTANCE TO CYTOSPORA MALI UNDER HIGH POTASSIUM STATUS

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Text

Cytospora canker, caused by *Cytospora mali*, is the most destructive disease in production of apples (*Malus domestica*). Adding potassium (K) to apple trees can effectively control this disease. Here, we found that series of resistance events were active in high K (HK, 9.30 g/kg) apple tissue, especially upregulation of resistance genes, callose deposition and formation of ligno-suberized tissues. Further multi-omics revealed that the phenylpropanoid pathway was reprogrammed by HK status, leading to increases of 18 antifungal chemicals. Among them, the physiological concentration of coumarin became sufficient to inhibit *C. mali* growth, and its exogenous application could improve the apple resistance. The transgenic apple calli with overexpression of *MdBGLU40* that encoded beta-glucosidase for coumarin synthesis contained higher levels of coumarin and exhibited high resistance to *C. mali* even under low K conditions. Meanwhile, suppression of *MdBGLU40* through RNAi reduced coumarin content and resistance in HK apple calli, supporting the importance of coumarin in apple resistance. Moreover, we found that the upregulation of transcription factor *MdMYB1r1* directly activated

MdBGLU40, and the binding affinity of MdMYB1r1 to the *MdBGLU40* promoter increased in HK apple tissue, leading to high levels of coumarin and resistance in HK apple. Overall, our results highlight the optimization of K content in fertilization practices as a novel disease management strategy.

P5.7-003

HOW WILL ATMOSPHERIC CO2 CONCENTRATION IMPACT THE VIRAL SUSCEPTIBILITY/RESISTANCE? : PHASEOLUS VULGARIS AS A MODEL PLANT

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Text

One major concern of this century is the impact of climate change, notably on crop cultures. Experts of climate change have forecast an increase in atmospheric CO_2 level from 400 µL.L-1 in 2014 to ~1000 µL.L-1 in 2100 in the worst predictive scenario, as well as a raise of 3.3-5.7°C in temperature. Plants are directly impacted by these changes as well as pathogen populations including viruses.
In that context, an important question is to what extent the increase in CO_2 will affect plant-virus interactions, whether susceptibility or resistance?
The objective of our work is to study the impact of elevated CO_2 (eCO_2) level on viral susceptibility/resistance using common bean (*Phaseolus vulgaris* L.) as a model plant. We used the *P. vulgaris*/*Bean pod mottle virus* (BPMV, *Comovirus*) pathosystem to investigate the impact of eCO_2 on the level of susceptibility/resistance to BPMV using two natural genotypes BAT93 (resistant to BPMV) and Black Valentine (susceptible to BPMV).
In that aim, we analyzed viral titers in plants grown under eCO_2. We also monitored the accumulation of salicylic acid (SA). Moreover, we studied the expression of genes encoding enzymes of the SA pathways, as well as genes encoding PR proteins and components of the RNA silencing pathway.
Our preliminary results show that both genotypes are more resistant/less susceptible under eCO_2 and this seems correlated with a higher accumulation of SA and with an increased expression of defense genes.

P5.7-004

CORONATINE ORCHESTRATES ABI1-MEDIATED STOMATAL OPENING TO FACILITATE BACTERIAL PATHOGEN INFECTION

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Text

Closure of stomata on plant epidermal cells can prevent the invasion of pathogenic bacteria and plays an important role in plant innate immunity. Abscisic acid (ABA) can induce plants

to close stomata, while bacterial infection can antagonize ABA by secreting coronatine (COR), reopening the stomata to promote infection, but the internal molecular mechanism is not clear. Our previous experiments found that COR treatment could upregulate the content of ABI1, a negative regulator of ABA signaling pathway, to reduce plant sensitivity to ABA signaling. We also identified a nucleoplasmic transporter that may mediate the nucleoplasmic shuttling of ABI1 to regulate its function. Therefore, this project can reveal the molecular mechanism of COR antagonizing ABA function and opening stomata, and finally provide new ideas and theoretical basis for the improvement design of drought-resistant and water-saving crops.

P5.7-005

IDENTIFICATION OF HEAT-TOLERANT BLAST RESISTANCE GENES IN RICE

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Text

Rice is the staple food of more than half of the world's population. With economic development and population growth, global warming has become a problem that cannot be ignored. The high-temperature stress caused by climate warming will seriously affect rice growth and development and reduce rice yield. Previous studies have shown that high temperatures may increase the risk of the destructive rice blast caused by *Pyricularia oryzae* in subtropical regions and tropical winters. However, little is known about the interactions between *P. oryzae* and rice under higher temperatures. We first tested the effects of different temperatures on the mycelial growth and sporulation of 25 representative *P. oryzae* isolates from different years and regions in Taiwan. A heat-tolerant isolate was selected to evaluate the effectiveness of three blast resistance genes, i.e., *Pi2*, *Pi9*, and *Ptr*, in the genetic backgrounds of ‘Lijiangxintuanheigu (LTH)’ and a heat-tolerant cultivar ‘Kaohsiung 145 (KH145)’. Plants with *Pi2* and *Pi9* showed resistance no matter at normal (28°C), higher (32°C), or the highest temperature (35°C); however, KH145 with *Ptr* showed unstable resistance at 35°C. How high temperatures affect gene expressions in *P. oryzae* and the resistance regulatory networks of different *R* genes in rice are worth further investigation.

P5.7-006

EFFECT OF DROUGHT ON GRAPEVINE WOOD FUNGAL PATHOGEN COMMUNITIES USING A METATRANSCRIPTOMICS APPROACH.

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Text

Crops are facing increasing biotic and abiotic stress pressures due to global changes. However, trade-off mechanisms between these stresses and the underlying physiological processes are still poorly understood, especially in perennial crop species. To better understand these trade-offs, we studied the effect of drought on grapevine (*Vitis vinifera*) physiology and esca-related wood fungal communities. Esca is a vascular disease caused by a community of wood-infecting pathogenic fungi, and characterized by trunk necroses, leaf scorch symptoms, yield losses, and mortality. This grapevine disease leads to leaf symptoms associated with xylem hydraulic failure, and leaf symptoms are inhibited by severe drought. To characterize the molecular processes underlying the interactions between drought and esca, we conducted two greenhouse experiments on 30-years-old Sauvignon blanc vines. Esca leaf symptoms expression was monitored and vines were subjected to drought stress or not under controlled conditions. Sapwood samples from the trunks were used to perform a community-level transcriptomics (i.e. metatranscriptomics) analysis. Results will be also related to metabolomic and ecophysiological data acquired on wood and leaf samples. This integrative approach will provide new insights into the understanding of the grapevine/esca pathosystem under drought, in terms of physiological and functional responses in both host and pathogens.

P5.7-007

STRATEGIES FOR BIOTIC STRESS MANAGEMENT IN PULSES UNDER CHANGING CLIMATE

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Text

An increasing agricultural productivity is a critical first step in meeting the Sustainable Development Goals of ending hunger and poverty by 2030. Majority of poor farmers survives in Sub-Saharan Africa and South Asia where an overall loss of attainable yield from pest & disease (P&D) is much greater. In particular, significant damage has been caused globally by transboundary pests against the background of climate change and globalized movement of people and goods. Climate change, disruption of pest & diseases biological synchrony, promotion of minor pest into major, soil moisture deficit and lack or apparent failure of resistance cultivars seems to be major reasons for the production loss in pulses like chickpea and pigeonpea. Tailored research information on transboundary and emerging P&D, climate vulnerabilities for epidemics, crop resistant and new management technologies could be essential to bring the transformation in poor farming families. Accelerated pest's damages prejudice the obligation of pest prioritization and risk assessment for optimization of the preemptive breeding in demarcated target population environments, determination of socio-economic condition of farmers and rapid action on climate smart crop management and

adaptation. New tools and technologies linked to the advisory will help in providing rapid response to the farmers for managing their crops in a real time manner.

P5.7-008

ROLE OF SHARED INTERGENIC REGULATORY ELEMENTS IN ENHANCED RESISTANCE TO COMBINED STRESSES IN RICE.

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Text
Crop plant adaptation to stresses, including heat and disease, affects chromatin accessibility and expression of numerous genes by exposing short sequences in promoter areas, specifically cis-regulatory elements (CRE) or combinations of CRE that are organized as modules (cis-regulatory modules or CRM). Increasing evidence demonstrates that conserved CRE/CRM are found in promoters of genes that are co-activated by a single stress and/or are common to genes co-activated in plants with enhanced tolerance to different stresses. Moreover, genetic polymorphisms modifying CRE/CRM can significantly impact gene activity and plant responses to stresses. We have shown that many genes involved in broad-spectrum disease resistance (BSDR) share a set of CRMs in rice. Also, accumulating genes with the most ‘active’ form of CRMs in several BSDR genes leads to enhanced disease resistance in the field. Presently, we are testing modifications of specific CRE/CRM via genome editing and transient assays to analyze their relevance. Based on these results, we propose a strategy to simultaneously breed for crop resilience to heat and disease through the identification of shared CRE/CRMs. The identified elements, and detected polymorphisms affecting them, can be used to develop conserved molecular markers for breeding. Our goal is to enable genome-wide selection of complex traits governed by multiple genes with a reduced number of markers rather than the traditional one marker-one gene approach.

P5.7-009

UNDERSTANDING THE IMPACTS OF HEAT STRESS ON BACTERIAL BLIGHT RESISTANCE GENES IN RICE

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Text
By the year 2100, average air temperatures are predicted to increase by 2°C to 6°C around the world. This impacts the day-to-day conditions in which plants grow and is a threat to production of many cereal crops, including rice. When rice plants are exposed to combined stresses, such as high temperatures and disease, there is often a reduction in the effectiveness of bacterial disease resistance mechanisms within the plant. These

mechanisms include the protective actions provided by resistance genes (*R* genes), which have previously been effective at controlling the spread of bacterial blight, a yield-reducing disease caused by *Xanthomonas oryzae* pv. *Oryzae*, in rice. Diminishing disease resistance in the face of increasing temperature trends will expose growers to additional acceleration of predicted yield losses in the coming decades. Interestingly, one rice *R* gene, *Xa7*, shows milder disease phenotypes under heat stress, indicating improved *R* gene efficacy under these conditions. Transcriptome analysis predicts that alternative stress response signaling involving hormonal crosstalk may be occurring in this phenotype, but the enhanced efficacy and molecular changes observed in *Xa7* interactions have not previously been compared in interactions with other *R* genes. The impacts of high temperatures on rice and bacterial blight disease for other *R* genes including *Xa3*, *xa5*, *Xa10*, and *Xa21* will be presented.

P5.7-010

RASPBERRY PHYSIOLOGY AND LATE LEAF RUST SEVERITY IN PLANTS UNDER WATER STRESS AFTER THE PATHOGEN INFECTION

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Text

Aculeastrum americanum is the causal agent of late leaf rust. The fungus penetrates raspberry leaves only through the stomata. In raspberries under water deficit, the penetration capacity of *A. americanum* is drastically affected, due to stomatal closure, leading to less disease severity. The study aimed to investigate what happens with the plant and the disease when water limitation occurs after pathogen penetration. For this, we compared epidemiological parameters of the disease and the gas exchange of inoculated plants ($5x10^4$ mL^{-1} urediniospores of *A. americanum*) submitted to two water conditions: 30% and 60% of soil water storage capacity (WSC) after inoculation. The plants grown without water limitation exhibited higher disease severity and lesion density than plants grown at 30% WSC. The production of urediniospores per lesion did not differ between treatments. Our results revealed that under water stress there was reduction in the leaf gas exchange since the beginning of the experiment. Consequently, the development of these plants was drastically affected, which could have interfered with the severity of the disease.

P5.7-011

APOPLASTIC METABOLIC AND PROTEOMIC CONTENTS ARE AFFECTED BY ABIOTIC STRESS AND CONTROL PATHOGEN VIRULENCE.

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Text
Plant responses to pathogen invasion leads to the production of defence proteins, metabolites and hormones. Since the apoplastic space represents the first layer of defence, important changes take place in this compartment. These changes have not been described yet in the case of necrotrophic fungal pathogens that kill host cells rapidly and proliferate on dead tissues. However, the initial phase of the interaction is critical since these pathogens have to cope directly with plant defenses, in particular in the apoplastic space. In this study we describe for the first time the modifications that occur in the apoplast of Arabidopsis thaliana in this initial phase of infection by two necrotrophic pathogens, E. amylovora and B. cinerea. Our results showed that important changes are triggered in the first hours of infection at the proteomic and metabolomic level. In parallel, we analyzed the impact of nitrogen nutrition on apoplast content. N is an essential nutrient not only for plant development but also for defense against abiotic and biotic stress. Changes in the plant apoplast status in response to E. amylovora colonization are highly beneficial to the hrp-dependent bacterial cycle under low N. Taken together, this cartography of the early apoplastic molecular dialogue between plants and pathogens revealed putative defence, susceptibility and virulence factors that play a critical role in the outcome of interactions and on the impact of abiotic stress on these interactions.

P5.7-012

EFFECTS OF COMBINED ABIOTIC AND PATHOGEN STRESS IN POMEGRANATE (PUNICA GRANATUM L.)

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Text
Plants are often challenged by stresses acting in combination and the response to combinatorial stress differs from that triggered by each factor individually. Pomegranate cultivation in Italy is currently limited by the emerging *Coniella granati* and *Phytophthora palmivora* pathogens. It is ignored if they benefit from previous stress caused in pomegranate by periods of heavy rainfall or intense drought. We hypothesized that drought and waterlogging experienced by plants will increase damage caused by subsequent infection. In June 2022, two-year-old plants were subjected to regular watering (C), drought (D) and waterlogging (W) conditions and stem-inoculated, individually or simultaneously, with *C. granati* and *P. palmivora* (n=10) or with a PDA plug as control (n=10). Lesion length in the stem was assessed and used as a proxy of plant susceptibility. W prior to *C. granati* infection significantly increased lesion length (140%), and D prior to *P. palmivora* infection significantly decreased lesion length (60%), in relation to C-infected plants. Lesion length caused by *C. granati* significantly increased if plants were co-infected by *P. palmivora* and this was dependent on conditions experienced by plants before infection (ca. 100, 50 and 30% for C, D and W plants, respectively). Lesion length caused by *P. palmivora* was not altered if plants were co-infected *C. granati* irrespective of the conditions experienced by plants before infection.

P5.7-013

ANALYZING THE GENOMIC VARIATIONS IN THE PATHOGENIC POPULATION UNDER HOST GENOTYPE X ENVIRONMENT X PATHOGEN INTERACTIONS

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Text

Climate change accompanied by modern agricultural practices has presented a threat of emerging novel pathogen lineages capable of compromising host resistance or expanding host range. Here, we tested the influence of altered ozone levels on plant disease development and pathogen evolution under otherwise natural field conditions using *Xanthomonas perforans*, a causal agent of bacterial leaf spot disease, on resistant and susceptible pepper cultivars. We observed significantly higher disease severity, but with more variations, under concurrent stress conditions, which led us to hypothesize that pathogen populations adapt by accommodating higher plasticity. It would reflect in altered ecological interactions among pathogen genotypes and overall pathogen diversity at the genomic levels. Our data revealed high strain turnover in the pathogen community under concurrent stress by the end season, accompanied by high and variable nucleotide diversity and mutation rates in contrast to the individual stresses. The signatures of parallel evolution with positive selection of different alleles at high frequency along with a high degree of flux in dispensable genes and reduction in certain high-cost effector genes to possibly limit the fitness constraints. Together, increased ecological and evolutionary plasticity was obsered for pathogen adaptation to resistant host and altered ozone, indicates to further examine the outcomes of this plasticity in evolution of virulence.

Plant protection potential of persistent (cryptic) viruses in fungi, plants and insect vectors of plant disease

C7.2-1

EXPLORING MYCOVIRUS-MEDIATED HYPOVIRULENT STRAIN AS PLANT VACCINE TO CONTROL CROP DISEASE

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Text
Mycoviruses can be found in almost all kinds of fungi, they are rich in diversity and have been assigned into 29 families by ICTV in 2022. A few of mycoviruses that infect plant pathogenic fungi could cause hypovirulence, and can be potentially used to control fungal diseases. One of the strategies to control diseases with mycoviruses is to target on fungal pathogen, using mycovirus to weaken fungal virulence. However, because the transmission of mycoviruses is limited by host vegetative incompatibility system, this fungi-target strategy has not been widely adopted in field. Previously, we isolated a hypovirulence-associated DNA mycovirus (SsHADV-1) from a hypovirulent strain of Sclerotinia sclerotiorum. We found that SsHADV-1 could convert its host from a necrotrophic pathogen to a mutualistic endophyte; when virus-infected-strain growing on plants, it could enhance plant resistance and promote plant growth. We then, developed virus-infected strain as plant vaccine and used it to inoculate rapeseed seeds, and found that the seed yield of rapeseed was significantly improved in the field. The vaccine strategy targets on both plant and pathogens and overcomes the biocontrol efficiency issue limited by mycovirus transmission. Importantly, the vaccine strategy can be used widely in the field since more and more hypovirulence associated mycoviruses have been found to have ability to convert its host to mutualistic endophyte on plants.

C7.2-2

EFFECTS ON THE SPOROGENESIS AND BIOCONTROL FUNCTIONS OF TRICHODERMA SPP. BY THE MYCOVIRUSES

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Text
Trichoderma spp. are known to impact host plant growth and defense activities. With the findings of the mycoviruses from Trichoderma spp., more and more effects on the sporogenesis and biocontrol functions of host induced by mycoviruses were known. In our researches, Trichoderma harzianum partitivirus 2 (ThPV2) from *T. harzianum* strain (T673) showed higher conidiospore, and chlamydospore production than the virus-infected strain T673-F, and moderately but statistically significantly improved biocontrol activity when compared with the T673-F in the experiments with cucumber seeds inoculated with *Fusarium oxysporum* f. sp. *cucumerinum*. Trichoderma harzianum mycovirus 1 (ThMV1) from *T. harzianum* strain (T525) not only affected the phenotype of the host strain but also reduced its biocontrol function on *F. oxysporum* f. sp. *cucumerinum*. The yeast two hybrid nuclear system screened the hexokinase1 (HXK) also was the interacted protein with ThMV1-CP, Then the hexokinase gene knockout mutant (T525^{-hxk}) and reverse mutant (T525^{Rhxk}) of T525 were obtained. connecting the results of transcriptomic analysis, Real-time RT-PCR and the phenotype of cucumber, the interaction of ThMV1-CP/HXK in T525 was proved to negatively regulate the disease resistance pathway of T525 and positively regulate the growth promotion effect of T525 in the cucumber.

C7.2-3

CONDITIONAL MUTUALISM BETWEEN GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS AND VITIS VINIFERA CONFERS TOLERANCE TO DROUGHT AND FUNGAL DISEASES

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Text
Plants and phytobiome (composed by microbiome and macrobiome) interact with each other and with the environment, modulating plant responses to biotic and abiotic factors. In this framework, plant–virus interactions can be addressed not only from the point of view of a classical binary relationship (host–pathogen) but also as a single microecosystem adapting to the external environment. Viruses are not necessarily detrimental in all situations, indeed, in specific cases they could display a mutualistic relationship according to their host and the environment. An interesting example of this phenomenon is related to grapevine rupestris stem pitting-associated virus (GRSPaV), one of the most prevalent viruses that infect grapevines, usually found in Vitis vinifera cultivars in a latent state, without the development of macroscopic phenotypic alterations. Eco-physiological analyses proved that GRSPaV-infected grapevines exposed to drought stress had higher rates of photosynthesis and stomatal conductance, low hydraulic resistance to water transport, and increased ability to extract water from the soil. GRSPaV also alters the profiles of several genes and microRNAs that act as important players in the regulation of the interplay between GRSPaV and drought in grapevine. Furthermore, multiple interactions among grapevine, GRSPaV, powdery and downy mildews revealed an interesting cross-reaction among different pathogens that could be useful for implementing pest management strategies.

C7.2-4

INVESTIGATING PLANT PERSISTENT VIRUSES IN PEPPER

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Text
Plant viruses have been mostly studied for their role as pathogens. However, research on plant-viral interactions in populations of wild plants has shown that some viruses have non-pathogenic effects on their hosts. Plant persistent viruses (PVs) are vertically transmitted, i.e., inherited through pollen and seed, and are not infectious. PVs cause little or no disease symptoms in their hosts, and it has been hypothesised that these RNA viruses are mutualists rather than pathogens. Building on these ideas, PV-mediated effects on host phenotypes are being investigated in pepper (Capsicum annuum). Using RT-PCR, genomic RNAs of Partitiviridae and Endornaviridae were detected in eight accessions of pepper. PV RNAs were observed in all tissues of PV-harbouring lines. Virus-virus interactions between PVs and pathogenic viruses were investigated in pepper using tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV). Preliminary experiments show that TMV and CMV RNA levels were lower in PV-harbouring pepper varieties compared to PV-free varieties, possibly due to interaction between PVs and pathogenic viruses. To improve future analyses, virus-induced gene silencing using tobacco rattle virus vectors has been used successfully to

generate isogenic PV-harbouring and PV-free pepper plant lines. The effects of PVs on a broad range of host traits, from seed yield, resistance to aphids and aphid-vectored pathogens, to drought tolerance are being examined using these isogenic lines

C7.2-5

LATENT VIRUS INFECTION IN INSECT PESTS: THE CASE STUDY OF IFLAVIRUSES AND PHYTOPLASMA VECTORS

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Text

The advent of Next-Generation Sequencing (NGS) technologies applied to viral metagenomic analyses of insect pest populations has significantly accelerated the rate of virus discovery and opened new avenues for the exploitation of virus-based tools to counteract plant diseases and insect pests. Among the vast number of newly identified viruses infecting insect vectors, members of Iflaviridae, a family of nonenveloped, single-stranded RNA viruses that infect arthropods, were frequently detected. Iflaviruses often cause mild or asymptomatic infections and are frequently found in mixed infections within the same host. Investigating the effects of covert infections on insect transmission capabilities and fitness, alone or in combination with other stress factors, is an important step to describe the interactions between insect viral communities, the host insects, and the plant pathogens they transmit. The case study of the Euscelidius variegatus Virus 1 (EVV1), an iflavirus causing covert infection in the leafhopper *Euscelidius variegatus*, vector of phytoplasmas, provides valuable insights into the role of latent infections in shaping insect ecological traits and possibly phytoplasma transmission ability. Finally, EVV1 may be used as a tool of Virus-Induced Gene Silencing (VIGS) to alter the expression of insect genes involved in plant pathogen transmission, thus paving the way to transmission suppression.

C7.2-6

THE MISCELLANEOUS MYCOVIROME ASSOCIATED TO THE PLANT PATHOGENIC FUNGUS ERYSIPHE NECATOR

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Text

Erysiphe necator is a biotrophic fungus causing powdery mildew in grapevine, affecting different parts of the plant. Grapes and leaves with powdery mildew were collected in the

major grapevine areas of Spain and Italy. A total of 220 samples were collected and 101 samples were selected. Total RNA extracted from the 101 samples were mixed in 17 pools for next generation sequencing. The results of each pool were analysed separately to identify the virome associated to each one, resulting in 17 distinct lists of viruses. The degree of contamination with plant and other organisms was determined, but all samples were mainly represented by E. necator. To avoid erroneous host assignment, all viruses were named as “Erysiphe necator associated” to maintain open the possibility that the real host could be grapevine or some other organisms present in the samples. A total of 514 viruses were identified in our samples, 429 with a genome of positive single strand RNA (ssRNA (+)), 41 with double-stranded RNA (dsRNA) and 44 with negative-sense RNA (ssRNA (-)). Most of the ssRNA (+) viruses belong to the phylum Lenarviricota while ssRNA (-) viruses were placed in the order Bunyavirales inside the phylum Negarnavircota. Grapevine viruses, such as Grapevine leafroll associated viruses, Grapevine rupestris stem pitting associated virus or Grapevine fanleaf virus, and other plant viruses were also associated to the analysed samples. The phylogenetic relationship of the viruses will be discussed.

F7.2-1

DISTINCT PERSISTENT INSECT VIRUSES CHARACTERIZE LOCAL POPULATIONS OF TOSPOVIRUS-TRANSMITTING THRIPS SPECIES

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Text

Frankliniella occidentalis (WFT) and *Thrips tabaci* (OT) are insect species that greatly impact horticultural crops through their transmission of tomato spotted wilt virus (TSWV) and iris yellow spot virus, members of the family *Tospoviridae* in the *Bunyavirales*. We have identified 64 viral segments in samples from 12 populations of OT and WFT from Italy, corresponding to 41 viruses. Fifteen were assigned to WFT, and 17 to OT, while 9 viruses could not be assigned to any species based on our stringent criteria for host association. All these viruses are putative representatives of new species, and some are the type members of new higher-ranking taxa. Repeated sampling in a subset of locations, and further virus characterization in a subset of four populations, reared in laboratory on a controlled diet for more generations, provided evidence of a locally persistent thrips core virome that characterizes each population.

Two WFT populations differentially infected by a virgavirus, a mononegavirus and a densovirus were tested for their efficiency in transmitting TSWV; some preliminary results indicate that densovirus infection inversely correlates with TSWV accumulation and transmission. Furthermore, the only virus that persisted over one year in an OT population is a member of the family *Mitoviridae*, among the first viruses of this family associated with insects: here we present different approaches to determine its host, tissue and subcellular localization unequivocally.

P7.2-001

THE DIVERSITY OF VIRAL COMMUNITY IN APHIDS, VECTORS OF THE BARLEY YELLOW DWARF VIRUS, REVEALED BY METATRANSCRIPTOMICS

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Text

Plant viruses are closely linked to aphid virome and plant virome because plants and aphids regularly and efficiently exchange and transmitted viruses. However, few studies on aphid viromes have been reported. Therefore, high-throughput sequencing (HTS) was used to monitor viral community in three species (Rhopalosiphum maidis; RM, Rhopalosiphum padi; RP and Sitobion avenae; SA) of aphids known to transmit barley yellow dwarf virus (BYDV). The paired end sequencing of the library with Illumina HiSeq 4000 resulted in raw data of 7.68 Gb for RM, 7.65 Gb for RP and 7.65 Gb for SA, respectively. In this study, virus populations were identified from three aphid libraries, and barley virus G (BVG), BYDV-PAV and sugarcane yellow leaf virus in RM, BVG in RP and BYDV-PAS and BYDV-PAV in SA. Most viral genomes were successfully assembled de novo using metagenomic analysis. To confirm the presence of the plant viruses identified in the three aphids by HTS, specific primers for each virus were designed and RT-PCR was performed to confirm of the viruses. In addition to plant viruses, hubei wuhan insect virus, wuhan aphid virus, wuhan house centipede virus, wuhan insect virus were also identified in the aphid virome. These results show that the HTS-based metatranscriptomic analysis was a reliable and powerful tool for detection and identification of viruses in aphids.

P7.2-002

INVESTIGATING MYCOVIRUS-MEDIATED SYSTEMIC RESISTANCE IN OILSEED RAPE

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Text

Oilseed rape, *Brassica napus*, is an important and popular food crop. Fungal diseases of oilseed rape, namely light leaf spot (caused by *Pyrenopeziza brassicae*) and phoma stem canker (caused by *Leptosphaeria maculans* and *L. biglobosa*), inflict >£100M annual yield losses in the UK alone.

This project involves screening and characterising mycoviruses in the three fungal pathogens and quantifying how the plant host recognises and responds to each fungus. Virus-infected and virus-free isogenic lines of a Chinese *L. biglobosa* isolate (W10) have been resuscitated to inoculate plants of the oilseed rape cultivar Charger. Samples were collected from distinct parts of the plants and will be compared in terms of gene regulation using RNA-seq and quantitative PCR.

Field isolates across Europe and Canada were collected and, to date, 48 *L. maculans*, 63 *P. brassicae* and 19 *L. biglobosa* isolates have been screened. Six UK *L. biglobosa* isolates have been confirmed to contain LbQV-1, and one Canadian isolate contained a novel

polymycovirus, as shown by Sanger sequencing. Nine UK *L. maculans* isolates showed the first incidence of a large dsRNA species. None of the *P. brassicae* isolates from six countries across northern Europe appeared to be virus-infected.
Further work involving genome walking will be done to fully characterise the novel viruses identified. Virus-infected and virus-free isogenic lines are also being produced using cycloheximide treatment to investigate their pathogenicity.

P7.2-004

EXPANDING THE SPECTRUM OF HOSTS FOR FUSARIUM POAE VIRUS 1 TO OTHER FUSARIUM SPECIES

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Text
Fusarium poae virus 1 (FpV1), is one of mycoviruses that is discovered earlier. Due to the vegetative incompatibility barrier that often exists between different species or strains of filamentous fungi, FpV1 is thought to be limited to its host, F. poae, as a non-hypovirulence mycovirus in the past 20 years. Here, a novel strain of FpV1 (FpV1-Fa) with two dsRNA segments (2157- and 2080-nt), was consistently identi?ed in *F. asiaticum* isolates in the field. FpV1-Fa induced abnormal morphology and hypovirulence of *F. asiaticum*, along with a high viral load. FpV1-Fa was detected only from the *F. asiaticum* and *F. tricinctum* strains at FpV1-Fa sampling site (119.014289, 33.8261), while the other strains from other sites were not identified FpV1-Fa. Horizontal transmission experiment showed FpV1-Fa can be transformed from *F. asiaticum* to *F. poae* and *F. tricinctum* but not *F. graminearum*. The selection analysis of FpV1-Fa revealed RdRP and CP were under strong purifying selection, and the C-terminal side of RdRP was under positive selection. In these regions, 9 amino acid mutations in RdRp and 21 mutations in CP appeared to cause the variation of host range and virulence in FpV1-Fa.

P7.2-005

DIFFERENCES IN MYCOVIRAL CONTENT UNDERLIE PHENOTYPE AND VIRULENCE CHANGES IN BOTRYTIS CINEREA ISOLATES

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Text
Botrytis cinerea is one of the most important plant-pathogenic fungi, causing the gray mold disease in more than 200 crops worldwide. In recent years, mycoviruses gained interest as potential biocontrol agents, since they can induce hypovirulence in its fungal hosts. In a previous study, a total of 248 B. cinerea field isolates were collected from vineyards in

different main regions of Italy and Spain. These field isolates were mixed in 29 pools and their mycovirome was analyzed by next generation sequencing (NGS). Mycoviruses, putative related to hypovirulence in B. cinerea or other different fungal hosts, were identified in these analyses. Several mycoviruses were selected for further studies based on biological, molecular and evolutionary aspects. The presence of these mycoviruses was confirmed by PCR detection in field isolates. Subsequently, the mycoviral content of these independent field isolates, and the new obtained single-spore and single-protoplast isolates was analyzed by NGS. These isolates showed differences in growth and virulence, then, further analyses are in progress to determine if the observed differences can be associated to the presence or absence of specific mycoviruses.

P7.2-006

RELEVANCE OF CAPSID PROTEIN ON BOTRYTIS VIRUS F REPLICATION AND DISPERSION INSIDE THE HOST

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Text
Mycoviruses infect filamentous fungi, yeast and oomycetes and some of them have been shown to affect the phenotype and virulence of the infected hosts. The induction of hypovirulence in their host makes them a very attractive target to be used in biocontrol strategies. Then, most research efforts have been focused on understanding the phenotypic effects they cause in the fungus and not on their molecular biology. Although much has been studied regarding the function of capsid proteins in plant viruses, not much is known about the specific function in mycoviruses, particularly regarding the transmission and maintenance of mycoviral infection. In plants, viral capsid proteins have been shown to be essential for extracellular infection, but cell-to-cell movement relies on movement proteins, exemplified by the ability of a capsidless tobacco mosaic virus to infect and spread through a plant. Botrytis virus F (BVF) is a positive-sense, single-stranded RNA virus within the Gammaflexiviridae family of the plant-pathogenic fungus Botrytis cinerea. BVF genome contains two open reading frames encoding an RNA dependent RNA polymerase (RdRp) and a coat protein (CP). We addressed the effect of several mutations on the CP gene to determine whether these modifications affect or not the ability of BVF to replicate or move inside the fungal host.

P7.2-007

MOLECULAR CHARACTERIZATION OF THE FIRST PARTITIVIRUS FROM A CAUSAL AGENT OF SALVIA MILTIORRHIZA DRY ROT

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Text

The root rot of *Salvia miltiorrhiza* is a common root disease caused by *Fusarium* spp., which has become one of the main diseases affecting the production of *S. miltiorrhiza*. Currently, several hypovirulence-related mycoviruses have been identified in many phytopathogenic fungi including *Fusarium* spp., which showing potential as biological control. In this study, we report a new mycovirus, Fusarium oxysporum partitivirus 1 (FoPV1), isolated from *F. oxysporum* strain FCR51, a causal agent of *S. miltiorrhiza* dry rot. The FoPV1 genome contains two double-stranded RNA segments (dsRNA1 and dsRNA2). The size of dsRNA1 is 1773bp, and it encodes a putative RNA-dependent RNA polymerase (RdRp), The dsRNA2 is 1570bp in length, encoding a putative capsid protein (CP). Multiple sequence alignments and phylogenetic analyses based on the amino acid sequences of RdRp and HP indicated that FoPV1 appears to be a new member of the family *Partitiviridae* that is related to members of the genus *Gammapartitivirus*. Assay of pathogenicity showed that FoPV1 confers hypervirulence to its host, *F. oxysporum*. This is the first report of a partitivirus infecting *F. oxysporum* and the first hypovirulence-related mycovirus from causal agent of *S. miltiorrhiza* dry rot.

P7.2-008

ENGINEERING OF THE CANNABIS CRYPTIC VIRUS: FIRST SUCCESSFUL CLONING OF A DSRNA VIRUS IN A PLANT SYSTEM

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Text

Cannabis cryptic virus (CanCV) belongs to the Betapartitivirus genus within the *Partitiviridae* family. Its genome consists of two double strand (ds) RNA molecules, each of which is encapsidated in independent non-enveloped icosahedral particles. Each single positive sense strands RNA (ssRNA (+)) is monocistronic: the longer one (2397 nts) codes for the RNA dependent RNA polymerase (RdRp) while the shorter one (2266 nts) codes for the coat protein (CP). CanCV life cycle is restricted to the cytoplasm and the virus propagates in the plant exclusively during cell division of meristematic cells. Furthermore, CanCV is transmitted only vertically, it is persistent and asymptomatic. Such biological characteristics make this virus a good candidate viral vector for plant genetic engineering and functional genomics in *Cannabis* spp. through the virus mediated expression of heterologous sequences. For this purpose we cloned the cDNA copy of each CanCV ssRNA(+) into an *Agrobacterium tumefaciens* binary expression vector. Agroclone infectivity was tested in *Nicotiana benthamiana* by their co-infiltration in mature leaves with or without the transient expression of Tomato bushy stunt virus p19, used to suppress the sense transgene-induced post-transcriptional gene

silencing. CanCV particles were then purified and observed by transmission electron microscopy. RNA extraction, DNase I and RNase T1 digestion were performed to quantify viral dsRNA in the purified particles.

P7.2-009

THE GENE FUNCTION OF BDCV1-DERIVED SRNA5636 IN BOTROSPHAERIA DOTHIDEA WITH MYCOVIRUSES

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Text

Pear ring rot disease induced by Botryosphaeria dothidea is one of the important diseases, which has seriously hindered the development of pear industry. Mycovirus-mediated hypovirulent strain is a novel idea and an effective measure used for the biological control of pear ring rot disease. Increasing evidences showed that vsRNAs play important regulatory roles in disease-resistant defensive response. Therefore, it is helpful to explore the possibility of mycovirus to prevent and control the disease by manipulation vsRNA genes. In this study, obtaining the hypovirulent LW-C with BdCV1?and the candidate vsR5636 involved in the interaction between mycovirus and B. dothidea. It is verified that the existence of vsRNA5636 was dependent on BdCV1 in B. dothidea strains, whose expression level was affected by BdAgo3. The predicted targets of BdCDKc (Cyclin-dependent protein kinase complex) mediated by vsRNA5636 in B. dothidea was verified by 5' RLM-RACE. It showed that gene expression levels of BdCDKTc mRNA and BdCV1 RdRp were decreased, meanwhile LW-1 mycelia vegetative growth rate became fast, the pathogenicity was enhanced. It was more sensitive to cell wall and cell membrane stress in LW-1/OE-vsRNA5636. It indicated that vsRNA5636 mediated BdCDKc and BdCV1 involved in antiviral response and host biological regulation. It provided valuable genetic resources with the mycovirus-mediated biological control of fungal diseases in practice by manipulation vsRNA genes.

P7.2-010

MYCOVIRUSES IN FUSARIUM GRAMINEARUM - HOST TRANSITION OF FUSARIUM POAE VIRUS 1

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Text

Mycoviral infections alter the metabolism of their hosts. This can have direct effects on the aggressiveness of phytopathogenic fungi. When the infection results in a reduced aggressiveness, this is referred to as hypovirulence.
In this study, we report the occurrence of four RNA viruses of the genera *Ambivirus*, *Mitovirus*, *Sclerotimonavirus*, and *Partitivirus* in a single isolate of *Fusarium graminearum*. Coinfection of the wild type suppressed the production of the type B trichothecenes deoxynivalenol, 3- and 15-acetyl-deoxynivalenol *in vitro*. This effect was confirmed by transfection of a virus-free strain via hyphal anastomosis. After transfection, a reduction in the production of aurofusarin, a bis-naphthopyron pigment that protects *Fusarium* fungi from a variety of animal predators, was also observed. In addition, infection of the wild type suppressed the production of an unknown volatile sesquiterpene and increased the release of 1-butanol compounds. Increased concentrations of 3-octanone and sylvestrene were also measured after transfection. Food preference experiments with springtails (*Folsomia candida*) revealed a preference for the infected wild type of *F. graminearum.*
Combining the studies on the relationship between virus infection, secondary metabolism, and food preference provides valuable information on the modulation of secondary metabolism by viruses, hypovirulence in mycotoxin-producing fungi, and changes in feeding behavior of fungivorous arthropods.

P7.2-011

INVESTIGATING THE ADAPTATION MECHANSIMS OF MITOVIRUS-INFECTED CHENOPODIUM QUINOA PLANTS TO BIOTIC AND ABIOTIC STRESS

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Text

The promotion of quinoa is part of a broader FAO strategy to encourage the cultivation of traditional, resilient crops. Other than for its high nutritional properties, quinoa is an interesting plant since it is adapted to a wide range of marginal agricultural soils, including those with high salinity and those tending to drought. We recently provided evidence of a mitovirus named Chenopodium quinoa mitovirus 1 (CqMV1) accumulation in symptomless *Chenopodium quinoa* lines. We compared two mitovirus-infected lines (Regalona and IPSP1) and two mitovirus-free lines (BO78 and BO25). The virus is apparently cryptic (no obvious symptoms) in normal growth conditions. We therefore looked at the effect of some physiological parameters in abiotic stress condition (water stress and mechanical damage) and some biotic stress (virus infections, herbivore damage, aphid colonization). We looked and characterized the volatilome and leaf ionomic profile. Furthermore, since the mitovirus limits its replication inside the mitochondria, we looked at the differential mitochondrially-enriched proteome of mitovirus-infected and mitovirus-free leaf extracts, showing some specific virus-caused down-regulation or up-regulation of a number of interesting proteins.

Finally, we began the process of obtaining mitovirus-infected and mitovirus-free quasi-isogenic lines developing markers to monitor reciprocal crosses of virus-infected and virus free lines.

P7.2-012

DIPLODIA SAPINEA IS HOSTING MULTIPLE MYCOVIRUSES

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Text

Diplodia shoot blight is an emerging disease caused by *Diplodia sapinea* which becomes pathogenic under stressful conditions. Gaining knowledge about the mycovirome of D. sapinea could help identify biological control agents. Double-stranded RNA were extracted from 110 isolates obtained from *Pinus* spp. in Europe, South Africa and America. Electrophoresis profiles of the dsRNA elements indicated the presence of mixed infections and/or potential segmented viral genomes. Twelve isolates from distinct locations in France were characterized by high throughput sequencing of purified ds RNA. Over 20 mono-and multi segmented new mycoviruses with dsRNA, ssRNA+ and ssRNA- genomes were identified. All the isolates had mixed infections except one that was single infected. On the other hand, one isolate was found to be infected with up to 14 viruses, some of which were present as several variants. The two dsRNA viruses already described in D. sapinea were found in coinfection in two isolates and individually detected in one and two isolates respectively. Three new viruses belonging to the families Polymycoviridae, Partitiviridae and Botourmiaviridae showed the highest prevalence. Specific primers were designed to assess the presence of some of the identified mycoviruses by RT-PCR. They will enable us to screen the *D. sapinea* isolates collection and study the prevalence and diversity of *D. sapinea* mycoviruses. Our results provide new hopes for potential biological control candidates.

Plant responses to pathogens

C1.3-1

SECRETION OF VIRULENCE FACTORS DETERMINES THE INTERACTION OF OPPORTUNISTIC XANTHOMONAS BACTERIA WITH THE HOST AND THE MICROBIOTA

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Text

Xanthomonas bacteria are widespread plant pathogens that cause disease on a variety of plants. We have identified opportunistic Xanthomonas in the leaf-associated microbiota of Arabidopsis thaliana, that cause disease in immunocompromised rbohD knockout plants resulting in an altered microbiota composition. However, the virulence factors of such opportunistic Xanthomonas and how they affect the overall microbial community are currently not known. Here, we found that these Xanthomonas secrete a cocktail of cell wall degrading enzymes via the type-2 secretion system (T2SS) that disintegrate leaf tissue and promote Xanthomonas growth during plant infection. Both disease and leaf degradation activity were stronger in rbohD compared to Col-0 plants, substantiating the opportunistic behaviour of these Xanthomonas strains. Gnotobiotic plant experiments using a synthetic bacterial community and drop-in of Xanthomonas wildtype and non-virulent mutants showed that the T2SS is required for plant disease and for the shift in microbiota composition. Overall, our data suggest that opportunistic Xanthomonas cause tissue damage in leaves thereby creating an environment for specific commensal bacteria to thrive.

C1.3-2

INFLUENCE OF ELEVATED TEMPERATURES ON RESISTANCE AGAINST PHOMA STEM CANKER

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Text

Cultivar resistance is an important tool for controlling pathogen-related diseases in crops. As temperatures increase due to global warming, temperature-resilient disease resistance will be important for crop protection. However, mechanisms behind temperature-sensitivity of disease resistance responses remain poorly understood in crop species, and little is known about the effect of elevated temperatures on quantitative disease resistance (QDR). The influence of elevated temperatures on both QDR and R gene-mediated disease resistance was analysed using field experiments and controlled environment (CE) inoculation assays. The impact of increased temperatures in spring or summer on the severity of phoma stem canker was of special interest, and high maximum June temperature was associated with increased phoma stem canker severity. Although higher June temperatures did decrease resistance of *Brassica napus* lines with *R* genes (*Rlm7*, *LepR3*, *Rlm4*) and with or without QDR in field experiments, resistance of cv. ES Astrid with only QDR was not affected by temperature. In contrast, resistance of cv. ES Astrid decreased when temperature increased to 25oC under CE conditions, suggesting that sustained periods of higher temperatures can weaken QDR. In the absence of QDR or R gene-mediated resistance, increased temperature decreased susceptibility to *Leptosphaeria maculans* in the field and CE conditions. Transcriptomics was used to study temperature responses of different *WAKL10* genes.

C1.3-3

IMPACT OF GLOBAL WARMING ON SUSCEPTIBILITY OF EUROPEAN MAIZE CULTIVARS TO CORN SMUT INFECTIONS

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Text

Maize is the most grown staple crop worldwide. Yet, severe yield losses are forecasted for the next decades due to man-made global warming. Rising temperatures can negatively impact yield directly and also are predicted to increase infections with plant pathogens. However, experimental data regarding the influence of climate change on plant-pathogen interactions are still missing. Therefore, we addressed this issue by infecting 17 European dent or flint maize cultivars with the corn smut fungus *Ustilago maydis* at different temperature conditions: i) Bavaria in 1985, ii) modelled conditions for Bavaria in 2050, iii) a short heat wave of three days. Besides scoring for symptoms, we measured leaf length as a proxy for biomass production, determined time of spore formation, and performed RNAseq experiments to identify differences in the transcriptional response of maize.
Our data showcase the serious consequences a small temperature increase of ~1.5 °C, as it is predicated within the next 30 years resulting from climate change, will have on plant-pathogen interactions and subsequently yields

C1.3-4

ARABIDOPSIS NAD KINASE C (NADKC): A MISSING LINK BETWEEN CA2+ SIGNALING, METABOLISM, AND PLANT STRESS RESPONSE

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Text

Plant perception and propagation of stress signals depend on calcium ions (Ca2+) and reactive oxygen species (ROS). The current model establishes that, in stressed cells, Ca2+ binds to NADPH oxidases localized on the plasma membrane, generating apoplastic ROS that can move to the neighboring cells. In turn, ROS trigger the activation of Ca2+ permeable channels, allowing the influx of Ca2+ that again stimulates ROS production. Thus, a long-distance signal is established thanks to a ROS/Ca2+ positive feedback loop, leading to signal propagation.
By a combination of proteomics, enzymology, and physiological observations, we have shown that the pathogen-triggered ROS burst in Arabidopsis seedlings is dependent on a new NAD kinase (NADKc) whose enzymatic activity is dependent on Ca2+-Calmodulin (CaM). In vivo analyses showed that the ROS burst induced by the pathogen elicitor flagellin 22 is absent in nadkc seedlings. In parallel, in concomitance with the ROS burst, wild type but not nadkc seedlings, show an increase in NADP(H)/NAD(H) ratio. The NADKc-dependent NADP/H increase is therefore required for the apoplastic ROS production in response to bacterial elicitors.

Analysis of NADKc homologues from a variety of plant species showed that this tight Ca2+-CaM control over the NADP pool is a recent evolutionary acquisition. Furthermore, a combination of plant sensor lines for Ca2+, ROS and the NADP pool are being used to unravel the long distance signaling orchestrated by NADKc1.

C1.3-5

STOMATA, KEY PLAYERS IN WHEAT RESISTANCE TO ZYMOSEPTORIA TRITICI

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Text

Septoria tritici blotch (STB), caused by *Zymoseptoria tritici* represents a threat for European wheat production as the very high natural genetic diversity of *Z. tritici* and its plasticity make this fungus capable of rapidly bypassing most disease management strategies such as fungicides and resistant cultivars. Therefore, there is an urgent need to deepen our understanding on wheat resistances to STB to develop sustainable knowledge-driven wheat resistant cultivars. We previously showed that the major *Stb16q* gene mainly stops an avirulent isolate during its penetration attempts through the stomata. Here, we investigated the resistance mechanisms of cultivars showing qualitative to quantitative resistance to *Z. tritici*. Following inoculations that either mimic natural infection or bypass the penetration stage with virulent and avirulent *Z. tritici* isolates, we studied the temporal dynamics of symptoms on quasi near-isogenic lines for five major *Stb* genes, on cultivars carrying quantitative resistances and on non-host species. The use of avirulent and virulent *Z. tritici* isolates constitutively expressing GFP allowed the quantification of the different steps of the infection cycle from spores germination to pycnidia formation. Finally, we investigated if stomatal closure plays a prominent role in these resistances. We will present how stomata, one of the most important structures for wheat growth, are most likely key determinants of the wheat/*Z. tritici* interaction.

C1.3-6

SNTOX5 MODULATES THE HOST IMMUNE SYSTEM TO INDUCE PROGRAMMED CELL DEATH AND FACILITATE MESOPHYLL COLONIZATION

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Text
Parastagonospora nodorum, that causes Septoria nodorum blotch of wheat, is known to secrete multiple necrotrophic effectors that interact with corresponding wheat susceptibility genes to induce programmed cell death (PCD). To date, necrotrophic effectors SnToxA, SnTox1, SnTox267, SnTox3 and SnTox5 have been cloned and functionally characterized. SnTox5 is the latest to be characterized and showed that in addition to induction of PCD, SnTox5 facilitates the colonization of the mesophyll tissue even in the absence of the susceptibility gene Snn5. To further investigate the role of SnTox5, we generated RNA-Seq data sets for Snn5 differential line LP29 inoculated with the P. nodorum strain Sn2000, that harbors SnTox5, and its SnTox5 disruption mutant. Samples were collected at 0,4,12,24,48,72, and 96 hours post inoculation (hpi) and differentially expressed genes of LP29 between the two treatments were evaluated. Differentially expression of 0,0,1,17,1666,11380, and 6000 genes were observed between the two treatments at each time point respectively. Gene ontology enrichment analysis showed that host defense related genes including WRKY transcription factors, WAKs and PR genes were up regulated, whereas other defense related transcription factors and negative regulators of PCD were downregulated in LP29 inoculated with Sn2000 compared to the mutant. The data suggest that SnTox5 facilitates PCD as well as mesophyll colonization by modulating components of the host immune system.

F1.3-1

HOMEOSTASIS OF AN INNATE AVR MIMIC PROTEIN MEDIATED BY SCFOSFBX388 COMPLEX BALANCES THE GROWTH AND IMMUNITY OF RICE

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Text
The ubiquitin-proteasome system is a selective protein degradation pathway, which plays a vital role in the regulation of disease resistance in plants, such as the homeostasis of NLR proteins. However, most E3 ligases, the key subunits of ubiquitination enzymatic cascade, have not been functional characterized in rice, whose genome encodes large number E3 ligases. Here, we identified an E3 ligase OsFBX388, whose downregulation resulted in cell death, enhanced resistance against fungal and bacterial pathogens, and retardation of growth. OsFBX388 interacts with an Skp1 homolog OSK25 through its N-terminal F-box. Further, we showed that OsFBX388 targets an HMA domain-containing protein OsHIPP56 for ubiquitination and degradation via 26S proteasome. Disruption of OsFBX388 resulted in the overaccumulation of OsHIPP56. Overexpression of OsHIPP56 also resulted in enhanced disease resistance without yield penalty. In addition, both OsFBX388 and OsHIPP56 are required for pattern-triggered immunity. Furthermore, OsHIPP56 interacts with another HMA-domain containing protein RGA5, the sensor NLR protein of the NLR pair RGA4/RGA5. Co-expression of OsHIPP56 with RGA4/RGA5 induces cell death in both tobacco cells and rice protoplasts, suggesting OsHIPP56 functions as an innate mimic AVR protein. Together, our results provide a new mechanism in which the E3 ligase OsFBX388 balances the growth and immunity of rice by at least partially mediating the homeostasis of the AVR mimic OsHIPP56.

F1.3-2

IDENTIFICATION AND FUNCTIONAL VALIDATION OF SOFT-ROT SUSCEPTIBILITY GENES IN THE SPICE CROP GINGER - A STEPPING STONE TOWARDS GENOME EDITING FOR SOFT ROT RESISTANCE.

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Text

Little is known about host susceptibility genes that facilitate the infection process of soil-borne, necrotrophic oomycete pathogen Pythium myriotylum Drechsler, which cause soft rot disease in spice crop ginger (Zingiber officinale Roscoe). From the differential accumulation of jasmonic acid (JA) and salicylic acid (SA) between the pathogen inoculated ginger and the highly resistant wild congener Z. zerumbet (L.) Smith, we presumed a susceptibility function for JA in Zingiber-Pythium pathosystems. This presumption was vindicated when the exogenous application of Z. zerumbet with JA produced high susceptibility to P. myriotylum. Consistent with this, the application of SA prior to JA application in Z. zerumbet reverted the susceptibility. The susceptibility function of JA was confirmed following the real time expression analysis, phytohormone quantification and histopathological methods. With this background, in ginger, we silenced four key genes involved in the JA signalling pathway using virus induced gene silencing. The silenced plants showed tolerance to P. myriotylum. Histopathology of the silenced plant showed no pathogen ingress as compared to unsilenced inoculated and mock inoculated plants. The study depicted a key role for the relative concentration of SA/JA in deciding the host response to P. myriotylum and helped to identify four putative susceptibility genes for the CRISPR based genome editing in ginger for soft rot tolerance.

F1.3-3

ZAP1 AND STE12: ANTAGONISTIC ROLES IN THE VIRULENCE OF THE DUTCH ELM DISEASE FUNGUS OPHIOSTOMA NOVO-ULMI.

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Text

Dutch elm disease, caused by the highly virulent ascomycete fungus *Ophiostoma novo-ulmi*, has devastated the American elm (*Ulmus americana*) and caused catastrophic losses throughout the range of this species. In order to better understand the molecular bases of the interaction between *O. novo-ulmi* and *U. americana*, we performed an in planta analysis of the transcriptome during the host-pathogen interaction. The results obtained for *O. novo-ulmi* enabled us to undertake the functional study of over 20 candidate genes, including the gene which encodes the transcriptional regulator Zap1 controlling the expression of genes sensitive to zinc, as well as the gene encoding the Ste12 transcription factor found exclusively in the fungal kingdom. Null mutants were generated by CRISPR-Cas9 genome editing and

inoculated into young elm saplings in a greenhouse. The delta-Zap1 mutant became more virulent than the wild-type *O. novo-ulmi* H327 strain from which it was derived, whereas the delta-Ste12 mutant was avirulent. Moreover, elm saplings first inoculated with the delta-Ste12 mutant showed increased resistance when challenged with the wild-type strain two weeks later. Ongoing work aims to identify, in these mutants, the other genes whose expression has been altered by the inactivation of the zap1 and ste12 genes.

P1.3-001

RESISTANCE OF ABACA HYBRID BC2-7 (BANDALA) TO BUNCHY TOP VIRUSES IN EASTERN VISAYAS REGION OF PHILIPPINES

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Text
Abaca (*Musa textiles* Nee) is an important fiber crop in the Philippines. Infection of bunchy top viruses (BTVs) causes significant economic losses on the abaca plantations in Eastern Visayas region. The Institute of Plant Breeding-University of the Philippines Los Baños (IPB-UPLB) developed the abaca hybrid to tolerate the BTVs that is prevalent throughout the Philippines archipelago. In Eastern Visayas, this abaca hybrid needs to be tested with the different isolates of BTVs in the region to assess their resistance thus, the resistance and reaction of abaca hybrid to BTVs was evaluated and compared to Inosa and Pacol in this study. Based on disease incidence, all BTVs isolates infected 100% of Inosa plants but none of the abaca hybrid and Pacol. BTVs symptoms appeared in Inosa 26 days after inoculation (DAI) in Leyte isolate, Southern Leyte (30 DAI), Biliran (32 DAI), and Samar (38 DAI), while no symptoms were observed in abaca hybrid and Pacol. In terms of disease reaction, abaca hybrid and Pacol showed high resistance to BTVs, whereas Inosa is highly susceptible. BTVs were detected using Polymerase Chain Reaction (PCR) with primers BBT-1 and BBT-2 in Inosa from Leyte and Southern Leyte isolates at 5 DAI, Samar (6 DAI), and Biliran (7 DAI). The use of *Musa* tagged microsatellites primes AGMI025 and AGMI026 confirmed the accuracy of BTVs detection by PCR. The study confirms that abaca hybrid and Pacol are highly resistant to all BTVs isolates in Eastern Visayas.

P1.3-002

SCREENING OF ADVANCE OIL PALM PLANTING MATERIALS FOR GANODERMA DISEASE RESISTANT VARIETIES

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Text
Ganoderma boninense, identified as a major threat to the palm oil industry in Southeast Asia. TTo date, the only practical and sustainable approach to controlling *G. boninense* is by breeding disease resistant or tolerant palm lines as one of the approaches in Intergrated

Ganoderma Management programme. However, the success of this strategy depends on the variability of *G. boninense* isolates as the pathogen is ideally suited to cope with this selection pressure through outcrossing and prolific spore production to adapt for aggressiveness traits. Therefore, the effect of the most aggressive *G. boninense* isolate was studied. One hundred and twenty-eight oil palm progenies have been tested for their resistance / tolerance or susceptible and based on the data obtained, two progenies namely TUP 1319 and TUP 1269 have been identified as potential tolerant to Ganoderma disease with infection recorded at 11.1% (TUP 1319) and 20% (TUP 1269) respectively. Two potential susceptible were also identified namely TUP 1309 and (86.67%) and TUP 1364 (83.33%). It was noted that for the potential tolerant progenies were derived from Chemara Dura background. TUP 1319 was from parental background of Chemara Dura X Ekona pisifera and TUP 1269 was derived from parental background Chemara Dura X Nigerian Pisifera. For potential susceptible progenies, TUP 1309 was derived from parental background Ulu Remis Dura X Ekona pisifera and TUP 1364 was derived from Ulu Remis Dura X Nigerian Pisifera.

P1.3-003

RESEARCH HIGHLIGHTS ON THE EVALUATION OF SOYBEAN GENOTYPES FOR RESPONSES TO PATHOGENS IN THE SOUTHERN STATES OF THE USA

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Text

Soybean is one of the most important crops in the world. However, various soybean diseases cause substantial losses annually. Analysis of plant responses to pathogens is an important step towards breeding disease resistant soybeans. Phenotyping soybean genotypes for their responses to Phakopsora pachyrhizi, the causal agent of soybean rust, was carried out using both seedling and detached leaf assays. PI 200492 (Rpp1) had near immune reaction when tested with a Mississippi isolate MS06-1b, but resistant RB lesions to four Florida isolates and one Louisiana isolate. PI 230970 (Rpp2), PI 462312 (Rpp3), PI 459025B (Rpp4), PI 567102B (Rpp6) and PI 605823 (Rpp7) had RB lesions to isolate MS06-1b, whereas Williams 82 (Susceptible) and PI 200526 (Rpp5) had susceptible TAN reactions with high disease severity and sporulation ratings to isolate MS06-1b. Screening hundreds of soybean lines for their responses to Diaporthe longicolla, a causal agent of Phomopsis seed decay (PSD), identified new sources of resistance. Although PSD is a seed disease, a cut-seedling inoculation technique was developed to rapidly evaluate soybeans for reaction to D. longicolla and identify PSD resistant genotypes without waiting a whole growing season. Nine accessions having resistant reactions to Cercospora spp., causing purple seed stain of soybean, were also resistant to PSD. These accessions could be useful in breeding programs to develop soybean cultivars with improved resistance to both seed diseases.

P1.3-005

TERATOSPHAERIA DESTRUCTANS-RESISTANT EUCALYPTUS GENOTYPE PRODUCE A WAX COMPOUND THAT INHIBITS PATHOGEN GERMINATION IN-VITRO AND IN-VIVO

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Text

Waxes on leaf surfaces are amongst the first anatomical barriers that foliar pathogens must overcome in order to initiate infection. We analysed the wax composition of *Eucalyptus* genotypes leaves having contrastanting levels of resistance to Teratosphaeria leaf blight disease (TLB) caused by *Teratosphaeria destructans*, an important disease of *Eucalyptus* in tropical and sub-tropical environments. Analysis of cuticular waxes of four *Eucalyptus* genotypes using gas chromatography-mass spectrometry revealed significant differences in the wax composition. A triterpenoid, resembling cycloartanol (CAS) was identified only in the most resistant genotype and not in the other host genotypes. The effect of five concentrations of CAS on *T. destructans* spore germination was evaluated *in-vitro* and *in-vivo* using light and scanning electron microscopy. Spore germination was significantly inhibited with increasing CAS concentrations ($P < 0.01$) in Petri dishes as well as on healthy *Eucalyptus* leaves. These results show that waxes are an important component of resistance to infection of *Eucalyptus* by *T. destructans* and that CAS inhibits spore germination and subsequent foliar infection by the pathogen via stomata.

P1.3-006

THE IMPACT OF LOW-TEMPERATURE PLASMA ON SECONDARY METABOLITES AND ANTIOXIDANT ENZYMES IN DIFFERENT HALF-SIB FAMILIES OF PINUS SYLVESTRIS SEEDS

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Text

Due to climate change, the expansion of tree pests and pathogens are expected. The novel studies are focused on anticipatory strategies that could help to strengthen tree itself resistance. Concentrations of secondary metabolites and antioxidant activity have an impact on tree resistance. In our study we used seed treatment with low-temperature plasma (CP), which induced physical stress for plant.Eleven different half-sib families of Pinus sylvestris L. were selected. The seeds were treated with CP 1 min (CP1) and 2 min (CP2). We found that seed treatment with CP had an impact on concentrations of biological activity compounds and antioxidant enzymes in 2-year-old P. sylvestris needles. We evaluated that CP1 increased (from 0.29 to 1.28 mg/g) total phenolics (TPC) in needles in five and (from 0.57 mg/g to 2.15 mg/g) total flavonoids content (TFC) in four ($p < 0.05$) half-sib families. However, in one family CP1 reduced TPC and TFC. The seed treatment with CP2 increased TPC and TFC in lower number of half-sib families than CP1. Estimation of antioxidant enzymes (CAT, APX, and POX) also showed changes between treated (CP) and control seeds in 2-year-old needles which depended on half-sib family. Our results confirmed that

short-term stressor (CP) might increase secondary metabolites (TPC and TFC), as well as concentrations of antioxidant enzymes. The results are relevant as guides for forestry industries to increase the quality of P. sylvestris seeds in danger of climate change.

P1.3-007

DISEASE RESISTANCE SCREENING IN A SUGARCANE PLANT BREEDING PROGRAMME: CAN WE DO THINGS DIFFERENTLY?

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Text

Smut caused by Sporisorium scitamineum is an important fungal disease of sugarcane. Screening for resistance in the plant breeding programme is limited by cost, difficulty, time, labour, number of genotypes and field requirements. Due to these factors, screening is delayed to later selection stages, closer to new cultivar release. Consequently, susceptible genotypes are “carried” before they can be screened and discarded. Application of new screening tools at earlier stages will result in cost savings, productivity benefits and increased numbers of resistant clones progressing to later selection stages. Near Infra-red Spectroscopy (NIRS) is a potential means of examining the interaction between sugarcane and its’ attackers. Resistance to smut comprises two separate mechanisms, external (constitutive, lateral bud based) and internal (responsive, physiological). Previous NIRS models have predicted external resistance using spectra gathered from intact dormant lateral buds. Here we describe novel methodology for screening internal resistance using leaf discs inoculated with smut teliospores, despite S. scitamineum not normally being considered a leaf pathogen. Predictive chemometric models were derived using samples with known resistance ratings from field trials and spectral data. These models are superior to those developed for external resistance. One technician can make predictions for 100 genotypes per week whereas only 300 genotypes can be screened per year in the field.

P1.3-008

IDENTIFICATION OF LEAF RUST RESISTANCE GENES IN WHEAT CULTIVARS FROM GANSU PROVINCE IN CHINA

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Text

Identification of leaf rust resistance genes in wheat cultivars from Gansu Province in China

Wheat leaf rust is one of the most important wheat diseases worldwide, which is a fungal disease caused by Puccinia triticina. It can reduce production by up to 40% in susceptible cultivars. Over the past decade, leaf rust has periodically caused severe yield losses in China. Five Chinese provinces, including Gansu, recorded high yield losses in 2012. Resistant wheat cultivars are the most economical, effective and environmentally friendly

way to control leaf rust. So, 37 main wheat cultivars from Gansu province of China and a set of 35 near isogenic lines with Thatcher background and 7 lines with known Lr genes were inoculated in a greenhouse with 22 Pt pathotypes to identify seedlings effective Lr genes. By comparing the infection types (ITs) produced on the 37 cultivars by the 22 Pt races with the ITs on the differential sets, the Lr genes were postulated. The results show that the six leaf rust-resistant genes Lr2B, Lr13, Lr16, Lr22A, Lr30 and Lr14B have been postulated in seven cultivars, either singly or in combination. No Lr gene was detected in fifteen cultivars. Fourteen cultivars were deduced containing other genes different from the 42 known Lr genes used in this study. Identification of leaf rust resistance at the adult stage indicates that 11 cultivars are highly adult–plant resistant, with potential applications in wheat production and wheat resistant cultivar breeding.

P1.3-009

DEVELOPMENT OF TESTS FOR RESISTANCE/TOLERANCE TO VIRAL YELLOWS IN SUGAR BEET

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Text

In order to find alternative solutions to the suppression of neonicotinoids in the fight against viruliferous aphids, the National Plan for Research and Innovation (PNRI) must find alternative solutions to chemical control, which are both effective and environmentally friendly.

The PNRI Yellows Resisbeet project (2021-2024), led by GEVES, in partnership with ITB, aims to develop a protocol for assessing varietal resistance/tolerance to 4 virus species responsible for virus yellow in EU: Beet Yellows Virus (BYV), Beet Chlorosis Virus (BChV), Beet Mild Yellowing Virus (BMYV) and Beet Mosaic Virus (BtMV). The ambition is to rapidly promote the inclusion of tolerant/resistant varieties in the French Catalogue and their availability to farmers.

This project has made it possible:

- to develop a method to produce inoculum, from viruliferous aphids (Myzus persicae),
- to define the parameters of inoculation in the field and under controlled conditions ensuring a homogeneous infestation of viruses and a significant discrimination of symptoms and yield between inoculated and non-inoculated modalities,
- to develop a multiplex RT-qPCR method to detect and identify these 4 viruses,
- to study the most relevant criteria for assessing varietal tolerance based on productivity data, visual or RGB ratings of symptoms, and viral load.

This genetic lever will be proposed to the Experimental Pilot Farms to develop an integrated control method in an agro-ecological context

P1.3-011

DEVELOPING A DISEASE SCREENING PIPELINE FOR RESISTANCE TO FUSARIUM HEAD BLIGHT OF WHEAT

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Text

Host resistance is the most sustainable and affordable means to manage Fusarium head blight (FHB) of wheat and other small grains. This study aimed to establish a disease-screening pipeline to effectively evaluate local wheat germplasm for resistance to FHB in South Africa. A greenhouse screening technique was developed by evaluating the pathogenicity, virulence and mycotoxigenic potential of several isolates of F. graminearum, F. pseudograminearum and F. equiseti. Three wheat cultivars were selected for greenhouse evaluation namely SST 806 (susceptible), Sumai 3 (resistant) and SST 0166 (resistance unknown). Disease parameters were determined following artificial inoculations (either a centre- or top-floret point-inoculation). Breeding line Sumai 3 demonstrated the greatest tolerance with SST0166 being moderately tolerant and SST 806 being most susceptible. Fusarium graminearum was the most virulent on all cultivars, except for Sumai 3, where F. equiseti was more virulent. Additionally, thirteen published primer pairs were selected for the screening of seven QTL positions on three chromosomes using DNA from Sumai 3, SST 0117, SST 0127 and SST 8154 (tolerant to FHB); SST 806 and SST 0166. Ten of the 13 primer pairs positively detected the target regions present in the plant material. An optimised disease assessment protocol with molecular markers provides an efficient and effective tool for the identification of plant resistance to FHB pathogens.

P1.3-012

FEEDING BEHAVIOR OF SHARPSHOOTER VECTORS OF XYLELLA FASTIDIOSA AND EMISSION OF VOLATILE COMPOUNDS EXPLAIN RESISTANCE TO LEAF SCALD DISEASE IN PLUM GENOTYPES IN BRAZIL

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Text

Plum leaf scald (PLS) is the main obstacle to plum expansion in Brazil. This disease is caused by *Xylella fastidiosa*, which colonizes the foregut of xylem-sap feeding insects and the xylem of the plants. Breeding programs identified new plum genotypes that showed no leaf scald symptoms in the field. However, the mechanisms that confer this resistance are not fully elucidated. We hypothesized that the performance of these genotypes may be related to the

vector feeding behavior influenced by the emission of volatile organic compounds (VOCs) by the host. To verify this hypothesis, we investigated the feeding behavior of sharpshooters on the PLS-resistant plum genotypes (SC7 and Zafira) and on the naturally infected cultivar (Laetitia), using the EPG technique. Also, we investigated the volatile compound profile from six plum genotypes with different resistance levels to PLS. Probing and feeding activities differed between individuals held on Zafira and Laetitia. Specifically, duration of xylem sap ingestion events differed for *B. xanthophis* held on Zafira and Laetitia. We observed that the VOCs emitted from resistant genotypes are different from susceptible cultivars. An example, cedrol was not detected in susceptible genotypes, but was found in the resistant ones. We will test these substances in the olfactory assays. If these compounds prove to be efficient, foliar applications can be carried out in order to repel the vectors, preventing the *X. fastidiosa* transmission.

P1.3-013

UNDERSTANDING PYRENOPEZIZA BRASSICAE POPULATIONS FOR EFFECTIVE CONTROL OF LIGHT LEAF SPOT IN WINTER OILSEED RAPE

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Text

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is the most economically damaging disease of oilseed rape (*Brassica napus*) in the UK. Fungicide insensitivity development highlights the need for non-chemical controls like host resistance. Currently, there is limited information on virulence of UK *P. brassicae* populations, which is crucial for effective use of host resistance. To study pathogen populations, isolates from different regions and different hosts were tested in glasshouse experiments. *P. brassicae* isolates were obtained from oilseed rape and kale cultivars across England, and other European isolates were acquired through Rothamsted Research. A total of 24 *P. brassicae* isolates were tested on a differential set of nine oilseed rape cultivars/lines. In addition, field experiments were done in England for the 2021/2022 cropping season at Hereford and Huntingdon with cultivars Aquila and Flamingo. Disease severity was assessed by measuring disease score (scale 1-8, with 1 being resistant), percentage area with sporulation on leaves and presence of necrotic flecking (collapsed epidermal cells). Results from glasshouse experiments showed differences in disease severity between both cultivars and isolates. Results from field experiments differed between locations and cultivars, suggesting variations in pathogen populations between locations. Genotypic differences between *P. brassicae* isolates will be studied using molecular techniques.

P1.3-014

A NOVEL RESISTANCE PATHWAY IN N-GENE TOBACCO AGAINST TMV IDENTIFIES AN OLD INHIBITOR AND AN EARLIER FUNCTION

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Text
The transcription factor (TF) SHE1 is induced by TMV infection of *N* gene tobacco. SHE1 is involved in a novel resistance pathway and interacts with the inhibitor of virus replication (IVR), in vivo and in vitro. SHE1-overexpression (OEx-SHE1) in tobacco reduced susceptibility to TMV infection, whereas SHE1-silencing (si-SHE1) in tobacco had no local effect on TMV infection but resulted in a slow systemic infection. OEx-SHE1 tobacco plants constitutively expressed IVR, whereas si-SHE1 tobacco plants inhibited the expression of IVR. SHE1 expression occurs earlier than IVR expression. IVR was shown to comprise the C-terminal 34% of anaphase-promoting complex 7 (APC7), part of the cellular cyclosome, a 13-subunit E3-ubiquitin ligase controlling the progression of mitotic division. APC7 contains six tetratricopeptide repeats (TPRs, each containing a helix-turn-helix structure). The 199-amino acid IVR contains 10 helices (3.5 TPRs and three additional single helices), as well as an unstructured 28-amino acid C-terminus. The C-terminal half of IVR contains the SHE1 interaction site. The sequences of the tobacco *APC7* gene, upstream of the IVR coding region, contain several putative promoter sites for various TFs including four GCC binding elements for SHE1, upstream of putative transcription start sites. We propose that IVR binding to SHE1 acts to prevent further SHE1 expression, a classic example of end-product inhibition.

P1.3-015

TOBACCO MYB TRANSCRIPTION FACTOR NTMYB92 IS INVOLVED IN THE NEGATIVE REGULATION OF N GENE-INDEPENDENT VIRUS RESISTANCE

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Text
MYB transcription factors regulate gene expression by binding upstream cis-element in biotic and abiotic stress responses. However, the virus resistance-related MYB transcription factors in tobacco are poorly understood. In the present study, we isolated cDNA of an MYB transcription factor gene (Nt*MDP92*) whose transcript level decreased during *N*-gene-mediated resistance induction in tobacco mosaic virus-infected *Nicotiana tabacum*. Next, we examined the intracellular localization of NtMDP92 fused to YFP in *N. benthamiana* and showed that MDP92 localized in the nucleus. To further investigate the biological function of *NtMDP92*, we performed transient co-expression of *NtMDP92* cDNA and an *N* genomic sequence, together with a GFP-encoding infectious clone of tomato mosaic virus (*ToMV-GFP*) or potato virus X (*PVX-GFP*) in *N. benthamiana*. As a result, transient expression of *NtMDP92* increased the fluorescent area of GFP and the transcript level of *GFP*, with or without co-expression of the *N* genomic sequence. Based on these results, we suggest that

NtMYB92 is the transcription factor involved in the negative regulation of *N*-independent virus resistance.

P1.3-016

ASSESSMENT OF QUANTITATIVE RESISTANCE AGAINST PYRENOPEZIZA BRASSICAE IN BRASSICA NAPUS TILLING MUTANTS

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Text

Pyrenopeziza brassicae is a pathogenic fungus responsible for light leaf spot disease, one of the most important diseases affecting winter oilseed rape (*Brassica napus*) crops across the UK and Europe, currently considered the most economically damaging disease affecting the UK crop. Current cultural and chemical control practices are insufficient to manage epidemics of the disease, therefore genetic host resistance against *P. brassicae* has become of increasing importance as an effective control strategy. In a previous study, screening of 195 lines of *B. napus* against *P. brassicae* revealed eight gene expression markers (GEMs) significantly associated with infection. One of them, an HXXXD-type acyl transferase gene, was negatively correlated with quantitative disease resistance, suggesting its function as a potential susceptibility gene. The expression of this gene was induced during infection in susceptible, but not in resistant lines, of *B. napus*. Screening of a TILLING mutant with a D167N substitution in the HXXXD-motif was performed in a controlled environment via spray inoculation with *P. brassicae* and the resistance phenotype evaluated using visual assessment and the counting of spores released from acervuli. The D167N mutant was significantly more resistant than background cv Cabriolet when using visual assessment (P=0.0283) or spore counting (P=0.0190), supporting our hypothesis that the HXXXD-type acyl transferase acts as a susceptibility factor against *P. brassicae.*

P1.3-017

IS THERE A POTENTIAL FOR A GENETIC CONTROL OF THE BARLEY DISEASE, RAMULARIA LEAF SPOT?

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Text

Since the 1980's, barley production across many regions in the world, has been facing challenges posed by the fungus *Ramularia collo-cygni* (*Rcc*), which causes Ramularia leaf spot (RLS). The appearance of fungicide resistance in *Rcc* populations, together with the lack of known genetic resistance in widely grown barley varieties, indicates limited options to control this disease in the medium to long-term. This highlights the importance of investigating the potential control of RLS via host genetics and an improved understanding of the host/pathogen interaction. We identified one major QTL involved in RLS resistance at the end of the barley chromosome 4H, by using a genome-wide association study on 238 spring barley varieties. Based on this study, a subset of the identified spring barley cultivars was tested in field trials in both Scotland and Germany between 2021 to 2022. The results on disease development support the conclusion that resistant QTL carrying barley lines exhibit an increased tolerance to RLS. Moreover, results from controlled environment experiments further support this link. We further found that *Rcc*-DNA levels in planta did not correlate with symptom expression, under both controlled and field conditions, suggesting that endophytic colonisation by the fungus may not always lead to the appearance of symptoms. Future research will investigate what triggers symptom expression in barley and elucidate associated factors in both host and pathogen.

P1.3-018

PHILIPPINE MUSA BALBISIANA ACCESSIONS: KEY SOURCES OF RESISTANCE AGAINST BANANA BUNCHY TOP VIRUS

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Text

Cultivars with B genome component are reportedly less susceptible to infection by banana bunchy top virus (BBTV). In the Philippines, the 'Lakatan' (AAA) is very susceptible to BBTV while the 'Saba' (BBB/ABB) may escape field infection for years. Wild progenitors of bananas, two M. acuminata ssp. errans (AA) accessions and 34 M. balbisiana (BB) from the germplasm collections of the Institute of Plant Breeding, UP Los Banos were evaluated for disease response against BBTV through artificial inoculation. Symptoms were monitored and plants were indexed by PCR three and six months after inoculation. All M. acuminata ssp. errans and all Lakatan controls were infected with BBTV, whereas all plants of M. balbisiana accessions remained uninfected. M. balbisiana plants were transferred to a field under high BBTV inoculum pressure and remained uninfected for up to five years, while all Lakatan control plants were infected after two months. To our knowledge, this is the first report of apparent immunity to BBTV and these M. balbisiana accessions are further studied to uncover novel sources of BBTV resistance genes for functional genomics research, genome-wide association studies, and marker-assisted plant breeding applications.

P1.3-019

A MODERATE RESISTANCE IN OAT VARIETIES TO DON PRODUCERS DOES NOT GUARANTEE A MODERATE RESISTANCE TO HT2+T2 PRODUCERS

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Text

Oat harvested from plants infested with plant pathogenic fungi within the Fusarium head blight (FHB) complex may sometimes contain high levels of mycotoxins, which makes the grain unsuitable for food and feed. Fusarium graminearum, a deoxynivalenol (DON) producer, and Fusarium langsethiae, a T-2 toxin (T2) and HT-2 toxin (HT2) producer, are commonly occurring in Norwegian oats. We have analysed grains of Nordic oat varieties and breeding lines for the content of mycotoxins and DNA of Fusarium species belonging to the FHB disease complex (Hofgaard et al. 2022). The grains were harvested from field trials located in South-East Norway in the years 2011-2020. The ranking of oat varieties according to HT2+T2 levels corresponded with the ranking according to the DNA levels of F. langsethiae. However, this ranking did not resemble the ranking for DON and F. graminearum DNA. Our results implies that a moderate resistance to DON producers does not guarantee a moderate resistance to HT2+T2 producers. Separate tests are therefore necessary to determine the resistance towards DON and HT2+T2 producers in oats. This creates practical challenges for the screening of FHB resistance in oats as todays' screening focuses on resistance to F. graminearum and DON. We identified oat varieties with generally low levels of both mycotoxins and FHB pathogens which should be promoted to mitigate mycotoxin risk in Norwegian oats.

Hofgaard, I.S., et al. (2022). Toxins. 14(5), 313

P1.3-020

AUSTROPUCCINIA PSIDII INTERFERES WITH GUAVA LEAF STOMATAL REGULATION

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Text

Several studies have shown that rust fungi negatively interfere with the host gas exchange. The objective of this study was to analyze the effect of *Austropuccinia psidii* infection, the causal agent of myrtle rust, on the stomatal control of guava leaf. Guava potted plants were inoculated with urediniospores suspension of *A. psidii* and kept under favorable environmental conditions to infection. As a control, guava plants were sprayed with distilled water and kept under the same conditions. The stomatal conductance (g_s) in healthy and diseased leaves was measured 15-20 days post-inoculation using a portable infrared gas analyzer. The measurements begun under photosynthetically active radiation of 800 µmol m^{-}

^{2} s^{-1} (30 min), which was reduced to 80 µmol m^{-2} s^{-1} (50 min) and then increased to 800 µmol m^{-2} s^{-1} (50 min), with data logging every 1 min. The analysis of the pathogen colonization was performed on leaf tissues prepared with histological techniques. Diseased leaves (mean rust severity of 20.4%) showed a reduction of 40% in g_s compared with healthy leaves. The maximum g_s values in healthy and diseased leaves were, respectively, 0.21 and 0.13 mol H_2O m^{-2} s^{-1}. Furthermore, diseased leaves closed and opened their stomata more slowly when exposed to low and high light, respectively. This stomatal impairment probably resulted from the obstruction of the substomatal chamber by pathogen hyphae, evidenced by histopathological analyzes.

P1.3-021

SCREENING RESISTANCE LOCI TO PLASMOPARA VITICOLA, THE CAUSAL AGENT OF GRAPEVINE DOWNY MILDEW, IN VITIS AMURENSIS WITH FOLIAR SPECTROSCOPY

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Text

European grapevine (*Vitis vinifera*) is highly susceptible to grapevine downy mildew (GDM, *Plasmopara viticola*) in humid regions, necessitating frequent fungicide applications for effective management at great financial and environmental expense. Breeding disease-resistant varieties can broadly improve sustainability and profitability, but progress has historically been slow due to a lack of phenotyping precision and throughput. The wild species *V. amurensis,* native to East Asia, has been identified as a source of disease resistance due to its numerous resistance quantitative trait loci (QTLs) for both GDM and other diseases, including powdery mildew, anthracnose, and white rot. We crossed *V. amurensis* '588634' with the interspecific hybrid 'NY84-0101-03' to produce 229 grape seedlings segregating for moderate and stable QTLs for GDM resistance. The goal of this project was to quickly and non-invasively screen these offspring with GDM resistance QTLs using handheld foliar spectroscopy in the visible to shortwave infrared range (SWIR, 400-2400nm) and compare speed and accuracy to traditional phenotyping methods. Our preliminary results found that wavelengths spanning the near to SWIR are associated with GDM resistance QTLs. These spectral regions are known to be associated with plant chemistry and secondary metabolite concentration. Thus, hyperspectral sensing could potentially accelerate development of disease-resistant grape varieties through rapid GDM-resistance screening.

P1.3-022

CHARACTERIZATION OF HT-RESISTANCE GENES AGAINST EXSEROHILUM TURCICUM

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Text

Northern corn leaf blight (NCLB) is one of the important maize diseases worldwide. Disease control is mainly based on the use of fungicides and resistant varieties. Curiously, the resistance reaction expressed against Exserohilum turcicum infection may differ between the major genes. Histological, epidemiological, physiological, and biochemical studies have been conducted on maize plants of the line B37 carrying the genes *Ht1*, *Ht2*, *Ht3* and *Htn1*. The histological characterization using the Chlorazol Black E staining indicates that main differences are in xylem and mesophyll colonization and strong necrosis was observed in plants carrying *Ht1*. According to the epidemiological parameters, sporulation has higher for plant carrying *Ht2* gene. Physiological measurements demonstrate that CO_2 assimilation, transpiration, and stomatal conductance were not decreased in plants carrying *Ht3*. The biochemical parameters indicate an increase in the peroxidase activity for *Htn1*, whereas H_2O_2 was localized in penetration sites in all resistant lines. Cluster analysis using histological and epidemiological parameters indicates that *Ht1* and *Ht2* are in the same group, whereas *Ht3* and *Htn1* form a second group. For the biochemical and physiological parameters, a cluster was formed within *Ht2* and *Ht3* whereas the second cluster was formed within *Ht1* and *Htn1*. In conclusion, the resistance conferred by the *Ht* genes is distinct and influences photosynthesis and sporulation.

P1.3-023

THE ROLE OF EAR DOMAIN IN PLANT IMMUNITY

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Text

Plants have evolved well-orchestrated defence strategies against pathogen attacks.Consequently, pathogens have evolved strategies to overcome these defences.A key early outcome is transcriptional reprogramming mediated pathogen effectors. this can be achieved by activation or repression.The latter involves the rapid and targeted removal of negative regulators, enabling induction of key response pathways.We previously undertook a 13-time point infection series designed to capture transcriptome changes associated with disease and defence following challenge of Arabidopsis thaliana with the virulent pathogen,P.syringae pv.tomato strain DC3000 or its non-pathogenic hrpA type III secret deficient mutant.Only1%of the transcriptome was differentially expressed between DC3000 challenged and mock treatment 3hpi, which rapidly increased to20%by7hpi and37% across the 13-time points.These represented~30%ofall the annotated Arabidopsis transcription factors in Arabidopsis. To understand the role of ABA in enhancing the susceptibility of plant to DC3000. we modelled theseTFs against genes encoding proteins involved inABA perception and signalling.The model identified as a core hub a MybTF hub encoding a transcriptional repressor domain with unusual expression dynamics, predicted to influence knownABA signalling. The key objectives are characterise this novel Myb transcription factor

and elucidate its role in DC3000's virulence strategy using a variety of genetic and physiological methods.

P1.3-024

ARE PHYTOHORMONES THE MESSENGERS IN SYSTEMIC INDUCED RESISTANCE IN AUSTRIAN PINE?

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Text

Microbe-induced whole plant-level immunity, known as systemic induced resistance (SIR), and its underlying mechanisms, remain largely underexplored in trees. We conducted a study in which we elicited a systemic defense response by inducing lower stems of 4-6-year-old Austrian pine by either wounding or inoculating with Diplodia pinea. Then, we sampled stem phloem 15 cm upstream of induction at 12 h, 24 h, 36 h, 48 h, 72 h, or 7 h days post induction to quantify systemic accumulation of phytohormones using LC-MS/MS. The acquisition and quantification methods were optimized for (±)-jasmonic acid, methyl jasmonate, jasmonoyl-isoleucine, 12-oxo phytodieonic acid, dihydrojasmonic acid, salicylic acid, methyl salicylic acid, abscisic acid, gibberellic acid (GA3), gibberellin (GA4), 3-indole acetic acid, and indole-3-carboxylic acid. We found higher concentrations of several stress hormones and intermediates, such as jasmonic acid and abscisic acid, alongside elevated levels of auxin intermediates, in response to pathogenic induction. We are now conducting gene expression analyses to further validate these findings. These results will be discussed in relation to the potential role of phytohormones in SIR as mobile signaling molecules.

P1.3-025

PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF SWEET PEPPER FRUITS INFECTED WITH ALTERNARIA ALTERNATA

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Text

Sweet pepper (*Capsicum annuum* L.) is vegetable susceptible to many pathogen infections, including postharvest decay caused by fungus *Alternaria alternata*. Pathogen inoculation and mechanical damages could provoke the accumulation of defensive phenolic compounds and increase antioxidant activity in plants.The aim of this study was to evaluate total phenolics and total flavonoid content and antioxidant capacity in the fruits of two sweet pepper genotypes: line 112/16 and line 134/16 infected with *A. alternata*. The fruits were grown in the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia, and harvested at the

technological maturity. Fruits of each genotype were divided into three experimental groups: the intact fruits (control group), the fruits injected with sterile water and fruits inoculated with a fungal spore suspension. For the inoculation monohyphal isolate (K-93) of *A. alternata* was used. The fruit assessment was performed 10 days after inoculation. Content of phenolics, flavonoids and antioxidant capacity measured by three different assays (2,2-Dyphenyl-1-picrylhydrazyl (DPPH) assay, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and Ferric Reducing Antioxidant Power (FRAP) assay) were determined in 70% methanol extracts of fruits. Both genotypes manifested low tolerance to *A. alternata* infection. Content of phenolic compounds and antioxidant capacity decreased in infected sweet pepper fruits.

P1.3-026

PLANT EPIGENETICS FOR FOREST RESILIENCE AGAINST INVASIVE PATHOGENS

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Text

Plant diseases, both endemic and recently emerging, are spreading and exacerbated by climate change. For example, in the last years, Acute oak Decline (AOD) and Ash Dieback Disease (ADD) are emerging diseases endangering oak and ash landscapes. Plant epigenetics has recently acquired extraordinary interest as it has been shown to contribute to both short-term phenotypic plasticity and the longer-term adaptive capacity of plant responses to abiotic and biotic stresses, including the capacity to transmit these marks to progenies. Our work aims to study how disease pressures alters DNA-methylation imprinting in oak and ash. For this, trees were scored, classified in disease severity levels and sampled. Leaf-DNA was extracted and subjected to Whole Genome Bisulfite Sequencing (WGBS). Bismark software and R scripts (DSS., DMRcaller) were employed to analyse methylomes. Differentially Methylated Regions (DMR's) were observed in different C-context. Correlation analysis identified global and targeted changes in DNA methylation with AOD and ADD disease severity. The identification of epigenetic mechanisms marking oak and ash disease resilience could be used to reforestation and conservation of future forests in a hostile environment.

P1.3-027

PROFILING MOLECULAR RESPONSES OF NICOTIANA GLUTINOSA TO INFECTION WITH LETTUCE NECROTIC YELLOWS VIRUS SUBGROUPS TO UNDERSTAND VIRUS DISPERSAL

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Text
We have analysed the molecular responses of the model dicot species *Nicotiana glutinosa* to the type cytorhabdovirus, lettuce necrotic yellow virus (LNYV). LNYV infects a broad range of monocot and dicot hosts, yet the impacts of cytorhabdoviruses are understudied. In addition, the responses of *N. glutinosa* have not been well explored. The LNYV population exists as two subgroups, SI and SII; SII appears to be dispersing more rapidly than SI, particularly in Australia where SI appears to have become extinct. Interactions with the host may influence the rate of dispersal, and there are concerns that SII may overtake SI in NZ to cause more devastating disease. We have compared the molecular responses of *N. glutinosa* to infection with LNYV SI and II at 28 dpi to identify candidate genes and metabolites that may cause differences in subgroup dispersal. Metabolomic analyses have identified subgroup specific sugar, amino acid, organic acid, and fatty acid profiles. RNA seq, and RT-qPCR analyses have identified differentially expressed genes associated with responses to biotic stimulus, defense, photosynthesis, and metabolic processes affecting primary and secondary metabolism. Findings of this study will significantly contribute to our understanding of the complicated mechanisms of plant responses to cytorhabdoviruses in general, but also provide clues as to the mechanisms this virus uses to improve its spread.

P1.3-028

THE ROLE OF DORMANCY-ASSOCIATED (DRM) DISORDERED PROTEINS IN PLANT – PATHOGEN INTERACTIONS.

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Text
Intrinsically disordered proteins (IDPs) are surprisingly common in biological systems and comprise approximately 25% to 30% of eukaryotic proteomes, with additional data suggesting that over 50% of eukaryotic proteins and 70% of signalling associated proteins have regions of long disorder. Due to their inherent plasticity and lack of ordered structure, IDPs rapidly undergo conformational changes when bound to their biological partners. Indeed, it is this plasticity that lends itself to the characteristic promiscuous nature of an IDP, often occupying salient roles as hub proteins in signalling cascades. Amino acid composition analyses of the highly conserved plant-specific Dormancy-associated (DRM) protein family clearly demonstrates that members of this family are enriched in major disorder-promoting residues and depleted in major order-promoting residues; in a manner typical for structurally characterized IDPs. Using the DRM protein family as an exemplar, we present data that this protein family plays a pertinent role in a plant's response to both biotic and abiotic stresses, with individual members displaying potential antagonistic functions to each other, including the regulation of reactive oxygen species (ROS) following pathogen infection. We will also discuss further the importance of protein disorder in biological signalling cascades within plant-pathogen interactions.

P1.3-029

EVALUATION OF GREEK OLIVE CULTIVARS FOR SUSCEPTIBILITY TO FOMITIPORIA MEDITERRANEA

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Text

In the present study, five of the most significant Greek olive cultivars (Amfissis, Chalkidikis, Kalamon, Koroneiki and Mastoidis) were evaluated for their susceptibility to *Fomitiporia mediterranea*. Artificial inoculations were carried out by drilling a hole into the trunk and inserting mycelial plugs of the fungus into the wood. Tissue reactions were evaluated by carrying out longitudinal and transverse sections of the trunks and measuring the length of bark and wood lesions, 33 months post inoculation. Trunk scanning and image pixel analysis were employed to estimate the total discoloration and decay area of infected wood. Moreover, *F. mediterranea* re-isolation ratio from wood chips taken from different sites above and below the inoculation point was also estimated. Data indicated that the susceptibility of olive cultivars to *F. mediterranea* varied significantly. ‘Amfissis’ and ‘Chalkidikis’ were the most susceptible, whereas ‘Kalamon’ and ‘Koroneiki’ were comparatively resistant; ‘Mastoidis’ showed an intermediate level of susceptibility. Measurements of lignin content showed that the resistance of olive cultivars to *F. mediterranea* could be associated with the level of lignin in their trunk wood. This is the first experimental evidence of differential susceptibility responses of olive cultivars against *F. mediterranea*, and reveals the potential exploitation of host resistance as a promising approach in the effort to control wood decay disease of olive in practice.

P1.3-030

HERE COMES THE SUN! AN INNER NUCLEAR ENVELOPE PROTEIN THAT REGULATES PLANT NUCLEAR DYNAMICS AND TRANSCRIPTIONAL STRESS RESPONSES AGAINST PATHOGENS

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Text

Nuclear repositioning and cytoskeletal rearrangements constitute the earliest plant cellular responses to pathogen invasion. Yet, their molecular aspects and biological significance remain underexplored, particularly in legumes, which are hosts to symbiotic and pathogenic fungi. We investigated the host nuclear dynamics during compatible *Pisum sativum* (pea)-powdery mildew (PM) interactions and observed that the host nucleus moves towards the fungal appressorium before penetration and subsequently positions itself adjacent to the newly formed primary haustorium. Actin depolymerization inhibits nuclear movement and haustorium formation suggesting that actin-mediated host nuclear repositioning is crucial for PM establishment. For further insights, we investigated the role of the pea inner nuclear envelope

(NE) SUN protein, an integral component of LINC (Linkers of Nucleoskeleton and Cytoskeleton) complexes known to regulate nuclear dynamics. *PsSUN* knockdown alters the nuclear shape and hinders PM-induced host nuclear movement and fungal growth in pea. *PsSUN* stable expression in Arabidopsis causes NE deformation and up-regulation of defense-related genes involved in the salicylic acid (SA), pipecolate, and camalexin pathways. Notably, *PsSUN* overexpression lines accumulate elevated levels of SA and exhibit reduced susceptibility to a bacterial pathogen. Overall, our results suggest that PsSUN may regulate plant immunity through its impact on nuclear movement and gene expression.

P1.3-031

CHARACTERIZATION OF OLIVE FRUIT RESISTANCE TO COLLETOTRICHUM GODETIAE

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Text

Anthracnose, caused by *Colletotrichum spp.*, is the olive's most severe fruit disease. The pathogen causes fruit rot that negatively impacts the oil quality. Marked differences in resistance to the pathogen are observed between cultivars during the ripening in the field, but little is known about the defense mechanisms involved. Here, we used microbiological and chemical approaches to fill part of this gap. Inoculations showed that developing fruits were immune to the pathogen independently of the cultivar and the presence or absence of wounds on the peel. Conversely, fruit resistance was depended on the peel wounds once the fruit reached its maximum size at the beginning of the ripening process. Peel and mesocarp EtOH extracts of developing fruit caused a high inhibition of spore germination (> 80%) independently of the olive cultivars. On the contrary, in mature fruits, peel and mesocarp extracts, and to a lesser extent fruit wax, of resistant cultivars significantly affect the viability of the pathogen spores and their capacity to form appressoria compared with susceptible fruit cultivars. The current experiments point that the speed of transformation of the phenolic compounds in the ripe fruit, from primary compounds (ligstroside and oleuropein) to aglycones and, then, sercoroids, together with the concentration of terpenes in its peel, may contribute to cultivar resistance.

P1.3-033

A DEAD-BOX RNA HELICASE REGULATES IRON HOMEOSTASIS AND IMMUNE RESPONSE IN ARABIDOPSIS

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Text

Abstract
Acquisition of nutrients from plants is essential for pathogen colonization. As a key mineral nutrient for nearly all organisms, iron plays an important role in plant growth and disease resistance. However, how plant regulates iron homeostasis to enhance plant immunity remains unclear. Our previous studies showed that iron sensor protein BTS (BRUTUS), a key regulator of iron homeostasis, positively regulated plant disease resistance and is an important disease-resistance factor in plant immunity. Therefore, we proposed that there is an important relationship between iron homeostasis and plant immunity. To identify the immune elements regulated by BTS, we performed EMS (ethyl methane sulfonate) mutagenesis screening on *bts-2* seeds. A key gene that regulates BTS-mediated iron metabolism was identified by map-based cloning, which encodes a DEAD-box RNA helicase (RHa). Our data showed that RHa is an ATP-dependent RNA helicase that negatively regulated the resistance of plants to pathogens and the susceptibility to iron deficiency. RHa interacts with BTS in the nucleus and regulates the activities of the downstream iron deficiency regulatory proteins, leading to the accumulation of iron in plants, thereby facilitating pathogen proliferation. Our findings suggest that RHa regulates iron homeostasis through BTS and thus affects plant immune response.
Key word: *Arabidopsis thaliana*, plant innate immunity, BRUTUS, DEAD-box RNA helicase, iron homeostasis

P1.3-034

DIFFERENTIAL RESPONSE OF SUGAR BEET GENOTYPES TO THE LOCAL AND SYSTEMIC BEET CURLY TOP IRAN VIRUS INFECTION: VIRUS ACCUMULATION AND TRANSCRIPTOME ASSAY IN RESISTANT AND SUSCEPTIBLE GENOTYPES

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Text

Curly top disease caused by geminiviruses including Beet curly top Iran virus (BCTIV) is a limiting factor for sugar beet production. The most economical control of curly top disease in sugar beet would be through resistant cultivars though, most commercial cultivars possess only low to moderate resistance. A doubled haploid Line KDH13 showed resistance to Beet curly top virus (BCTV) and produced mild symptoms. However, the response of Line KDH13 to BCTIV which is genetically different from BCTV was not studied before. Here we tested the response of the resistant line and two susceptible genotypes (KDH19 and 9B) to the BCTIV local and systemic infection. Real-time PCR showed that BCTIV replicated in the locally agroinfiltrated cotyledons in all tested genotypes. However, in systemic assay, the virus was detected only in susceptible genotypes. Transcriptome analysis for the BCTIV infected plants showed a higher number of genes were deregulated in the locally infected tissues compared to the systemic infection. Among them, DNA recombination and defense response genes were the most upregulated candidates. However, in the systemic infection, genes related to the gibberellin and salicylic metabolic were the most upregulated groups. This work demonstrates the response of sugar beet plants to BCTIV infection at both local and systemic infection and highlights the metabolic pathways and defense-related genes for their contribution towards BCTIV resistance.

P1.3-035

THE RICE HVA22 PROTEINS IN RICE IMMUNITY

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Text

The endoplasmic reticulum (ER) is the largest intracelluar endomembrane system. ER-phagy removes excessive ER and the associated cargo proteins, thereby remodeling ER morphology and functions. ER-phagy receptors selectively target ER subdomains and induce autophagic sequestration. Despite multiple ER-phagy receptors have been reported, their functional counterparts in plants remain largely unexplored. Here, we report a HAV22 family protein OsHLP1 that is a novel ER-phagy receptor in plants. OsHLP1 interacts with ATG8b and recruits ER subdomains and the cargo protein OsNTL6, a negative immune regulator, to autophagosomes upon the fungal pathogen Magnaporthe oryzae infection, which substantially actives the disease resistance in rice. OsHLP1 orthologous induced similar ER-phagy in Arabidopsis thaliana. We therefore discovered a conservative protein family that act as ER-phagy receptors and highlight their roles in plant immune response.

P1.3-036

CHANGES IN TERPENE PROFILING OF PINUS PINASTER AND P. RADIATA IN RESPONSE TO WOUNDING AND INFECTION WITH FUSARIUM CIRCINATUM

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Text

Fusarium circinatum is a fungal pathogen causing Pine Pitch Canker (PPC). In Europe, it is an invasive pathogen established in northern Spain and Portugal where the disease affects forest stands of *Pinus radiata* and *P. pinaster*. Of these, *P. radiata* is considered one of the most susceptible pine species to PPC disease while *P. pinaster* has shown a moderate disease resistance. *P. pinaster* is also the Iberian species from which resin is collected. Defence mechanisms employed by conifers include the production of terpenoid oleoresin, which act as physical and chemical barriers against insect and pathogen attack. The aim of this work is to identify the most relevant terpenes produced in response to *Fusarium circinatum*, which may explain the differences in PPC disease susceptibility, and to determine the effect of these terpenes on the in vitro growth of the pathogen. For this, a controlled experiment was carried out by inoculating with *F. circinatum* or wounding seedlings of both species. The terpene profile (170 metabolites) was analysed by GC-MS

after 11 and 18 dpi. To determine terpenes produced in the presence of *F. circinatum* colonizing seedlings, a comparison of inoculated vs. mock-inoculated seedlings in an OPLS-DA model was performed for each dpi (all models were significant at p-value < 0.05 for *P. pinaster* and *P. radiata*). The inhibitory or enhanced effect of these terpenes on mycelial growth of *F. circinatum* was studied using turbidimetric measurements with Bioscreen C.

P1.3-037

COMBINED TRANSCRIPTIONAL AND METABOLIC PROFILING TO DETERMINE PHYTOHORMONE RESPONSE IN PINUS-FUSARIUM CIRCINATUM INTERACTION

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Text

Pine pitch canker (PPC) disease, caused by the fungus Fusarium circinatum Nirenberg and O'Donnel, poses a serious threat to several Pinus species affecting plantations and nurseries worldwide, with important economic losses. In plant pathogen interactions, phytohormones play a crucial role in determining the progression of diseases. In the present work, we explore the phytohormone profile in two Pinus species with different degrees of susceptibility to F. circinatum, Pinus radiata (highly susceptible) and P. pinaster (moderately resistant), at different stages of infection. Dual-RNA sequencing assay showed that the moderate resistance of P. pinaster can be explained by the early activation of defence-related genes and a complex phytohormone signalling at 3, 5 and 10 days post-inoculation, which includes mainly salicilyc acid, jasmonic acid, ethylene and to a lesser extent auxins. We also hypothesise the key steps in which the pathogen could be manipulating host defence. Given that metabolism is driven by specific enzymatic products of gene expression, the phytohormone metabolome of both pine species by GC-MS at 5 and 10 dpi was explored. Abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), indole acetic acid (IAA), gibberellic acid (GA1 and GA4) and cytokinins (DHZ, iP, tZ) were quantified. Integration of "omic" data and the validation of key genes by qPCR will allow the core pathways that explain differences between Pinus species to be identified.

P1.3-038

INTRASPECIFIC DIVERSIFICATION OF PATHOGEN DEFENCE SIGNALLING IN THE WILD TOMATO SPECIES SOLANUM CHILENSE

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Text
Natural plant populations need to adapt to their environment. What the implications are of such adaptations for the functioning of the pathogen defence signalling network is not known.

We study the species-wide diversity in pathogen defence mechanisms in the wild tomato species Solanum chilense. It grows in Chile and Peru and its specific demography, allows us to learn how evolution of resistance properties happens in new diverse habitats.

We performed large-scale phenotyping and measured defence responses in plants representing 9 different wild populations. Variations in defence properties are not limited to responses triggered by major resistance genes like NLRs or RLPs and also affect the often assumed assumed conserved mechanisms involved in quantitative defence responses (QDR).

We established that QDR components, such as ROS or phytohormone regulation, are highly variable within the species. Quantitative resistance can only be explained by effects of multiple components. The effect of each component differed dependent on the population's origin. We also find that genes in diverse defence pathways are under differential selection, dependent on the location of origin of the population and are now further studying the transciptomic and metabolimic response triggered in the plant.

This work thus shows that QDR is more variable in nature than previously assumed. It also highlighs the importance of looking beyond model plants and single accessions to understand defence signalling.

P1.3-039

HLB TOLERANCE IS ASSOCIATED WITH ENHANCED CARBOHYDRATE DYNAMICS IN THE LEAVES, UPREGULATION OF DEFENSE ACTIVATORS, AND PRESERVATION OF XYLEM CONNECTIVITY

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Text
While HLB is still one of the most severe citrus diseases worldwide, improved tolerant cultivars, such as the Sugar Belle® [SB] mandarin, have been developed in Florida-USA. To understand the responses that this tolerant material is undergoing upon infection, we compared leaf carbohydrate dynamics, callose accumulation in the phloem, relative gene expression of plant defense activators, and anatomical sections of vascular elements between healthy and infected SB trees and susceptible sweet orange (SO). Physiologically, SB showed a 250% increase in 14CO2 fixation and a 13% decrease in 14C-carbohydrate export whereas SO presented a decrease of 33% and 50%, respectively. Localized callose accumulation was differentially encountered in infected SB. Pathologically, expression of papain-like cysteine proteases (PLCPs) known for interacting with the SDE1 effector of Candidatus Liberibacter asiaticus was upregulated in SB but downregulated in SO. Anatomically, SB showed minor alterations in the total area of the midribs pith and xylem, but 29% fewer and 19% smaller cells in the phloem. Contrastingly, SO showed a 200% and

181% increase in cell number and 156% and 136% growth of cell lumen area in the xylem and phloem, respectively. Three mechanisms of tolerance in SB are hypothesized: i) increase carbohydrate availability induced by differential carbohydrate dynamics in the leaves, ii) increase in the defense response activated by PLCPs, and iii) maintenance of xylem connectivity.

P1.3-040

AVOCADO SUNBLOTCH VIROID: THE SMALLEST AVOCADO PATHOGEN CAUSING BIG CHANGES IN HOST GENE EXPRESSION

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Text
Avocado sunblotch disease is caused by avocado sunblotch viroid (ASBVd) - a small, single-stranded molecule of RNA. This economically important disease is associated with symptoms such as the formation of coloured, sunken lesions on avocado fruit, yellow streaks on stems and chlorosis of leaves. Under field conditions, infection by ASBVd may remain asymptomatic for long periods of time, though avocado trees without chlorotic symptoms have been known to display altered growth habits and decreased fruit yield. Despite the global prevalence of the viroid and its impact on the avocado industry, molecular mechanisms underlying ASBVd infection remain unknown. In this study, we used RNA-seq to determine transcriptomic changes in avocado triggered by ASBVd infection. RNA was extracted from leaves of asymptomatic Hass grafted seedlings, and infection status of individual nursery trees was determined. The mRNA of ASBVd-infected and uninfected replicates was sequenced using Illumina NovaSeq 6000. RNA-seq data indicated that ASBVd infection induced significant changes to avocado gene expression, despite asymptomatic infection. Pathway analyses revealed that host processes such as plant defense response and phytohormone signalling pathways were significantly affected by ASBVd infection. These findings represent the first global transcriptomic study in ASBVd-infected avocado; an important advancement in improving our understanding of the interactions between this viroid and its host.

P1.3-041

EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF A NEWLY EMERGING MONOPARTITE BEGOMOVIRUS IN SOUTH AFRICA, CAPABLE OF INDUCING SEVERE SYMPTOMS IN COMMERCIAL TOMATO CULTIVARS WITH MULTIPLE TY RESISTANCE GENES.

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Text

Tomato curly stunt virus (ToCSV) is a tomato yellow leaf curl disease present in southern Africa. Since its introduction in South Africa (1998), ToCSV has become one of the most destructive tomato viral diseases in the country. Over the last three years, commercial tomato cultivars known to be ToCSV-resistant, have been found to show particularly severe symptoms characteristic of begomovirus infection. The increasing occurrence in tomato growing areas in the northern parts of the Limpopo province, prompted further investigation. Tomato plants with TY-resistance, showing severe symptoms of leaf curling and stunting were collected (field isolate) and used as source material in a whitefly-mediated inoculation greenhouse trial. The disease symptom severity of susceptible and resistant cultivars were compared to a severe variant of ToCSV, maintained via agroinoculation. The symptoms induced by the field isolate was much more severe in both susceptible and resistant cultivars, in contrast to the ToCSV isolate. Rolling circle amplification (RCA) and PacBio, single molecule, long read sequencing was used to characterize the begomovirus isolates present in the field isolate. Sequence comparison and phylogenetic analyses revealed the presence of multiple begomovirus isolates, with 85 – 99% sequence identity with known begomovirus species. Ongoing epidemiological and further molecular characterization of these viral isolates will be discussed.

P1.3-042

BREEDING FOR RESISTANCE AGAINST PHOMOPSIS HUSK ROT DISEASE IN THE AUSTRALIAN MACADAMIA ORCHARDS

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Text

Macadamia is an evergreen tree nut that produces high-value kernels used in the food and cosmetic industries. Major production areas are found in the tropical and subtropical regions worldwide. Australia is one of the top producers of macadamia with a farm gate value of USD$204 million. Annually, macadamia orchards undergo major yield losses due to pests and diseases, prompting the development of new resistant cultivars with marketable characteristics. One of the major fungal diseases in macadamia orchards is Phomopsis husk rot (PHR) caused by Diaporthe species, which provokes the premature drop of the immature nut reducing orchards profitability. Here, we investigated the diversity of PHR resistance in 14 macadamia cultivars, 7 open-pollinated (OP) progeny and their parents. In vivo wound inoculation assays of the fruit pericarp were used to evaluate host resistance to D. australiana over two years. Significant differences among cultivars were observed, but not in the OP and parent populations. This suggests breeding for resistance for PHR is a viable option for disease control. Studies on heritability of resistance to PHR in macadamia are currently underway.

P1.3-043

RESISTANCE AND RESPONSE TO FUSARIUM HEAD BLIGHT DISEASE IN PIGMENTED WHEAT GENOTYPES

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Text

Fusarium head blight (FHB) is a severe disease of cereals, including bread (*Triticum aestivum*) and durum wheat (*Triticum turgidum* subsp. *durum*) caused by *Fusarium* spp. The main effect of FHB is a drastic reduction of yield and mycotoxins contamination in the raw materials (flours). Pigmented wheat genotypes have flavonoid-rich kernels, especially in the external layers (pericarp and/or aleurone) and polyphenols are known to be antioxidant and antimicrobial agents in plants. Main aims of present work were: to verify if genotypes rich in anthocyanins are more resistant to FHB; to elucidate if *Fusarium* spp. infection affects the phenylpropanoid pathways in wheat. Six pigmented wheat genotypes were artificially inoculated, taking into account type II and type IV resistance, AUDPC and fungal biomass quantification. The results showed that some pigmented genotypes are very susceptible (Purple durum, Skorpion and Rosso), other are quite resistant (Purendo and Indigo) or resistant (Vanilnoir), but pigmentation and resistance seems to be not correlated, even if total phenolic content in mature spike is the parameter more related to the infection. The relative expression of 10 genes related to phenylpropanoid pathways were screened in the pigmented genotypes. In addition, for the bread wheat genotypes an RNA-seq experiment was carried out comparing infected and control plants.

P1.3-044

EVALUATION OF SOYBEAN GENOTYPES FOR RESPONSE TO CONIOTHYRIUM GLYCINES, THE CAUSE OF RED LEAF BLOTCH

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Text

Red leaf blotch (RLB) is a significant fungal disease of soybean caused by the fungus Coniothyrium glycines. Currently, up to 50% loss in yield can be attributed to RLB infection in sub-Saharan Africa. Until now, there are no known varieties with resistance to RLB. 230 soybean lines were screened under screen-house conditions to identify sources of resistance. RLB ZS20B isolate and line SB25 were used as the fungi isolate and susceptible check, respectively. Seeds from each line were germinated in trays filled with a sterile potting mixture of red soil, sand, and manure (2:2:1). Seedlings were maintained in the screen-house (25°C) for two weeks before infection. Infection with RLB was achieved by spraying a spore suspension (2.08×109 spores/ ml) on plant leaves using a spray bottle until runoff. Plants were then transferred into a humid chamber at 25°C. Disease severity was assessed three weeks post-infection based on a 1–5 scale, with 1 representing no disease and 5 representing 66-100% severity. 54 lines had a disease severity ranging between 1-1.9, 103 in the range of 2-2.9, 54 in the range of 3-3.9, and 19 had a severity of 4-5. Lines with a severity of 1-2.5 were re-screened under similar conditions. 38 of these had a severity

ranging from 1-2. Four lines had a severity of 3, while two lines had a severity of 4. The 38 lines are regarded as potential sources of resistance and will be further evaluated to ascertain the presence of resistant genes.

P1.3-045

DETECTING BIOCHEMICAL CHANGE IN CASSAVA LEAF SPOT TISSUES CAUSED BY CURVULARIA LUNATA HC-04 BY USING SYNCHROTRON FOURIER-TRANSFORM INFRARED SPECTROSCOPY

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Text

Cassava leaf spot is a complex disease caused by many fungi. The objective of this study was to detect the biochemical change in epidermis and mesophyll tissues leaf spot disease by using Synchrotron Fourier-transform infrared spectroscopy (SR-FTIR). After inoculation of *Curvularia lunata* HC-04, the cassava leaf containing spot symptoms was collected and frozen in optimal cutting temperature. Then the samples were cut-cryosectioned with a thick 12 microns, which were placed on BaF_2 windows and measured by SR-FTIR. In epidermis tissues, the PC1 and PC2 loadings were explained by 33 and 12%, respectively. Similarly, in mesophyll tissues, the PC1 and PC2 loadings were 56 and 12%, respectively. In the second derivative analysis, the lipid (2919, 2850 cm^{-1}), pectin (1733 cm^{-1}), amide I (1652, 1606 cm^{-1}), amide II (1540, 1513 cm^{-1}) and lignin (1442, 1374, 1311 cm^{-1}) were significantly decreased in epidermis tissue when *C. lunata* HC-04 infected. Furthermore, the amide I (1654, 1608 cm^{-1}), amide II (1542, 1513 cm^{-1}), lignin (1446, 1342 cm^{-1}), were significantly decreased in mesophyll tissue when *C. lunata* HC-04 infected while the pectin (1735 cm^{-1}), polysaccharide (1278, 1203, 1145, 1114, 1060, 1037, 993, 962 cm^{-1}) were significantly increased. Mean the polysaccharide and lipid were not significant in the epidermis and mesophyll tissue between control and *C. lunata* HC-04, respectively. In conclusion, the SR-FTIR was an effective tool for detecting biochemical changes in plant tissue.

P1.3-046

INFLUENCE OF SALICYLIC ACID ON ENZYME ACTIVITY TO CONTROL ANTHRACNOSE DISEASE IN CASSAVA BY IN VITRO AND IN VIVO STUDIES

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Text

The aim of this study to investigate the influence of salicylic acid formulation (SA) on total chlorophyll content, reactive oxygen species (ROS) accumulation, phenylalanine ammonia-lyase (PAL) enzyme activity, and endogenous salicylic acid accumulation in cassava. The results found that cassava after enhanced with SA three times has the highest total chlorophyll content in cassava leaves treated with SA 0.155±0.011 µg/mm2. In addition, the production of ROS has a higher accumulation of O2- and H2O2 in leaves tissue reached a maximum at 12 and 24 hours after inoculation (HAI) and then reduce to a low level in 48 HAI. Moreover, PAL activity of cassava leaves showed the increased response of PAL activity level at 24 HAI at 6.93 µmol trans-cinnamic acid min-1 mg-1 protein and decreased to low-level in 48 HAI. Furthermore, endogenous salicylic acid showed that the SA formulation can enhance the higher level of endogenous SA content at 24 HAI to approximately 16.77% and decrease in 48 HAI. These data could be con?rm the enhanced ROS response before SA biosynthesis can trigger systemic acquired resistance (SAR) in cassava to control anthracnose pathogen. The interaction of amino acids ARG31, LEU32, ALA93, and GLN97 resulted in a strong hydrogen bond with SA. According to this theory, salicylic acid was bound to the active site and formed a complex with the active portions of PAL. According to this mode of binding, salicylic acid was bound to the active site of PAL.

P1.3-047

GENOME-WIDE ASSOCIATION ANALYSIS IDENTIFIES RESISTANCE LOCI FOR BACTERIAL BLIGHT IN DIVERSE EAST AFRICAN RICE GERMPLASM

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Text

Xanthomonas oryzae pv. *Oryzae*, the causal agent of rice bacterial blight disease is now a threat to rice production in Africa. Resistance break-down has accelerated because *Xoo* evolves rapidly. The continuous evolving *Xoo* and breakdown of resistance in cultivated rice varieties needs discovery of new loci to enable breeding broad-spectrum resistant elite rice . East African germplasm holds useful genetic variation for bacterial blight (BB) resistance. This study was conducted to identify loci associated to BB resistance and new genetic donors for breeding program.
To identify candidate sources of resistance for advancing breeding, we used highly four virulent strains of *Xoo* (*PXO99*, *MAI1*, *BAI3* and *Xoo3-1*) to screen 78 East African accessions by GWAS. The core genetic base of the diverse accessions exhibited high degree of resistance

to the Xoo strains. 50.63% of the accessions were highly resistant to the Philippines strain PX099, while 20.25% were highly susceptible to the virulent West African strain MAI1. Two novel resistant loci significantly associated hotspots were identified using 1901 SNPs. The two hits were located on Chr12 (*Xa25*) and Chr6 (*Xa7*, *Xa27*, *Xa33*).
Our finding have identified novel loci that gives a useful basis for more investigation and a wide core genetic pool of high resistance for broad-spectrum resistance genetic improvement.
Keywords: genome-wide association, Oryza sativa, bacterial blight (BB), Xanthomonas oryzae, disease resistance

P1.3-048

TREATMENTS WITH A VOLATILE ORGANIC COMPOUND CAUSE METABOLIC CHANGES IN GRAPEVINE LEAVES AGAINST DOWNY MILDEW

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Text
Plant volatile organic compounds (VOCs) play a crucial role in plant responses against plant pathogens. Previous studies revealed that the abundance of terpenes was higher in resistant than in susceptible grapevine genotypes upon Plasmopara viticola inoculation, indicating their possible involvement in defense mechanisms. This work aims at identifying the metabolic response of terpene-treated grapevine leaves and the potential activation of VOC-mediated defense mechanisms against P. viticola. Functional analyses showed that terpene treatments reduced downy mildew severity on leaf disks. An untargeted metabolomics approach was performed on VOC-treated leaf disks at one and six days post inoculation with P. viticola. A principal component analysis was carried out on the detected features and it discriminated samples according to treatment and time point, indicating global metabolite changes after VOC application. Features with significant increases in abundance were selected, annotated by searching mass spectra in different chemical databases, and validated with analytical standards. Results showed that terpene treatment increased the abundance of phenylpropanoids, terpenoids, and lipids, suggesting that VOC treatments can activate a metabolic response in grapevine leaves that include defense-related compounds.

P1.3-049

CLONING AND MECHANISM ANALYSIS OF ADULT PLANT RESISTANCE GENES TO RUST IN WHEAT

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Text
Growing resistant cultivars remain the most economical, environmentally friendly and

effective method to manage wheat leaf rust and stripe rust. In the present study, a QTL on chromosome 6BL form durum wheat explained 13.1-30.7% of leaf rust severity variation and was designated as Lr84. We confirmed TRITD6Bv1G225630 as the gene that underly Lr84 and it encodes a classic nucleotide binding leucine rich repeat (NLR) protein that is typical of classic disease resistance immune receptors through transgenic and VIGS. Interestingly, Atred#2+Lr84 and overexpressed T2 plants showed susceptibility up to the three-leaf stage but the necrosis associated with a resistance reaction occurred at the four-leaf stage and near immunity was expressed after the five-leaf stage. Furthermore, we found thicker, more rigid leaf cell walls and greater penetration resistance against fungal invasion, in wheat lines harboring the Lr34/Yr18/Sr57/Pm38 gene than in lines lacking it, indicating the involvement of a cell wall-associated defense mechanism. Yeast accumulation assay confirmed the role of Lr34/Yr18/Sr57/Pm38 in the transportation of sinapyl alcohol in vitro. Both genetic and virus-induced gene silencing (VIGS) approaches revealed that the disease resistance conferred to wheat by Lr34/Yr18/Sr57/Pm38 could be enhanced by the presence of the TaCOMT-3B gene. The above studies played an important role in improvment rust resistance for both durum wheat and bread wheat.

P1.3-051

IMMUNITY MODULATION BY DISTINCT RHIZOSPHERE MICROBIOTA IN RICE UNDER NITROGEN SUPPLY

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Text

Nitrogen (N) fertilizers not only maximize the crop yield but also modify the plant's ability to resist pathogen infections. The interaction between *Magnaporthe oryzae* (*M. oryzae*) and rice is one such example where excess N fertilization results in nitrogen-induced susceptibility (NIS). We hypothesized that N supply would dictate the rhizosphere microbiota of rice, which may in turn modulate susceptibility to the rice blast fungus. To test our hypothesis, we established a system in which 2N compared to 0N increased susceptibility to *M. oryzae* without causing apparent physiological aberration. Community structure was assessed by 16S rRNA (V3-V4) and ITS2 region using Illumina MiSeq. Our results showed that community structures are significantly affected by both N supply and pathogen inoculation. The defense related genes of 2NI were weakly induced. SA levels significantly reduced in leaves of 2N plants at 24- and 48—hpi compared to 0N plants, resulting in increased susceptibility. To recapitulate NIS, MF separated from 2NI regime was treated to 0N plants in MF-treated experiment. This showed increased susceptibility of 2N-MF treated plants. We conclude that both N and *M. oryzae* inoculation impacted microbiome assembly as well as plant defense. Our work would provide not only novel glimpse into what pathobiome of the rice blast disease looks like but also the empirical basis to build a model for predicting and intervening the outcome of interaction between plants and microbes.

P1.3-052

EVALUATING THE EFFECT OF PLASMODIOPHORA BRASSICAE VIRULENCE ON GLUCOSINOLATE PROFILES IN CLUBROOT-RESISTANT AND SUSCEPTIBLE OILSEED RAPE CULTIVARS

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Text

Clubroot is a disease caused by the soil-borne pathogen Plasmodiophora brassicae, which affects cruciferous plants. Previous studies have shown that clubroot infection can change the glucosinolate profiles of affected plants. Depending on the case, specific glucosinolate levels may either increase or decrease. This study evaluated changes in glucosinolates in the roots and leaves of different clubroot-resistant and susceptible oilseed rape cultivars following artificial inoculation with P. brassicae isolates of varying virulence. The results showed significant differences in clubroot incidence and severity, as well as the amount of total and individual glucosinolates between oilseed rape cultivars in response to the pathogen's virulence. Both single and total aliphatic and indolic glucosinolate contents were significantly lower in the leaves and roots of susceptible cultivars compared to resistant ones. The different isolates of P. brassicae analyzed differ in their ability to reduce gluconasturtiin contents in the host. The more aggressive isolate, P1 (+), may suppress gluconasturtiin synthesis of the host more significantly than isolate P1. Furthermore, a potential interplay between aliphatic and indolic glucosinolates, which may be involved in water homeostasis in resistant cultivars, is discussed.

P1.3-053

CHARACTERIZATION OF HORMONAL RESPONSES IN TWO CLONES OF CUPRESUSS SEMPERVIRENS WITH DIFFERENTIAL SUSCEPTIBILITY TO SEIRIDIUM CARDINALE

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Text

Common cypress (*Cupressus sempervirens* L.) represents one of the major features of the Mediterranean landscape. Unfortunately, since the first half of the last century, cypress trees came under heavy attack by *Seiridium cardinale* (Wagener) Sutton & Gibson, the main causal agent of the Cypress Canker Disease. Nowadays, among the management strategies to limit the spread of this lethal disease, the use of resistant cypress selections for new plantations seems the most promising. This pioneer study aims to elucidate hormonal responses, at bark and twig level, in resistant and susceptible clones of *C. sempervirens* inoculated with *S. cardinale*. In inoculated susceptible cypress, a high production of ethylene (Et) and jasmonic acid occurred (more than 2-fold higher than uninoculated ones) at 3- and 4-days post inoculation (dpi) in the bark, suggesting a not efficient attempt to counteract

fungal infection and its detrimental effect. In inoculated resistant cypress, Et biosynthesis raised at 3 and 13 dpi in the bark (more than 2-fold higher). Conversely, an increase of salicylic acid levels (more than 2-fold) occurred at 1 dpi in the foliage, concomitantly to an accumulation of abscisic acid, that reached the maximum values at 9 dpi. The early activation of phytohormones and signaling molecules may counteract oxidative burst and prevent lipid peroxidation by offering protection to bark tissues and foliage in resistant cypress.

P1.3-054

EVALUATION OF RESISTANCE AND PRESENCE OF SECONDARY METABOLITES IN ALMOND CULTIVARS DURING INFECTION BY THE WILT FUNGUS VERTICILLIUM DAHLIAE

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Text

Infestation by the soil-borne fungus Verticillium dahliae involves a multitude of key crops worldwide, including almond. In this context, four commercial almond cultivars (Ferragnes, Vairo, Marinada, Texas), all budded on GF-677 rootstocks were evaluated for their sensitivity against V. dahliae infection. Potted plants were inoculated with V. dahliae conidia and disease symptoms were recorded in time. Moreover, the distribution of the fungus in different parts of the plant was determined by molecular detection methods. The amount of disease (expressed as relative AUDPC) presented a positive correlation with nested PCR results indicating that the most susceptible cultivars are Ferragnes and Texas, while the most tolerant cultivars are Vairo and Marinada. In addition, the concentration of specific secondary metabolites (total phenols, flavonoids, flavanols, o-diphenols) as well as the total antioxidant capacity (TAC) was determined in wood samples corresponding to different sections of the plants. After statistical processing of the results, it was possible to make the appropriate comparisons and determine the way in which the disease affects the characteristics of the plant. Significantly higher concentration of phenols was detected in the stem of the rootstock on which the tolerant Marinada cultivar, was grafted. In the same treatment, the concentration of phenolic components was positively correlated with detection of the pathogen in the different parts of the plant.

P1.3-055

IDENTIFICATION OF NEW SOURCES OF RESISTANCE TO BACTERIAL SPOT AND BACTERIAL CANKER IN WILD TOMATO VARIETIES

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Text
Bacterial diseases pose a major threat to tomato production around the world. The introduction of resistance loci into commercial varieties is an effective and environmentally friendly way to counter the damage caused by bacterial pathogens. To identify new sources for disease resistance and/or tolerance to bacterial canker and bacterial spot, we screened a library of more than 100 accessions of Solanum pimpinellifolium and Solanum lycopersicum var. cerasiform. Plants were independently inoculated with the bacterial canker pathogen Clavibacter michiganensis and bacterial spot pathogens Xanthomonas euvesicatoria pv. euvesicatoria, Xanthomonas euvesicatoria pv. perforans (Xeup) and Xanthomonas vesicatoria, and visually monitored for disease symptoms. Our screen identified multiple accessions that harbored high tolerance or partial resistance to the four pathogens. In particular, Solanum pimpinellifolium line P3031 exhibited little to no symptoms upon infection with each of the four pathogens. Additionally, syringe infiltration of Xeup into P3031 leaves, but none of the other pathogens, elicited a hypersensitive response (HR)-like cell death. Our screen identified Solanum pimpinellifolium P3031 as a potential source of tolerance and/or resistance loci against bacterial diseases in tomato.

P1.3-056

THE PLANT IMMUNE SYSTEM OF FERNS AND LIVERWORTS

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Text
Land plants evolved five major lineages: angiosperms, gymnosperms, ferns, lycophytes and bryophytes. All can interact with microbes. Angiosperm is by far the most diverse. Hence the angiosperm immune system has been deeply studied. It relies on cell surface and intracellular receptors. The genetic diversity and the signalling pathways of these receptors are well known, with remarkable breakthroughs in the recent years.
We hypothesised that other plant lineages also rely on different mechanisms to resist pests. To test this hypothesis, we explore the natural intraspecific diversity of Marchantia polymorpha (a bryophyte). We inoculated a collection of sequenced accessions with the oomycete Aphanomyces euteiches, the cause of root rot in legumes. We seek to map the genes associated with resistance using GWAS, further characterise them and assess their potential in root rot resistance.
In ferns, the genetic resources are limited due to large size genomes. We aim at developing pathosystems with several fern species. We found polymorphic responses to several microbes, including many ascomycetes. Upon further phenotypic description of the pathosystems, we will investigate the genetic base of immunity using comparative transcriptomic.
By characterising the immune system of liverwort and ferns, our goal is to propose novel solutions for crop protection and understand the evolution and diversification of the plant immune system for the last 450 million years.

P1.3-057

IDENTIFICATION OF PLANT GENOTYPE DEPENDENT MICROBIOME RECRUITMENT ASSOCIATED WITH DISEASE RESISTANCE AGAINST ROOT ROT IN PEAS

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Text

The cultivation of pea (Pisum sativum) is highly constrained by various soil-borne pathogens. Together these pathogens form a pea root rot complex (PRRC) and trigger soil fatigue. Microbiome-mediated disease resistance poses a possible mechanism to mitigate yield loss through PRRC. It is however largely unknown how the PRRC interacts with other members of the root microbiome and how this affects plant resistance. Here, we compared the root microbiome of 252 pea lines in a controlled soil-based phenotyping assay that was previously shown to predict field-relevant resistance against PRRC. Root bacteria and fungi were characterized by 16S rRNA and ITS amplicon sequencing. We analyzed alpha diversity and microbial community composition, and identified heritable hub OTUs. Based on differential abundance analysis we further identified heritable bacterial and fungal hub taxa that are associated with root rot resistance. Subsequent genome-wide association studies revealed plant genomic regions that are significantly correlated with beneficial hub taxa and overall microbial community composition. In a next step, the identified genetic markers will be used to select pea breeding material for field validation of microbiome-mediated resistance against PRRC. This work demonstrates the potential of microbiome-assisted breeding to promote sustainable farming practices.

P1.3-058

CANDIDATUS LIBERIBACTER ASIATICUS ATTENUATES PHLOEM DEFENSE RESPONSES TO ALLOW ITS PROPAGATION AND MOVEMENT IN CITRUS

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Text

Huanglongbing (HLB), caused by the Gram-negative bacteria Candidatus Liberibacter asiaticus (CLas), poses an existential threat to the industry worldwide. Developing more

tolerant or resistant HLB cultivars is needed, but our understanding of the basic biology of the disease is difficult due to the low levels and irregular distribution of the bacteria in the plant, and their restriction to the phloem sieve elements. We show that in HLB infected trees, callose accumulates and plugs the phloem, and that the accumulation of CLas and callose inhibits the translocation of sugars. These results explained some of the symptoms observed in the HLB trees but raised the question of how the bacteria can move in the occluded phloem. To explain this, we compared CLas-phloem interactions in the leaves and the seed coats. In the leaves, we saw that both phloem occlusion and ROS levels increased in the infected phloem, but in the bacterial titer was very low, and the cells examined were for the most part bacteria-free. We found that in the seed coats the bacteria accumulate to very high levels, and we demonstrate that when bacteria are present, either in the leaves or fruit phloem, they reduce both callose and ROS levels. Our results demonstrate a constant arms race between CLas and the phloem, and that during pathogen colonization, the bacteria inhibit the plant defence response, aimed to block bacteria movement, which allows the pathogen to move through the sieve pores and the plant.

P1.3-059

ATNPR1 BOOSTS THE BASAL IMMUNE RESPONSES AND ENHANCES TOLERANCE TO LIBERIBACTER INFECTION IN CITRUS

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Text

Citrus Greening, caused by Candidatus liberibacter asiaticus (CLas) is a devastating disease of citrus imposing a huge economic downfall in Florida. Most commercial citrus cultivars are susceptible to HLB, and there needs to be an effective disease management strategy to combat this pathogen. In susceptible varieties, strong activation of phloem defense responses leads to phloem collapse and reduction of phloem transport. Transgenic citrus expressing the Arabidopsis thaliana NPR1 (AtNPR1) protein exhibits enhanced tolerance against HLB. However, the mechanism underlying this tolerance is unknown. In this study, we analyzed the phloem defense responses in transgenic ‘Duncan’ grapefruit, ‘Hamlin’ sweet orange, and Arabidopsis thaliana lines expressing AtNPR1 protein. We discovered that AtNPR1 lines had elevated levels of callose in the phloem but did not over-accumulate callose or ROS like the wild-type plants upon CLas infection. Expression analysis of callose synthase genes (CalS), RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD), and systemic acquired resistance-related genes validated these phenotypes. Furthermore, microscopy analysis revealed thicker phloem fibers and reduced phloem collapse in the transgenic lines upon CLas infection. Our results suggest that AtNPR1 plants have elevated basal immune responses and may suppress CLas-triggered robust immune responses seen otherwise in the wild-type plants, resulting in reduced symptom development and high tolerance to CLas.

P1.3-060

GENE CO-EXPRESSION NETWORKS BASED ON TOLERANT AND SUSCEPTIBLE TRANSCRIPTOME ENABLE A BROAD VIEW OF PLANT RESPONSES TO DIFFERING PATHOGENS.

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Text

A total transcriptome provides knowledge of early plant-microbe interaction between sugarcane and three pathogens (2 bacteria and 1 fungus). Susceptible and tolerant sugarcane plants are inoculated with *Xanthomonas albilineans*, *Leifsonia xylii*, and *Sporisorium scitamineum* and 48 hours post-infection total RNA was prepared for comparative purposes. A NovaSeq run yielded 19 billion PE reads from depleted ribosomal genes of total RNA samples prepared from 16 experimental conditions in three biological replicates. After QC filters, reads were mapped to 398,353 sugarcane gene spaces from SP80-3280, a Brazilian cultivar, using Kallisto. Over a third of the genes are expressed in all conditions tested. Statistical analysis based on gene co-expression networks and functional GO categories discloses 63 connections shared by all tolerant inoculated cultivars and 56 connections shared by all susceptible cultivars, irrespective of the pathogen. A collection of previously selected 580 genes reveal that some genes are pathogen specific, others are bacteria specific and some are cultivar or condition specific. As few as 19 genes were not expressed at any conditions. In addition, non-mapped reads disclosed expression from the resident microbiome and yet to describe genes from sugarcane. The approach opens new avenues for breeding programs and a broader understanding of the commonalities and uniqueness of these plant-microbe interactions.

P1.3-061

PERFORMANCE OF COMMERCIAL VARIETIES AGAINST PHYTOPHTHORA SOJAE

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Text

Phytophthora stem and root rot of soybean, caused by Phytophthora sojae, is mainly managed with single or stacked qualitative disease resistance genes. As pathotype complexity of the population increases, management through the tolerance of the commercial varieties would be advisable. The goal of this study was to evaluate the response of commercial genotypes against the pathogen. We used the hypocotyl inoculation and infected rice techniques. Six commercial genotypes were used together with “Sloan” variety as susceptible control, and 3 pathotypes of P. sojae (which differed in virulence on 1 to 6 Rps genes and are the most representative on the pampeana region). In the first technique, the response of the genotypes was identified as susceptible (70 % or more seedlings killed) or resistant (30% or less seedlings killed). The infected rice technique was

carried out in a Randomized Design with 3 repetitions per treatment. The total length of the roots of the surviving plants in the pots was evaluated 21 days after sowing. The data obtained were subjected to ANAVA and Fisher's LSD test ($p<0.05$). The variety identified as commercial 4 is the only one that presented significant differences compared to the control and the rest of the varieties. It was classified as resistant and reached 316% more growth in length. We conclude that it could be possible to use this variety as a tolerant control for future field resistance trials against Phytophthora sojae in Argentina.

P1.3-062

FUNCTIONAL ANALYSIS OF SUGAR TRANSPORTERS IN GRAPE UPON BOTRYTIS CINEREA INFECTION

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Text

Understanding of plant defense mechanisms is essential for developing alternative solutions to fight against cryptogamic diseases. Among these mechanisms, increasing evidence indicate that sugar transporters and invertases play important roles in plant resistance to pathogens. We previously reported that the overexpression of PM-localized H+/ hexose symporters STP13 enhanced Arabidopsis basal resistance to the necrotrophic fungus *Botrytis cinerea*, whereas STP13-deficiency resulted in an enhanced susceptibility indicating that STP13 may improve resistance by depriving the pathogen from resources and fueling the plant defense responses. By contrast, STPs could also play a negative role in defense by promoting the proliferation of biotrophic fungi during infection, as previously demonstrated in wheat and barley. Based on these studies, we now expand our research to plant of agricultural interest such as grapevine. To explore the functional role of sugar transporter genes upon *Botrytis cinerea* infection, we used a gain-of-function approach. Here, we present the phenotypic analysis of the ectopic expression of a candidate gene. Our results indicate that the control of apoplastic sugars by the activity of PM-localized hexose transporters may constitute a host-defense strategy that limits fungal proliferation.

Plant virus and host interactions - from molecular mechanisms to crop protection

C1.2-1

UNDERSTANDING THE PLANT MANIPULATION BY GEMINIVIRUSES

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Text
Viruses, as intracellular parasites, need to subvert the host cell in order to enable viral replication and spread. Due to strict coding limitations, viruses commonly produce a reduced number of proteins; this is the case of geminiviruses, plant DNA viruses believed to contain only 4-8 translated ORFs in their circular single-stranded genome. Strikingly, geminiviruses, like other plant viruses, are still able to successfully infect host plants, dramatically altering plant development and physiology, and ultimately causing devastating diseases to crops worldwide. In our group, we are interested in understanding how geminiviruses manipulate the plant cell and lead to disease, for which we use a combination of approaches, including molecular biology, cell biology, and genetics. By studying individual viral proteins, our results have shed light onto the molecular mechanisms underlying the replication of viral DNA, plant anti-viral defence and geminiviral counter-defence, and symptom development; in addition, we are interested in the identification of novel virulence strategies potentially employed by geminiviruses to maximize their coding capacity and impact on the host cell. These strategies include a prevalence of intra-viral protein-protein interactions, which shape the localization of viral proteins and their effect on the infected cell. The next challenge to be tackled is the study of the potentially expanded viral protein functional repertoire in a context-dependent manner

C1.2-2

A NOVEL ILARVIRUS PROTEIN IS EXPRESSED VIA STOP CODON READTHROUGH AND SUPPRESSES RDR6-DEPENDENT RNAI

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Text
Due to the limited size of their genomes, RNA viruses often use non-canonical translation mechanisms to optimize their coding capacity. One of such mechanisms is stop codon readthrough. By applying coding potential prediction algorithms to the genome sequences of ilarviruses, we discovered that ribosomal read-through of the coat protein (CP) stop codon can lead to the production of a C-terminal extended version of CP named CP-RT. We found that CP-RT of Asparagus virus 2 (AV2) is expressed upon infection. AV2 CP-RT deficient mutants unlike the wild type virus could not establish persistent infection. Heterologous RT domain of TSV, an ilarvirus belonging to a different subgroup, complemented the lack of AV2 RT domain which suggests evolutionary conserved function of CP-RT within the ilarvirus genus. We found that CP-RT acts as a viral silencing suppressor (VSR). RDR6-deficiency compensated for the lack of CP-RT we anticipate that CP-RT allows persistent AV2 infection by suppressing RDR6-dependent RNAi. RDR6 is a key factor limiting virus entry to the meristems. AV2 is vertically transmitted and infects shoot apical meristem (SAM)persistently. Mutations of RT domain changed the infection pattern of SAM from persistent into transient in the wild type plants but not in RDR6 knockdown plants. Further investigation is needed to find out whether attenuation of AV2 CP-RT mutants is a consequence of transient invasion of

SAM or both are manifestation of the same process.

C1.2-3

ANALYSIS OF THE INTERACTOME OF 17K PROTEIN, AN IMPORTANT VIRULENCE FACTOR CONSERVED IN LUTEOVIRUSES AND POLEROVIRUSES

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Text

Luteoviruses and poleroviruses cause many severe diseases in plants. A deep understanding of the molecular pathogenesis of these viruses will facilitate the development of novel measures for controlling their damages to global crop productions. The two types of viruses carry similar but not identical genomes, with the 17K protein conserved between them. Studies conducted using barley yellow dwarf luteoviruses (e.g., BYDV-PAV and BYDV-GAV) suggest that 17K play multiple roles to promote viral infection by acting as a movement protein, a suppressor of gene silencing, and an inhibitor of host mitosis. However, host plants have evolved an ability to limit the function of 17K by phosphorylating it, with the phosphorylated 17K subverted to enhance antiviral defense by elevating vsiRNA abundance. Clearly, the functions of 17K in luteovirus pathogenesis are regulated in a complex manner, although the proteins interacting with 17K in host cells remain fully characterized. Therefore, we have taken three complementary approaches (i.e., co-immunoprecipitation coupled mass spectrometry analysis, yeast two hybrid experiment, and phospho-proteomics) to identify the proteins interacting with BYDV 17K. A large number of host factors interacting with BYDV 17K have been identified and several of them have been characterized in depth, which has provided new insights into the molecular pathogenesis of BYDVs, as well as innovative strategies for enhancing host resistance to BYDV infection.

C1.2-4

A PLANT VIRUS EXPLOITS ACTIN FILAMENT-BINDING KINESIN MOTORS TO ESCAPE XENOPHAGY

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Text

Autophagy has emerged as an integral part of antiviral immunity in metazoans and plants,

including the selective elimination of viruses or their individual components. Adapted viruses have evolved multiple strategies to antagonize xenophagic degradation. The mechanistic understanding of the complex interplay between viruses and host autophagy is still limited in plants. We have previously shown that the autophagy cargo receptor NEIGHBOR OF BRCA1 (NBR1) mediates the degradation of virus particles of cauliflower mosaic virus (CaMV). Intriguingly, CaMV-induced viral inclusion structures formed by the viral P6 protein protect against autophagic destruction by sequestering capsid proteins. To further dissect the molecular processes underlying NBR1-mediated xenophagy and its counteraction by CaMV, we have applied a proteomics approach to isolate proteins associated with ATG8 and NBR1. We found that the actin-binding kinesin-like protein KAC1 is enriched in both interactomes during CaMV infection. We show that KAC1 and its close homolog KAC2 suppress the xenophagy pathway and promote viral accumulation and insect transmission. We further demonstrate that KAC1 associates with P6 inclusions in an actin filament-dependent manner and interacts with viral capsid proteins and particles. Our findings indicate that CaMV recruits host kinesin motor proteins to evade NBR1 targeting and to sequester virus material in autophagy-resistant inclusions.

C1.2-5

INVESTIGATING THE ANTIVIRAL DEFENSES PROTECTING PLANT STEM CELLS AND GERMLINE FROM INFECTION

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Text

Stem cells are essential for the development and organ regeneration of multicellular organisms, so their infection by pathogenic viruses must be prevented. Accordingly, mammalian stem cells are highly resistant to viral infection due to dedicated antiviral pathways including RNA interference (RNAi). In plants, a small group of stem cells harbored within the shoot apical meristem (SAM) generates all postembryonic above-ground tissues, including the germline cells. Many viruses do not proliferate in these cells, yet the molecular bases of this exclusion remain only partially understood. We show that a plant-encoded RNA-dependent RNA polymerase, after activation by the plant hormone salicylic acid, amplifies antiviral RNAi in infected tissues. This provides stem cells with RNA-based virus sequence information, which prevents virus proliferation. Furthermore, we find RNAi to be necessary for stem cell exclusion of several unrelated RNA viruses, despite their ability to efficiently suppress RNAi in the rest of the plant. In parallel to this work we developed cutting edge live microscopy techniques to track virus movement in plant reproductive organs, which will allow unparalleled analysis of infection dynamics. Finally, we have developed biological tools to investigate the antiviral mechanisms preventing virus vertical transmission through the germline, which ensure most infections are not transmitted by seed.

C1.2-6

TWO VIRAL PROTEINS TRANSLATED FROM ONE OPEN READING FRAME TARGET DIFFERENT LAYERS OF PLANT DEFENCE

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Text
Multi-layered defence responses are activated upon pathogen attack. Viruses utilize a number of strategies to maximize the coding capacity of their small-sized genomes and produce viral proteins for infection, including suppression of host defence. Here, we uncover translation leakage as one of these strategies: two viral effectors, cC4 and mC4, encoded by tomato golden mosaic virus (TGMV) are translated from two in-frame start codons and function cooperatively to suppress defence. cC4 localizes in chloroplasts, to which it recruits NbPUB4 to induce ubiquitination of the outer membrane; as a result, this organelle gets degraded, and chloroplast-mediated defences are abrogated. However, chloroplast-localized cC4 induces the production of singlet oxygen (1O2), which in turn promotes the translocation of the 1O2 sensor NbMBS1 from the cytosol to the nucleus, where it activates the expression of the CERK1 gene. Importantly, an anti-viral effect exerted by CERK1 is countered by mC4, localized at the plasma membrane. mC4, similarly to cC4, recruits NbPUB4 and promotes the ubiquitination and subsequent degradation of CERK1, suppressing membrane-based receptor-like kinase-dependent defences. Importantly, this translation leakage strategy seems to be conserved in multiple viral species and is related with host range, underscoring its adaptive value.

F1.2-1

IDENTIFICATION OF A. THALIANA ALY PROTEINS AS NOVEL HOST INTERACTING PARTNERS OF THE TURNIP YELLOWS VIRUS (TUYV) CP AND CP-RT PROTEINS

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Text
As for all viral pathogens, the establishment of turnip yellows virus (TuYV) cycle, belonging to the Polerovirus genus and newly to the Solemoviridae family, requires the expression of viral proteins whose function depends on their dynamic interactions with host factors. The investigation of these protein-protein interactions by yeast two-hybrid screen identified ALY proteins as potential interactants of two TuYV proteins. In Arabidopsis thaliana, four ALY family proteins (AtALY1 to 4) have been described. They participate redundantly in the nuclear export of cellular mRNAs mediated by the THO-TREX multiprotein complex.
Using co-immunoprecipitation experiments, we confirmed the interaction of all four AtALY proteins with the major capsid protein (CP) and also with the CP-RT fusion protein, which is notably involved in virus movement and in the plant aphid transmission.
Confocal microscopy analysis of agro-infiltrated Nicotiana benthamiana leaves confirmed the

nuclear colocalization of the four AtALY:eGFP with the CP:RFP protein in viral context, while the CP-RT:tRFP protein expressed by the virus localized in perinuclear vesicles associated with viral infection.
Importantly, we have shown that the inhibition of expression of the four AtALY genes in a quadruple mutant of A. thaliana induced a significant increase in TuYV accumulation, proposing the AtALY as a potential target to elaborate a novel strategy to fight against TuYV infection.

F1.2-2

P13 PROTEIN OF CITRUS TRISTEZA VIRUS IS A KEY REGULATOR OF STEM-PITTING SYMPTOM DEVELOPMENT IN CITRUS MACROPHYLLA

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Text
Stem-pitting (SP), an important malady of many fruit trees, is caused by *Citrus tristeza virus* (CTV) in citrus. CTV mutant with deletion of p13 displays very mild SP symptoms in *Citrus macrophylla* (Cmac). To reveal the role of this protein in SP development, we performed a combined study of transcriptome and microscopy analysis and characterization of phloem cell occlusions in Cmac infected with infectious clone of CTVΔp13 and CTV wild type (WT). CTVΔp13 induced non-visible SP symptoms and few defense response and development related genes, while the transcriptome responses of CTV WT infected trees, that caused moderate SP, was significantly affected. Transcriptomic and microscopic analyses of the stem-pitted area revealed that phloem regeneration is a characteristic manifestation of CTV-SP disease, while phloem regeneration was not observed in CTVΔp13 infected trees. CTVΔp13 induced less callose accumulation and lower *PP2* gene expression comparing to CTV WT. To further define the role of p13 protein in SP, p13 transgenic Carrizo and Cmac were generated. In Cmac transgenic tree, we observed longitudinal stem-pit like symptoms and ropey-like appearance of the stem that mimics CTV-SP symptoms. Microscopic analysis of vasculature tissue of symptomatic stem indicated collapse of phloem cells. Collectively, p13 activates plant responses and induces phloem occlusion factors which disrupt phloem cells, resulting in regeneration of new cells that contribute to SP symptom development.

P1.2-001

PATHOSYSTEM DRIVERS OF CHANGE INFLUENCING FIRST REPORT OF PHASEY BEAN MILD YELLOWS VIRUS INFECTING GROUNDNUT IN KENYA

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Text
Pathosystem drivers of change parameters have impacted directly and indirectly on the declining economic and nutritive value of groundnut (Peanut, Arachis hypogaea) in Kenya. PBMYV naturally infects Phasey bean (Macroptilium lathyroides) and has not been reported in Kenya. Transmission of PBMYV is by the groundnut-cowpea aphid (Aphis craccivora), whitefly (Bemisia tabaci) and grafting with infected scions. A survey in Bungoma, Busia, Kakamega and Siaya Counties during the short and long rains of 2020-2021, collected symptomatic leaves with mild yellowing, chlorotic streaking, chlorotic spots, mottling, bunching, puckering, curling downwards, stunting and those with evidence of aphid colonies. They were subjected to RT-PCR diagnostics and the positive samples were pooled then sequenced on the Illumina MiSeq platform for complete genome studies. Phylogenetic analysis was done using MEGA X software and Cucurbit aphid-borne yellows virus polerovirus (MZ508305.1) was used as a rooting outgroup. The PBMYV full genome sequences from Kenya (PBMYV_6, PBMYV_7) clustered together with other PBMYV and had closest sequence identity (91-95%) with PBMYV (KT963000.2, MT966033.1 and MT966038.1) from the GenBank. The PBMYV Kenyan strain is similarly diverse to the genetically distinct PBMYV Australian variants by descent. Further studies are needed to understand the contribution of climate change on the new disease distribution and molecular diversity of PBMYV in groundnut pathosystems.

P1.2-002

PSAC AND ATPSYN-A GENES INDUCE THE RNA SILENCING PATHWAY AND CAUSE RESISTANCE AGAINST THE SOYBEAN MOSAIC VIRUS

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Text
Increasing lines of evidence indicate that chloroplast-related genes are involved in plant–virus interactions. However, the involvement of photosynthesis-related genes in plant immunity is largely unexplored. RNA-Seq analysis showed that several chloroplast-related genes were strongly induced in response to infection with the avirulent strain of soybean mosaic virus (SMV), G5H, but were weakly induced in response to the virulent strain, G7H. For further analysis, we selected the *PSaC* gene from the photosystem I (PSI) and the ATP-synthase α-subunit (*ATPsyn-α*) gene whose encoded protein is part of the ATP-synthase complex. Overexpression of either gene within the G7H genome reduced virus levels in the susceptible cultivar Lee74 (*rsv3*-null). Both proteins are localized in the chloroplast envelope and the nucleus and cytoplasm. Because the chloroplast is the initial biosynthesis site of defense-related hormones, we determined whether hormone-related genes are involved in the *ATPsyn-α*- and *PSaC*-mediated defense. Interestingly, genes involved in the biosynthesis of several hormones were upregulated in plants infected with SMV-G7H expressing ATPsyn-α and PSaC. Both chimeras induced the expression of several antiviral RNA silencing genes, which indicates that such resistance may be partially achieved through the RNA silencing

pathway. These findings highlight the role of photosynthesis-related genes in regulating resistance to viruses.

P1.2-003

A PLANT-SPECIFIC HOMOLOG OF DP1/YOP1 FAMILY PROTEINS PLAYS A PROVIRAL ROLE IN POTYVIRUS INFECTION

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Text

The *Potyvirus* genus is one of the largest genera of plant RNA viruses responsible for serious diseases in crops worldwide. As potyviruses hijack the host secretory pathway and plasmodesmata (PD) for their transport, the goal of this study was to identify membrane and/or PD-proteins that interact with the 6K2 protein, a potyviral protein involved in replication and cell-to-cell movement of turnip mosaic virus (TuMV). Using Split-ubiquitin membrane Y2H assays we screened an Arabidopsis cDNA library for interactors of TuMV-6K2. We isolated AtHVA22a (*Hordeum vulgare* abscisic acid responsive gene 22) that belongs to a multigenic family of proteins homologous to DP1/Yop1 family proteins in yeast and interactors of reticulons. The role of HVA22 proteins in plants are not well-known, except the role in blast disease resistance in rice. Interestingly, proteomics analysis of PD fractions showed that AtHVA22a is highly enriched in Arabidopsis plasmodesmata proteome. We confirmed the interaction between 6K2 and AtHVA22a in yeast, as well as *in planta* by using bimolecular fluorescence complementation (BiFC) and showed that the interaction occurs at the level of the viral replication complexes (VRC) during TuMV infection. Finally, we showed that the propagation of TuMV in plants is increased when AtHVA22a is overexpressed but slowed down upon mutation of *AtHVA22a* by CRISPR-Cas9. Altogether, our results indicate that AtHVA22a plays an agonistic effect on TuMV propagation.

P1.2-004

TRANSCRIPTOMIC RESPONSES TO THE BANANA BUNCHY TOP VIRUS IN BANANA

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Text

The banana bunchy top virus is the most important virus disease of banana and there is no natural resistance in any Musa spp. Towards identifying potential susceptibility factors that facilitate BBTV infection, the current study profiled transcriptomic responses to BBTV in banana using RNASeq. Using a time course approach, 563, 1052, and 818 genes were differentially expressed between infected and uninfected plants at 7, 30, and 45 days post-inoculation, respectively. Enrichment analysis revealed that genes involved in the regulation of the cell cycle, DNA replication, far-red light signaling, stomatal movement, and chromatin-associated regulation of transcription, were associated with BBTV infection. Upregulated differentially expressed genes (DEGs) included the proliferating cell nuclear antigen (a key factor in the cell cycle), DNA replication licensing factors mcm2-6 that are central to the initiation of replication, histone subunits involved in epigenetic regulation of transcription, and HY5 and MYB transcription factors. DEGs involved in defence, including a thaumatin-like protein, ethylene-responsive transcription factor ERF024, a germin-like protein, and chalcone synthase, were downregulated in BBTV-infected plants. CRISPR/Cas9-mediated knock-out of candidate susceptibility factors identified among DEGs in this study is currently being pursued towards determining their potential utility in engineering resistance to BBTV in banana.

P1.2-005

NMD-MEDIATED VIRUS RESTRICTION IS COMPROMISED BY VIRUS-INDUCED AUTOPHAGIC DEGRADATION OF SMG7 IN PLANTS

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Text

Increasing evidence has suggested that NMD acts as a general virus restriction mechanism in eukaryotes. However, whether NMD factors, including SMG7 and UPF3, regulate virus infection is largely obscure. Here we show that overexpression of NbSMG7 and NbUPF3 attenuates cucumber green mottle mosaic virus (CGMMV) infection by targeting viral internal termination codon (iTC) and knock-down of NbSMG7 and NbUPF3 cooperatively facilitates virus infection. CGMMV infection upregulates the NbSMG7 transcription level but decreases its protein accumulation. Furthermore, NbSMG7, rather than NbUPF3, is subjected to autophagic degradation, which is executed by interacting with one of the autophagy-related proteins, NbATG8i. Mutation of the ATG8 interacting motif (AIM) in NbSMG7 (SMG7AIM1) abolishes its interaction with NbATG8i and comprises its autophagic degradation. Silencing of NbSMG7 and NbATG8i, or NbUPF3 and NbATG8i, compared to silencing of an individual one, leads to more virus accumulations, but overexpression of NbSMG7 and NbATG8i fails to achieve more potent virus inhibition compared to overexpressing one of them. However, overexpressing NbSMG7AIM1 and NbATG8i, or NbUPF3 and NbATG8i with CGMMV, exhibits more aggravated virus symptoms and more virus titers compared to their expression. These data show that both NMD and autophagy restrict virus infection, while NMD-mediated virus inhibition could be impaired by virus-induced autophagic degradation of NbSMG7.

P1.2-006

TRANSCRIPTOMIC AND FUNCTIONAL ANALYSES REVEAL THE ROLES OF EXOGENOUS BORON IN ALLEVIATING CUCUMBER GREEN MOTTLE MOSAIC VIRUS INFECTION

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Text

Cucumber green mottle mosaic virus (CGMMV)-induced watermelon blood flesh disease (WBFD) severely reduces the yield and edibleness of watermelon. We have found that application of exogenous boron can suppress CGMMV infection in watermelon fruit and alleviate WBFD symptoms. Here, the combined analysis results of proteomic and metabolomic showed that the main metabolic pathways of watermelon resistance to CGMMV infection by boron were concentrated in pyrimidine metabolism, sulfur metabolism, steroid biosynthesis, pyruvate metabolism, propanoate metabolism and photosynthesis. The most up-regulated differentially expressed genes (DEGs) were related to polyamine and auxin biosynthesis, abscisic acid catabolism, defense-related pathways, cell wall modification, and energy and secondary metabolism, while the down-regulated DEGs were mostly involved in ethylene biosynthesis, cell wall catabolism, and plasma membrane functions in transcriptome analysis. Additionally, in virus-induced gene silencing assays, silencing of *SPDS*, *BG12*, *SBT*, and *TUBB1* expression in watermelon caused an inhibited CGMMV infection correlating with no WBFD symptoms. In contrast, silencing *XTH23*, *PE/PEI7*, *GST*, and *ATPS1* expression promoted CGMMV accumulation, and *UGDH*, *AST*, *4CL4*, *RAP2-3*, *MYB6*, *WRKY12*, *H2A*, and *DnaJ11* are likely to participate in host antiviral resistance. Our results provide a novel molecular mechanism on the roles of boron in watermelon resistance to CGMMV-induced WBFD.

P1.2-007

UNDERSTANDING THE ROLE OF WRKY1 TRANSCRIPTION FACTOR IN PLANT RESISTANCE TO GEMINIVIRUS INFECTION

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Text

Geminiviruses constitute the largest group of known plant viruses and cause devastating diseases and economic losses in many crops worldwide. Understanding plant antiviral defense against geminiviruses is critical for development of strategies for geminivirus control especially in the case of limited naturally occurring resistance genes. Here we identified NbWRKY1 as a positive regulator of plant defense against geminivirus infection. We found that NbWRKY1 was upregulated in response to tomato yellow leaf curl China virus/tomato yellow leaf curl China betasatellite (TYLCCNV/TYLCCNB) infection. Overexpression of NbWRKY1 attenuated TYLCCNV/TYLCCNB infection, whereas knockdown of NbWRKY1 enhanced plant susceptibility to TYLCCNV/TYLCCNB. We further revealed that NbWRKY1 bound to the promoter of the NbWHIRLY1 (NbWhy1) transcription factor and inhibited the

transcription of NbWhy1. Consistently, NbWhy1 negatively regulates plant resistance against TYLCCNV/TYLCCNB. Furthermore, we demonstrated that NbWhy1 interfered with the antiviral RNAi defense and disrupted the interaction between NbCaM3 and NbCAMTA3. Moreover, the NbWRKY1-NbWhy1 also confers plant resistance toward tomato yellow leaf curl virus infection. Taken together, our findings suggest that NbWRKY1 positively regulates plant resistance to geminivirus infection by repressing NbWhy1. We propose that the NbWRKY1-NbWhy1 cascade could be further employed to control geminiviruses.

P1.2-008

OCCURRENCE OF YELLOWING VIRUSES INFECTING CUCURBITS IN KOREA AND DEVELOPMENT OF MULTIPLEX RT-PCR ASSAY FOR SIMULTANEOUS DETECTION OF THREE CUCURBIT VIRUSES

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Text
Cucurbits are one of the highly cultivated and economically important crops worldwide but their production is often limited by plant viruses. In the recent years, yellowing diseases caused by viruses have been damaging to cucurbit crops in Korea. A survey of the incidence of yellowing viruses in melon, cucumber, and watermelon crops was conducted in Korea during 2022. Reverse-transcription polymerase chain reaction (RT-PCR) was performed with specific primers for cucurbit aphid-borne yellows virus (CABYV) and cucurbit chlorotic yellows virus (CCYV) in melon and cucumber and melon aphid-borne yellows virus (MABYV) in watermelon. The results showed that the infection rates of CABYV were 35.1% (n=259/738) for melon; 10.4% (n=78/747) for cucumber and the infection rates of CCYV were 9.2% (n=68/738) for melon; 11.2% (n=84/747) for cucumber. MABYV, which was first reported in 2021, was detected only in 6 greenhouses growing watermelon from 2 areas with an infection rate of 3.6% (n=20/562). Sequence analysis based on the complete genome sequences of CABYV, CCYV, and MABYV revealed 96~99% nucleotide identities, respectively, with previously reported sequences. Since the yellowing symptoms of the three viruses CABYV, CCYV, and MABYV are virtually identically and occur as mixed infections in cucurbits grown in Korea, we developed a multiplex RT-PCR method for rapid, sensitive, and simultaneous detection of the three viruses.

P1.2-009

EVALUATION OF SQUASH RESISTANCE TO LEAF CURL DISEASE AND THE DEVELOPMENT OF RELATED MOLECULAR MARKER

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Text
Squash (*Cucurbita moschata*) is an important crop worldwide. However, cucurbit leaf curl

disease (CuLCD), causing by whitefly-transmitted begomoviruses results significant yield losses. In Southeast Asia, three major bipartite begomoviruses, Tomato leaf curl New Delhi virus (ToLCNDV), Squash leaf curl China virus (SLCCNV) and Squash leaf curl Philippines virus (SLCuPV) are associated with CuLCD. Resistance squash cultivars are important for the disease control, but limited for resistant resources. So far, few squash lines were resistant to CuLCD. In Taiwan, SLCuPV is the major squash begomovirus. Two SLCuPV resistant lines, TOT8101A and Bi were screened by whitefly inoculation. The Bi line was also confirmed with SLCCNV resistance by agroinoculation of infectious virus. Furthermore, the resistance of TOT8101A is single recessive gene, and Bi is one dominance. Based on the F2 population generated by crossing of susceptible squash with Bi line, five resistance marker candidates were screened by sequence-related amplified polymorphism (SRAP). Consequently, one SRAP marker was verified in a F3 population. Following the sequence analysis of the resistance SRAP marker, it has 99.63% nucleotide identity with putative disease resistance protein at 3806932-3807467 nt of chromosome 6 of the squash whole genomic sequences. The co-dominant marker of the dominant resistance is under developing for marker-assessed selection in the squash breeding.

P1.2-010

EFFECT OF PHYSALIS RUGOSE MOSAIC VIRUS (PHYRMV) AND GROUNDNUT RINGSPOT VIRUS (GRSV), IN SINGLE AND DOUBLE INFECTIONS, ON THE DEVELOPMENT, PRODUCTION, AND POSTHARVEST FRUITS PARAMETERS OF PHYSALIS PERUVIANA PLANTS

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Text

Physalis peruviana (Solanaceae), native to the South America Andean region, is an exotic plant whose cultivation is still emerging in Brazil. Five viruses have been reported naturally infecting physalis plants in the country. However, two have been drawing attention due to the severity of symptoms: the sobemovirus PhyRMV and the orthotospovirus GRSV. The present work aimed to evaluate the damage caused by single and double infections of PhyRMV and GRSV in the development, yield, and postharvest fruits parameters of *P. peruviana* plants. Both viruses caused severe systemic symptoms in infected plants, and double infection caused plant death. Plant height and aerial dry weight mass were significantly affected by infection with PhyRMV and GRSV. Double-infected plants were the most affected, with an average height and aerial dry weight mass reduction of 70.9% and 89.7%, respectively. PhyRMV- and GRSV-infected plants showed an average fruit yield reduction of 66.4% and 85.2%, respectively, compared to healthy plants. Double-infected plants did not produce fruits. Fruits produced by infected plants were smaller than those produced by healthy plants. Virus infection also affected postharvest parameters such as titratable acidity, total soluble solids (ºBRIX), the concentration of phenolic compounds, and antioxidant activity. The viruses did

not affect fruit color but affected pulp firmness and palatability. Therefore, strategies for disease management should be studied.

P1.2-011

HAM1 IS PRESENT IN SECOVIRUSES AS WELL AS IPOMOVIRUSES AND DISPLAYS ITPASE ACTIVITY

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Text

The production of cassava, a staple crop for many people in Africa, Asia, and Latin America, is threatened by viral infections such as cassava brown streak disease (CBSD) and cassava torrado-like virus (CsTLV). These viruses encode atypical Ham1 proteins with a specific structural domain known as the Maf/HAM1, which has a highly conserved inosine triphosphate (ITP) pyrophosphohydrolase (ITPase). Maf/HAM1 domains in viral genomes are uncommon; only two other viral species that infect euphorbia hosts have been identified (EuRV) and (CBSD). This study investigated the role of HAM1 proteins and their ITPase activity in CsTLV, the cassava plant, Nicotiana benthamiana, and CBSV. Our results showed that all the Ham1 proteins studied had high ITPase activity against non-canonical nucleotides such as ITP. Interestingly, we found that the CsTLV-Ham1 and Cassava plant Ham1 proteins had higher pyrophosphohydrolase activity specifically for ITP but not for dITP. Furthermore, we observed that the CBSVD-Ham1 protein with a specific point mutation (SHA) significantly reduced ITPase activity against noncanonical and canonical nucleotides such as GTP. These findings suggest that the leucine residues in the GLR motif of CsTLV-HAM1 may be important for substrate selection and activity in HAM1 protein, and further research is required. This study provides a deeper understanding of the mechanism of ITPase activity in CsTLV and its potential role in reducing viral mutation rates during infection.

P1.2-012

A PLANT VIRAL PROTEIN PROMOTES PLANT DISEASE DEVELOPMENT VIA MODULATING AUXIN HOMEOSTASIS

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Text

Potato virus Y (PVY) is an economically important plant pathogen that reduces the productivity of a wide range of host plants. PVY causes symptoms such as stunted growth, severe chlorotic mosaic, and leaf curling affecting the lamina. To develop PVY-resistant cultivars, it would be essential to identify the plant-PVY interactome and decipher the biological significance of those molecular interactions. We performed a yeast two-hybrid (Y2H) screen of *Nicotiana benthamiana* cDNA library using PVY-encoded NIa-pro as the bait. The *N. benthamiana Indole-3-acetic acid-amido synthetase* (IAAS) was identified as an interactor of NIa-pro protein. The interaction was confirmed via targeted Y2H and bimolecular

fluorescence complementation (BiFC) assays. We have shown the subcellular localization of both NIa-pro and IAAS protein in the nucleus and cytosol. IAAS converts free (active) IAA to the inactive, conjugated form, which plays a crucial regulatory role in auxin signaling. Transient silencing of IAAS in *N. benthamiana* plants interfered with the PVY-mediated symptom induction and virus accumulation. Conversely, overexpression of IAAS enhanced the symptoms induction and virus accumulation in the infected plants. In addition, the expression of several auxin-responsive genes was found to be downregulated during PVY infection. Our findings demonstrate that PVY NIa-pro protein potentially promotes disease development via modulating auxin homeostasis.

P1.2-013

PATHOGEN-TRIGGERED METABOLIC ADJUSTMENTS TO POTATO VIRUS Y INFECTION IN POTATO

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Text

Potato virus Y (PVY) is an important viral pathogen of potato. So far, the molecular plant-virus interactions underlying this pathogenicity are not fully understood. Gas chromatography coupled with mass spectroscopy (GC–MS) was used to study the changes in leaf metabolomes of PVY-resistant cv. Premier Russet (PR), and a susceptible cv. Russet Burbank (RB), following inoculation with three PVY strains, PVY-NTN, PVY N-Wi, and PVYO. Analysis of the resulting GC–MS spectra showed several common and strain-specific metabolites that are induced by PVY inoculation. In PR, the major overlap in differential accumulation was found between PVY N-Wi and PVYO. However, the 14 significant pathways occurred solely due to PVY N-Wi. In contrast, the main overlap in differential metabolite profiles and pathways in RB was between PVY-NTN and PVYO. Overall, limited overlap was observed between PVY-NTN and PVY N-Wi. As a result, PVY N-Wi induced necrosis may be mechanistically distinguishable from that of PVY-NTN. Furthermore, 10 common and seven cultivar-specific metabolites as potential indicators of PVY infection and susceptibility/resistance were identified. In RB, glucose-6-phosphate and fructose-6-phosphate were particularly affected by strain–time interaction. This highlights the relevance of the regulation of carbohydrate metabolism for defense against PVY. Some strain- and cultivar dependent metabolite changes were also observed.

P1.2-014

THE TRIOSE PHOSPHATE/PHOSPHATE TRANSLOCATOR EXPORTS PHOTOSYNTHETIC GLYCERALDEHYDE 3-PHOSPHATE FROM CHLOROPLASTS TO TRIGGER ANTIMICROBIAL IMMUNITY IN PLANTS

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Text

Whether photosynthetic metabolites participate in host defenses against pathogens remains unclear. Here, we found the triose phosphate/phosphate translocator (TPT) located on the inner membrane of the chloroplast interacts with the movement protein (MP) of brassica yellows virus (BrYV) in vivo and in vitro, and its expression is significantly down-regulated by viral infection or transgenic expression of the MP. The loss-of-function mutants of tpt mutant shows increased accumulation of BrYV and conversely increased expression of TPT confers enhanced resistance. The antiviral activity of AtTPT requires its phosphate transport capacity, suggesting that it functions in fighting the virus via its transported substrate(s). To this end, we discovered that one of the TPT substrates, glyceraldehyde 3-phosphate (GAP), directly acts as an activator of the immune system to depress viral accumulation in leaves. At the mechanistic level, we revealed that exogenous application of GAP drastically triggers defense-related genes expression and prominently induces defense signaling pathways, such as MAPK. Both TPT and GAP demonstrate strong inhibitive activities against multiple types of plant pathogens. Collectively, we proposed that GAP exported by TPT to the cytosol triggers antimicrobial immunity and thus mediates broad-spectrum resistance to a variety of plant diseases by virtue of the chloroplast-to-nucleus/cytosol retrograde signaling.

P1.2-015

CHARACTERISTIC ANALYSIS OF CUCUMBER MOSAIC VIRUS INFECTED IN AUTOPHAGY-DEFECTIVE NICOTIANA BENTHAMIANA PLANTS

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Text

Plant viruses primarily consist of nucleic acids and proteins, and their degradation in host plants is considered as a defense mechanism against viruses. The RNA silencing mechanism, triggered by double-stranded RNAs derived from infected viruses, effectively degrade the viral genomic RNA. Meanwhile, growing evidences suggest that autophagy (AP), a major proteolytic system, plays a crucial role in antiviral defense in plants. Our earlier research demonstrated that RNA silencing and AP work in tandem against cucumber mosaic virus (CMV). To counteract antiviral RNA silencing, most plant viruses express RNA silencing suppressors; CMV has the 2b protein (2b). AP strengthens RNA silencing against CMV by targeting 2b through the calmodulin-like protein called rgs-CaM for degradation. In this study, we explored the interactions between CMV and AP by creating AP-defective *Nicotiana benthamiana* plants. Upon inoculating the CMV vectors that express foreign proteins by replacing 2b with the genes encoding these foreign proteins, we observed a great increase in CMV multiplication and accumulation of these foreign proteins. Our findings suggest that: 1) CMV-encoded protein levels may be affected by autophagy in addition to 2b, and 2) AP-defective *N. benthamiana* plants can serve as an excellent platform plant to produce useful proteins using the CMV vectors.

P1.2-016

THE ROLE OF 6K1 AND CI GENETIC REGIONS IN THE ADAPTATION OF POTATO VIRUS Y IN PEPPER

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Text

The *Capsicum annuum* / potato virus Y (PVY) pathosystem is characterized by an intriguing type of resistance as isolates belonging to most PVY phylogenetic groups are able to infect pepper only locally with virus remaining restricted at the sites of entry after mechanical inoculation. Previous studies using synthetic chimeras between infectious cDNA clones derived from adapted (C1 group) and non-adapted (N and C2 groups) PVY isolates showed that the infectivity of PVY towards *C. annuum* is effectively linked to the P3-6K1-CI genetic region of the viral genome. The P3 region, and specifically a nonsynonymous substitution in the P3 and P3N-PIPO cistrons (clone N605Y) was shown to be essential for the adaptation of the virus to *C. annuum*, as a 50% infection at the systemic level was established. To elucidate the effect of 6K1 and CI on PVY infectivity in pepper, a series of additional chimeras were constructed exchanging the 6K1 and CI regions of the N605Y clone with those derived from a C1-group isolate, individually or in combination. Infectivity experiments with the obtained infectious chimeras unveil the role of the 6K1 and CI regions in PVY adaptation to pepper increasing our understanding on this uncharacterized type of resistance.

P1.2-017

DEVELOPMENT OF FOSMID-BASED SYSTEM FOR CONSTRUCTION OF INFECTIOUS CDNA CLONE OF PAPAYA LEAF DISTORTION MOSAIC VIRUS ISOLATE FROM TAIWAN

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Text

Papaya is one of the important fruits in many countries, including Taiwan. The papaya ringspot virus (PRSV) is the major virus in the field. Although the use of PRSV-resistant transgenic papaya effectively controls PRSV, the papaya leaf distortion mosaic virus (PLDMV) is becoming a new threat to papaya production. In this study, we analyzed the symptoms, host range, and whole genome sequence of a new PLDMV isolate, PLDMV-CZ, from Taiwan. We found that PLDMV-CZ can cause more severe symptoms in PRSV-tolerant papaya cultivars. Since it is important to study the pathogenicity of PLDMV-CZ for the development of control strategies, the generation of the cDNA clone of the virus is essential. However, the large genome size of PLDMV-CZ (10,154 nucleotides) and instability in the bacterial cell make construction of the infectious clone difficult. Therefore, we developed a

fosmid-based system to generate stable infectious clones of PLDMV-CZ. We modified the nucleic extraction and reverse transcription approach for the generation of full-length viral cDNA. We further used a low-copy fosmid pCC1FOS as a vector for cloning viral cDNA. Our results showed that the cDNA clone of PLDMV-CZ was stable in Escherichia coli. The papaya seedlings inoculated with viral RNA transcripts derived from the PLDMV-CZ clone showed similar symptoms to the wild-type virus. The total time needed for construction is about one week, and this approach can be applied to other large and unstable plant viruses.

P1.2-018

SUGARCANE MOSAIC VIRUS-ENCODED NIA-PRO MANIPULATES PRE-MRNA SPLICING IN MAIZE

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Text

Sugarcane mosaic virus (SCMV), as the main causal agent of maize dwarf mosaic disease in major maize production regions of many countries, is a representative of monocot-infecting members of the genus *Potyvirus* in the family *Potyviridae*. Intriguingly, though potyviral replication only takes place in the cytoplasm, a few potyvirus-encoded proteins, such as the nuclear inclusion protein a protease (NIa-Pro) can accumulate in nuclei of infected cells. The significance of NIa-Pro nuclear localization remains largely unknown. In this study, using SCMV-encoded NIa as a bait, we screened and identified 77 unique maize proteins that could potentially interact with NIa through a TurboID-based proximity labeling approach followed by LC-MS/MS. The KEGG pathway enrichment analysis showed that potential maize NIa-interactors were significantly annotated in the ribosome, spliceosome, photosynthesis, and metabolisms. Further functional annotations identified nine conserved pre-mRNA splicing-associated proteins that have the potential to interact with SCMV NIa. Since NIa is cleaved into NIa-Pro and VPg in potyvirus infected cells, we subsequently determined the role of SCMV NIa-Pro and VPg on pre-mRNA splicing through a splicing reporter system *in planta*. We found that NIa-Pro along can regulate pre-mRNA splicing in cells. Together, these data suggest that upon SCMV infection, NIa-Pro possesses the capacity to manipulate pre-mRNA splicing through associating with splicing factors.

P1.2-019

DEVELOPMENT OF BARLEY YELLOW DWARF VIRUS (BYDV) INFECTIOUS CLONES

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Text

Barley yellow dwarf virus (BYDV) is widely distributed and can infect more than 150 Poaceae spp, causing yield losses in economically important cereals such as maize, wheat, barley, and rice. Studies exploring BYDV generally require already infected plants, and aphid colonies to maintain and transmit viral isolates. However, these can prove very costly due to space requirements and efforts to maintain them. Additionally, cross-contamination among aphid colonies can prove highly problematic. Virus transmission using aphids can also prove unreliable due to no guaranteed behavioural consistency, for instance, colonies can suddenly decrease, or viral transmission efficiency may vary. For these reasons, an effective method to maintain and transmit BYDV for relevant studies is of value. A practical alternative to maintaining the source(s) of BYDV and continual transmission of the virus is the development of infectious clones. These have many advantages; they allow the long-term storage of inocula in a cost-effective manner and eliminate the dependence on insect vectors for challenging target plants, as infectious clones can be reliably introduced into a plant via Agrobacterium-based methods. Infectious clones may prove key in different viral research where replication is fundamental, such as understanding the function and interactions of viral genes. The aim of work here is to develop BYDV infectious clones representing UK-wide viral diversity for future research applications.

P1.2-020

LOCALIZATION OF VIRAL PROTEINS ASSOCIATED WITH THE ADAPTATION OF POTATO VIRUS Y IN PEPPER.

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Text

The ability of a virus to infect a host is attributed to complicated interactions among viral and host proteins. Previous studies using hybrid virus constructs between adapted (C1 group) and non-adapted (N and C2 groups) potato virus Y (PVY) isolates showed that the adaptation of PVY to pepper is associated with the *P3-6K1-CI* genetic region of the viral genome, with *P3* region being essential. To further comprehend the role of each the four encoded proteins (P3, P3NPIPO, 6K1 and CI), their subcellular localization and co-localization were studied. The analyzed proteins derived from three PVY isolates: a pepper-adapted one, a non-adapted one that remains restricted to the sites of inoculation, and a point-mutant of the latter in the *P3* coding region with the ability to infect pepper systemically. Results indicate that the P3 protein is localized differently depending on the isolate. More specifically, the P3 of the adapted isolate is localized in the actin/ER network, whereas the one derived from the non-adapted isolate is found in the cytoplasm. On the other hand, the P3 of the point-mutant shows an intermediate phenotype with the formation of aggregates in the actin/ER system. The CI proteins analyzed are found in both the nucleus and the actin/ER network, whereas the 6K1 proteins are mainly targeted to chloroplasts. The full set of data obtained from the localization studies enlightens the complex cellular processes that regulate the adaptation of PVY to pepper.

P1.2-021

DIFFERENTIAL RESPONSE OF PEPPER AGAINST TWO EVOLUTIONARILY DISTINCT ISOLATES OF POTATO VIRUS Y

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Text

The present study aims to unravel the basis of an uncharacterized form of resistance observed in the *Capsicum annuum* / potato virus Y (PVY) pathosystem. Evolutionarily distinct PVY isolates exhibit a contrasted ability to infect pepper which is linked to at least a single amino acid substitution in the viral P3 coding region. The role of the early plant response in this phenomenon was assessed through transcriptomic analysis of pepper plants challenged with three PVY isolates: an adapted one, a non-adapted one that remains restricted to the sites of inoculation, and a point-mutant of the latter with the ability to infect pepper systemically. Using standard in silico methods, differentially expressed genes (DEGs), gene ontology (GO) terms and pathways were identified and evaluated as putative resistance factors and markers of infection. The analysis depicts the important role of genes associated with the defence regulation, the cell wall metabolic processes and the hormonal response. Furthermore, an immunoprecipitation assay using the P3 proteins of the three isolates pinpoints plant interacting proteins associated to this differential response of pepper. The data that emerge from this study significantly contribute to unravelling the host key factors associated with this type of resistance.

P1.2-022

IS THE GLYCOPROTEIN RESPONSIBLE FOR DIFFERENCES IN DISPERSAL RATES BETWEEN LETTUCE NECROTIC YELLOWS VIRUS SUBGROUPS?

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Text

The population of lettuce necrotic yellows virus (LNYV), a cytorhabdovirus, comprises two subgroups, SI and SII. The virus is vectored by aphids primarily by *Hyperomyzus lactucae*. It appears to be endemic to Australia and Aotearoa New Zealand (NZ) but shows different population structures in each country; S1 dominates in NZ, while it appears to have become extinct in Australia. It has been suggested that SII is outcompeting SI, possibly through greater vector transmission efficiency and/or higher replication rate in its host plant or insect

vector. Rhabdovirus glycoproteins are important for virus–insect interactions. Analysis of LNYV glycoprotein sequences from NZ shows the same subgroup structure as previous analysis of the nucleocapsid protein. Prediction of the 3D protein structures revealed domain architectures similar to Vesicular Stomatitis Virus (VSV). Importantly, amino acids at positions 244 and 247 of the post-fusion form of the LNYV SII glycoprotein influenced the predicted structure, glycosylation at N248 and the overall stability of the protein. These data support the glycoprotein as having a role in the population differences of LNYV observed between Australia and NZ.

P1.2-023

DETERMINATION OF PVY RESISTANCE AND EXPRESSION OF RESISTANCE ASSOCIATED GENES IN TOMATO PLANTS

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Text
Tomato is a well-developed model system for molecular genetic studies and a natural host of potato virus Y (PVY). In recent years, it has been reported that the eukaryotic translation initiation factor 4E gene family is associated with resistance to the Potyvirus genus. In Türkiye, the agent has been detected in many tomato production areas including diverse regions. The resistance status of some wild Solanum species and some tomato cultivars were evaluated for reaction to a PVYN:O isolate obtained from the ?zmir province of our country. Few amino acid changes in the eIF4E protein explain resistance to PVY. For this reason, the polymorphism of eIF4E in the genotypes was determined. Pot-1 locus, which plays a role in PVY resistance in tomato, was shown to be associated with eIF4E. Genetic linkage of them was investigated by using the dCAPS marker (eIF4E-SpeI) in the tomato genotypes used. Viral genome-linked protein (VPg) protein plays a role in breaking resistance genes in some PVY-resistant genotypes. The virulence status of the virus isolate was determined by sequencing VPg. The expression of eIF4E-related genes suggests their role in the tomato-PVY interaction. The expression status of eIF4E1, eIF4E2 and eIF(iso)4E genes during PVYN:O infection in Solanum arcanum LA2157 was analyzed at different time points by Real-time Quantitative PCR. GAPDH, UBI, UK and ACT genes were used to determine the reference gene which shows the least variation during PVY infection.

P1.2-024

DYNAMIC TRANSCRIPTIONAL PROFILES OF ARABIDOPSIS THALIANA PROTOPLASTS TRANSFECTED BY TOMATO SPOTTED WILT VIRUS

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Text
TSWV, belongs to the genus *Orthotospovirus*, is broadly distributed worldwide. It infects more than 1,350 species of monocotyledons and dicotyledons, and causes great economic loss. In order to identify early-expression genes related to TSWV resistance in *Arabidopsis*, *Arabidopsis* protoplasts transfected with TSWV were collected for differential gene expression analysis at 1, 2, and 4 hours post transfection. The results of transcriptome analysis showed that when compared with the buffer transfection control group, there were 16 genes showed an increase in expression level at 1 and 2 hours after TSWV transfection, while 37 genes showed a decrease in expression level. There were 3 genes showed an increase in expression level at 2 and 4 hours after TSWV transfection, while 18 genes showed a decrease in expression. The expression levels of 9 genes were confirmed by RT-qPCR and was consistent with the results of transcriptome analysis. TRV-based gene silencing system were conducted to characterize their functions. Results showed that silencing of plant viral-response family protein gene caused an increase of the TSWV N gene expression. Silencing of calcium-dependent phosphotriesterase gene, tasiR480/255 regulating target gene, G-box binding factor 4 gene, and Pam16 transporter protein gene caused a decrease of the N gene expression. The analyses of the resistance of transgenic *Arabidopsis* against TSWV are ongoing.

P1.2-025

THE ROLE OF CI HELICASE ACTIVITY IN POTYVIRAL MOVEMENT

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Text
Potyviruses are some of the most common and widely distributed plant pathogens which infect a wide range of both cultivated and wild plants. One of their distinctive features is the formation of cytoplasmic inclusions by their helicase, cylindrical inclusion protein (CI). CI is involved in many potyviral infection processes, most importantly, replication, and the spread of infection from one cell and tissue to another, also known as “movement”.
My poster will present the preliminary results of my doctoral thesis project which aims to better understand the role of CI helicase domain in potyviral infection. Six CI helicase domain mutations were selected from previous studies and introduced into Potato virus A (PVA) CI. One of these was deficient in replication and the other five in movement. The effect of these mutations on CI’s ATPase and helicase activity were measured in vitro. Additionally, their effects on PVA replication, translation, and movement in Nicotiana benthamiana were monitored. Levels of replication were measured by RT-qPCR, and translation by dual luciferase assays. Effects on movement were measured by fluorescent confocal microscopy using a dual fluorophore assay. The project will also map the CI interactome using proximity labelling and yeast two-hybrid.

P1.2-026

SNEAKY SUO: PLANT PROTEIN PROMOTING POTYVIRAL TRANSLATION

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Text
SUO is required for miRNA mediated translation repression, independent of miRNA biogenesis and stability. *suo* is a loss of function mutant which reduces the activity of miR156/miR157 and AGO1 causing early vegetative maturity in *Arabidopsis thaliana (A.t.)*. To study if *suo* has an effect on potyvirus infections, we infected *suo- A.t.* and *suo* knock-down *Nicotiana benthamiana (N.b.)* with turnip mosaic virus (TuMV:GFP) and potato virus A (PVA:Rluc), respectively. Col0 plants and empty hairpin-infiltrated *N.b.* were used as controls, respectively. Viral gene expression was quantified by GFP-ELISA for TuMV, while Dual luciferase assay was performed to quantify Rluc levels from PVA:Rluc. Viral RNA transcript levels were measured by relative quantification RT-qPCR. We observed a two-fold and three-fold reduction in viral protein levels when SUO was downregulated in TuMV-*A.t*. and PVA-*N.b*. pathosystems, respectively. Viral transcript levels in both pathosystems did not change compared to the controls. In the absence of SUO, viral translation was inhibited. This result suggests that the virus hijacks SUO to assist the infection by promoting viral translation. Interestingly, we did not observe any significant change in the transcript levels of the three SUO homologs and AGO1 during PVA infection in *N.b*. Based on our results, we put forward a conjecture that SUO likely contributes to translation-associated control of potyviral infection in a transcription independent manner.

P1.2-027

EVOLUTION OF PAPAYA LEAF CURL VIRUS IN INDIA: INTERPLAY BETWEEN MUTATION, RECOMBINATION AND SELECTION FORCE

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Text
Genome variability and virus evolution are fundamentally based on the rapid accumulation of mutations, which expedite the spread and survival of begomoviruses and its vector, allowing preferential replication of genes and the pattern of more complex infections by crossing geographical origin and host range. In this study, we studied the mutational and selection parameters of the complete genome of Papaya leaf curl virus (PaLCV) and associated betasatellite isolates from Gorakhpur, India, to comprehend how the virus is evolving in this geographical region. Consequently, phylogenetic and recombination analyses suggest the exchange of genetic material among and between the various begomovirus isolates infecting different crop varieties, implying the occurrence of natural inter- and intraspecies recombinations. 45% of the polymorphic sites show substitutions in the third nucleotide codon position, indicating that non-synonymous substitutions are more frequent. Although isolates indicate strong evidence of purifying selection at most polymorphic sites, considerable positive selection was also identified in some proteins of isolates, pointing to the adaptive evolution of PaLCV and its betasatellites. Therefore, these findings on evolutionary rates due to selection pressure and mutation of begomovirus isolates from Indian populations are imperative for the critical perception that will elucidate the dynamics of rapidly evolving begomoviruses.

P1.2-028

SCREENING PEPPER PROTEINS CONDITIONING INFECTION OF PEPPER MILD MOTTLE VIRUS

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Text

Pepper mild mottle virus (PMMoV) causes severe damage on pepper production worldwide. As a member in the genus *Tabmovirus*, PMMoV is highly transmitted and is stable in environment. It is urgent to develop new strategies and measures for PMMoV control. Since virus relies on host factors for infection and proliferation via direct or indirect interaction, and the virus-encoded replicase, coat protein (CP) and movement protein (MP) play essential roles in infection, it is of great significance to identify host proteins and their functions for virus infection cycle. In this study, we screened pepper proteins that might interact with PMMoV replicase, CP or MP by using TurboID-based proximity labeling approach followed by LC-MS/MS. The biologically function important proteins that might interact with replicase, CP or MP were evaluated through yeast two-hybrid and firefly luciferase complementation imaging assays. For PMMoV replicase, CP, and MP, interaction networks were separately constructed with screened pepper proteins. Functional studies are going on with focusing on interacting proteins involved in immunity and splicing regulation in pepper.
Junmin Zhang and Tao Zhou are corresponding authors.

P1.2-029

THE VIRULENCE FACTOR OF BEET NECROTIC YELLOW VEIN VIRUS (BNYVV) ACTS AS TRANSCRIPTIONAL REPRESSOR

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Text

Rhizomania is one of the most economically devastating disease of sugar beet and is caused by Beet necrotic yellow vein virus (BNYVV). The sugar beet taproot undergoes massive reprogramming of auxin-responsive genes upon BNYVV infection governed by P25, the virulence factor of the virus. P25 on its own localize to nucleus and in the cytoplasm, may associate with host chromatin and, thus, act as transcriptional activator/ repressor. To address this hypothesis, a genome-wide ChIP-seq was performed to identify P25-interacting promoter region of Beta vulgaris. ChIP-seq data highlighted genes of several pathways involved in cell cycle, osmoregulation, chloroplast function, auxin signaling, lateral root development etc.,

probably activated/repressed by P25 for the virus benefit. We assessed the transactivation activity of P25 on the promoters of those genes by dual-luciferase assay and P25 was found to repress the activity of selected ten candidate promoters. Interestingly, P25 repressed a promoter of a gene encoding UDP-glucuronosyltransferase (UGT). UGT negatively regulates auxin signaling by glycosylating and thereby sequestering IAA and inhibiting auxin-mediated responses such as lateral root development. BNYVV, on the other hand, seems to repress negative regulators of auxin signaling and auxin response. Thus, our work provided new insights on P25 action as a virulence factor. The mode of action of P25 and the effect of P25 target genes on virus replication will be discussed.

P1.2-030

A SEARCH FOR RESISTANCE BREAKING STRAINS OF TOMATO SPOTTED WILT ORTHOTOSPOVIRUS IN CROATIA AND SLOVENIA

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Text

Tomato spotted wilt orthotospovirus (TSWV, Orthotospovirus) is one of the most economically important emerging plant viruses. TSWV has an extremely broad host range, including more than 1000 species. The most efficient control strategy against TSWV so far is the use of available resistant varieties. In tomato, resistance is determined by the Sw-5b gene. However, the use of resistant varieties led to the emergence of TSWV isolates capable of overcoming the resistance genes (RB strains). Most of the tomato TSWV RB strains had one of the two amino acid changes (C118Y or T120N) in the non-structural protein (NSm gene). Recently, another mutation (D122G) was found to be associated with RB isolates. Symptomatic tomatoes in Croatia and Slovenia were screened for potential TSWV RB isolates. During the three-year period (2020-22), we collected a total of 93 tomato samples. For 13 representative samples, total RNA was isolated, depleted of rRNA and prepared for high-throughput sequencing using Illumina or Oxford Nanopore platforms. No specific amino acid changes characteristic of RB isolates were detected in any of these tomato samples. Our future work will focus on a more detailed molecular characterisation of TSWV isolates from Croatia and Slovenia.

P1.2-032

THE INTERPLAY BETWEEN VIRAL PROLINE/SERINE-RICH PROTEINS AND THE PLANT POSTTRANSLATIONAL MODIFICATIONS DYNAMICS

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Text
While plants activate a vast array of defense responses against virus pathogens, viruses utilize versatile strategies to manipulate cellular regulatory systems of a plant host for successful infection. Posttranslational protein modifications (PTMs) are crucial for rapid reprogramming of cellular signaling in response to virus infections; but viruses appear to hijack a variety of PTM dynamics to suppress host defense and promote infection. We have been investigating viral proteins that potentially undergo PTMs to increase their functional diversity during plant-virus interactions. Inspired by preliminary data, we focus on a protein of 16 kDa (p16), unknown function, and that has been predicted to be encoded by a small ORF located at the genomic 3' end of some members of the family *Tymoviridae*. From a systemic genome analysis across 197 virus accessions, 464 predicted ORFs (including multiple strains of the same virus and multiple ORFs of single strain) were selected for *in silico* analysis. The proteins predicted from the selected ORFs showed to have a very high proline/serine content as well as several conserved sites predicted as potential sites for kinases-mediated phosphorylation. Using a virus infectious clone and its predicted p16 protein as model of study, we showed that p16 targets chloroplast, interact with many chloroplastic proteins, potentially including chloroplastic kinases, and interfere with the plant hypersensitive response during virus infection.

P1.2-033

SPITFIRE – SCREENING OF PISUM SATIVUM ACCESSIONS FOR PNYDV RESISTANCE

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Text
Pea (*Pisum sativum*) is a high-value leguminous crop that is generally used for livestock feed and human consumption. Pea production in Germany and Austria suffers from the infection of a nanovirus, pea necrotic yellow dwarf virus (PNYDV). PNYDV is a multipartite, single-stranded, circular DNA virus that infects leguminous crops and is transmitted by aphids in a circulative, persistent manner. The first identification of PNYDV in peas was in 2009 in Germany; then subsequently detected in Austria, the Netherlands, and Denmark. Infected peas show symptoms of leaf rolling, chlorosis, stunted growth, poorly developed pods; sometimes complete plant death, thus leading to severe yield losses. Currently, the control of virus vectors using pesticides is often expensive and not efficient, especially with the concerns for sustainability and environmental issues. Moreover, some insect vectors developed resistance to pesticides. Therefore, virus-resistant plant varieties are needed for the sustainable production of pea crops. As to date, no commercial PNYDV-resistant pea lines are known, the SPITFIRE project aims to identify genetic resources of peas that may confer resistance or at least tolerance to PNYDV infection. In a collaboration between the German Genebank (IPK), Julius Kuehn Institute, the Austrian Agency for Health and Food Safety, and pea breeders, screening for potential PNYDV resistance pea varieties, landraces, heritage cultivars, and wild Pisum spp. are being performed.

P1.2-034

OAT STERILE DWARF

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Text

Oat sterile dwarf is a disease that can cause sever yield losses in oats. It is most found in the middle parts of Sweden where oat is an important crop. Oat sterile dwarf disease is caused by the Oat sterile dwarf virus (OSDV) and transmitted by a planthopper vector Delphacidea, Javesella pellucida. If disease outbreak tends to increase, then ability to make accurate predictions will be appreciated. This study aimed to develop a method that would - in a relatively simple way - test a large number of individual planthoppers for virus content. This method would be a tool in trying to predict disease outbreak in next year's crop.
OSDV is a Fiji virus, genus Reoviridae, which are double-stranded RNA viruses. It replicates in its vector and in the plant host and is transmitted in a persistent way by planthoppers. The virus stays in the insect when it moults but is not passed on to eggs. The planthopper acquires and transmits the virus by feeding on cereals and grass.
The methods used for virus detection were reverse transcription polymerase chain reaction (RT-PCR) and dot-blot hybridisation. Primers for RT-PCR were developed, the fragments cloned and sequenced. The sequenced fragments were used to synthesise a probe for hybridization.
Virus was detected in extracts of individual planthopper nymphs with RT-PCR and dot-blot hybridization and in oat plants with RT-PCR. Percentage of planthoppers containing virus range from 0-30 percent in the populations studied.

P1.2-035

GRAPEVINE FANLEAF VIRUS RNAS EXHIBIT A UNIQUE URIDYLATION PATTERN

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Text

RNA uridylation, the addition of one to several uridines at the 3' end of RNAs, is a post-transcriptional modification found on both mRNAs or non-coding RNAs in eukaryotes. Recently, uridylation was revealed as an antiviral defense mechanism in *Caenorhabditis elegans* and in human. In this study, we evaluated the diversity of uridylation patterns for representatives of positive-sense single-stranded RNA phytoviruses. Using 3' RACE-seq, a technique combining rapid amplification of cDNAs 3' ends with high-throughput Illumina sequencing, uridylation patterns were analyzed on viral RNAs of 21 phytoviruses from 11 families. Our results uncover important variations in uridylation profiles across plant viral RNAs.

Interestingly, viral RNAs of grapevine fanleaf virus (GFLV, *Nepovirus*, *Secoviridae*) are uridylated to very high levels (>81%) with a mono-uridylation pattern. To evaluate the evolutionary conservation of GFLV 3' terminal features among *Secoviridae*, we investigated RNA uridylation patterns from nine other viruses from this family. Interestingly, only RNAs from GFLV and its closed relative, arabis mosaic virus, had in common this remarkable high percentage of mono-uridylation.
The immediate perspectives of this work are to identify which plant or viral factor catalyzes the mono-uridylation in GFLV RNAs and to determine the importance of GFLV RNA 3' terminal uridylation for viral infection using uridylated or non-uridylated GFLV viral infectious transcripts.

P1.2-036

DEVELOPMENT OF A QUANTITATIVE PEA NECROTIC YELLOW DWARF VIRUS (PNYDV) SCREENING SYSTEM FOR THE SELECTION OF RESISTANT PEA (PISUM SATIVUM L.) ACCESSIONS

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Text
Pea (*Pisum sativum* L.) is a widely grown grain legume in temperate regions and contributes largely to protein rich food and feed and biological nitrogen fixation in the crop rotation. However, many biotic stresses, such as fungal and viral pathogens and insect pests are crucial constraints of successful pea production. Pea necrotic yellow dwarf virus (PNYDV), an obligate aphid transmitted nanovirus, emerged in Central Europe only recently during the last 10-15 years. In contrast to other viral diseases of pea, PNYDV leads to substantial yield reduction or even complete loss in highly epidemic years. Control of this virus is challenging particularly in organic agriculture, where insecticidal treatment against the aphid vector is very limited or not allowed. The selection and breeding of resistant pea varieties is therefore the most promising approach. We have established a screening system for the selection of resistant lines by employing a newly developed qPCR assay for the differential assessment of the virus load between pea accessions upon inoculation with aphids carrying PNYDV. This quantitative assessment will allow the identification of breeding lines able to limit or suppress the virus multiplication. Breeding lines will be selected based on qPCR assay and validated in the field. This novel screening approach can be translated to other obligate aphid transmitted virus in different crops and become an important selection tool for breeding and genomic analysis.

P1.2-037

GRAPEVINE FANLEAF VIRUS AVIRULENCE FACTOR 2AHP (HOMING PROTEIN) INTERACTS WITH SEVERAL PROTEINS OF NICOTIANA OCCIDENTALIS INVOLVED IN PLANT IMMUNITY

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Text

Plants rely on a variety of mechanisms to counteract infections. R factors encoded by dominant resistance R genes generally correspond to nucleotide binding-leucine rich repeat (NLR) proteins and recognize pathogen-derived avirulence (Avr) factors. Interaction between Avr and R factors activates a hypersensitive response (HR) characterized by a programmed death that more or less efficiently restricts the pathogen at its entry point.
Grapevine fanleaf virus (GFLV), of the genus Nepovirus in the family Secoviridae, is the main agent causing grapevine fanleaf disease which greatly impacts the harvest in vineyards, worldwide. When inoculated to *Nicotiana occidentalis*, GFLV strain F13 induces HR whereas strain GHu multiplies without inducing HR. The use of chimeric clones allowed to identify the 2A homing protein ($2A^{HP}$) as the Avr factor and to delineate the 50 C?terminal aminoacids as the viral determinant of the HR.
To identify proteins encoded by *N. occidentalis* and involved in HR we overexpressed EGFP tagged $2A^{HP}$ proteins i.e. $2A_{F13}$ and $2A_{GHu}$ originating from F13 and GHu strains or the recombinant protein $2A_{F209G}$ in which the 50 last residues are from the GHu strain. We hypothesized that cellular proteins important for the Avr recognition and HR should preferentially interact with protein $2A_{F13}$. Candidate proteins purified by co?immunoprecipitation were identified using LC?MSMS. The most relevant candidates and the ongoing validation work will be presented and discussed.

P1.2-039

CHANGES TO THE HOST TRANSCRIPTOME TRIGGERED BY BEGOMOVIRAL DNA-B: A CASE STUDY USING SRI LANKAN CASSAVA MOSAIC VIRUS.

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Text

Sri Lankan cassava mosaic virus (SLCMV), a whitefly-transmitted bipartite begomovirus infecting cassava (Manihot esculenta) in south Asian countries can exist as both monopartite and bipartite, with differing pathologies in the experimental host Nicotiana benthamiana, when the viral DNA is inoculated using agrobacterium. This presents a unique opportunity to study the transcriptomic changes due to DNA-B. Plants inoculated with SLCMV DNA-A only and DNA-A + DNA-B, when monitored at three time points showed a faster onset of symptoms and higher symptom severity when both the DNAs were used, compared to only DNA-A. The accumulation of the two DNAs in newly emerged leaves varied widely over time, the DNA-A showing a more rapid accumulation compared to DNA-B in plants inoculated with DNA-A + DNA-B. The differentially expressed genes due to DNA-B only were computed following a comparison between the two groups of plants at a stage when both groups showed a similar accumulation of DNA-A. The results suggested that the presence of DNA-B under the given experimental setup triggered expression changes in a limited but defined set of genes. The transcriptomic changes were confirmed by quantitative PCR studies. The study reveals new facets about the pathological roles of the two DNA components of bipartite begomoviruses.

P1.2-040

CANNABIS VIROME RECONSTRUCTION AND ANTIVIRAL RNAI CHARACTERIZATION BY SMALL RNA SEQUENCING

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Text

Hemp (*Cannabis* spp.) is a rich source of natural compounds and fiber for medicinal and industrial use. While fungal and bacterial pathogens of hemp are quite well characterized, virus infections have been reported and seen as an emerging threat for hemp cultivation only in recent years. In this work we used Illumina small RNA sequencing for virome reconstruction and characterization of the antiviral defense based on RNA interference (RNAi) in industrial hemp plants (monoecious) and dioecious plants cultivated for production of CBD/CBG cannabinoids. By de novo and reference-based assembly of small RNA reads we identified and reconstructed previously-reported viruses such as Cannabis cryptic virus (family *Partitiviridae*), Cannabis sativa mitovirus 1 (*Mitoviridae*) and Grapevine line pattern virus (*Bromoviridae*) as well as a putative new species of *Partitiviridae*. Members of both *Partitiviridae* and *Bromoviridae* families were targeted by antiviral RNAi generating predominantly 21 and 22 nt small interfering RNAs from both strands of the entire virus genome. In contrast, mitovirus-derived small RNAs belonged to a wider size range, with 16 and 21 nt size classes being the most abundant, and resembled *Cannabis* mitochondrion genome-derived small RNAs. Association of disease symptoms (if any) with identified viruses and/or relative abundance of viral sRNAs will be presented. To our knowledge, this is the first characterization of antiviral RNAi in hemp plants.

P1.2-041

OXIDATIVE STRESS AND ACTIVATED METHYL CYCLE-RELATED RESPONSES IN POTY-POTEXVIRUS SYNERGISM IN NICOTIANA BENTHAMIANA

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Text

Poty- and potexviruses are positive-sense RNA viruses that cause mixed infections leading to significant yield losses in important crop plants. We have studied molecular mechanisms underlying coinfection between potato virus A (PVA), a potyvirus, and potato virus X (PVX), a potexvirus, in Nicotiana benthamiana. Glutathione is a scavenger of radical oxygen species involved in relieving oxidative stress associated with virus infections. Glutathione biosynthesis pathway is tightly connected to the activated methionine cycle (AMC). PVA helper component proteinase (HCPro) interferes with the key enzymes of AMC, S-adenosyl-

L-methionine synthetase (SAMS) and S-adenosyl-L-homocysteine hydrolase (SAHH). A strong downregulation in the amount of the reduced form of glutathione (GSH) is observed upon HCPro expression in PVX infected cells. In our recent study, we analyzed the metabolites related to AMC and glutathione synthesis pathway in PVA-PVX infection. The analysis revealed that both S-adenosyl methionine / S-adenosyl-homocysteine (SAM/SAH) ratio and GSH/GSSG ratio were significantly reduced in PVA-PVX coinfected plants during systemic infection. Decreased SAM/SAH ratio compromises transmethylation reactions, which has many negative effects on host cell function and defense against viruses. Since AMC and glutathione pathways have an essential role in viral coinfection, further research in this area may help finding solutions to diseases caused by mixed infections.

P1.2-042

ALLELE MINING FOR EIF4G-MEDIATED RESISTANCE IN 3K RICE GENOMES, DETECTION OF SIGNALS FOR POSITIVE SELECTION, AND DEVELOPMENT OF PACE MARKERS FOR IDENTIFIED EIF4G ALLELE TYPES.

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Text

Genetic databases of agronomically important crops are rich resources of natural alleles for valuable traits such as stress resistance. Lee et al. (2010) conducted an association mapping analysis for RTSV resistance which suggested that resistance in rice cultivar Utri merah was controlled by a single recessive gene located at a 200-kb region of chromosome 7. Within this region is a gene coding for translation initiation factor 4 gamma (EIF4G), a protein involved in virus multiplication. Rice accessions with potential RTSV-resistance were identified among the 3000 rice genomes based on non-synonymous SNPs within a 30-nucleotide region spanning EIF4G. Examination of the 3,000 rice genotypes have shown that 10% carry natural EIF4G alleles. Phenotype analysis for RTSV resistance of rice accessions with natural EIF4G alleles have shown association of RTSV resistance with the presence of non-synonymous SNPs within the 30-nt region of EIF4G. Allele-types were designated as ARC, AS, BON, HAT, KG, KK, KM, KM-UR, NH, TKM, and UR. About 90% of the rice genotypes with homozygous EIF4G alleles were RTSV-resistant and those that are heterozygous have shown a segregating phenotype. Analysis of distribution of EIF4G allele types in terms of subpopulation and geographical location have shown over-representation of certain allele types to certain rice subpopulation and continental regions. Lastly, we used the identified allele-types to design PACE markers for high throughput SNP genotyping.

P1.2-043

THE CHARACTERIZATION OF THE HELPER COMPONENT PROTEINASE (HC-PRO) OF THREE TULIP-INFECTING POTYVIRUSES

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Text

Tulips are among of the most economically important ornamental plants. Due to their vegetative propagation and long juvenile phase, tulips are prone to viral infections. The most widespread viral disease among tulips is the color-breaking syndrome, which is primarily caused by three potyviruses: tulip-breaking virus (TBV), Rembrandt tulip-breaking virus (ReTBV), and lily mottle virus (LMoV). Since limited information is available on the HC-Pro protein of these viruses, we successfully isolated, cloned, and determined their nucleotide sequence. All three HC-Pro sequences are deposited in NCBI GenBank, ReTBV HC-Pro being the first available nucleotide sequence. In this study, phylogenetic analysis and amino acid sequence comparison of the three HC-Pros were carried out. Furthermore, the silencing suppressor activity was compared using Agrobacterium-mediated transient expression of TBV, ReTBV, and LMoV HC-Pro in Nicotiana benthamiana plants. According to the GFP visualization and real-time PCR quantification, TBV HC-Pro had strong, and ReTBV HC-Pro showed moderate silencing suppressor activity. Interestingly, LMoV HC-Pro failed to have suppressor activity in the N. benthamiana plants. Moreover, the in situ localization of the HC-Pro of these tulip-infecting viruses was also evaluated using GFP fusion proteins.

P1.2-044

GENOMIC CHARACTERIZATION OF PAPAYA RINGSPOT VIRUS (PRSV) ON CARICA PAPAYA AND ITS MANAGEMENT THROUGH APHID VECTORS IN PUNJAB PAKISTAN

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Text

Papaya (Carica papaya L.) belongs to the family Caricaceae widely known for its economic importance throughout tropical and subtropical areas. Papaya productivity is affected by different factors mainly viruses causing huge economic losses. Papaya ringspot virus (PRSV) is major devastating disease right now. In Pakistan, PRSV has not been well defined for its genomic reference. Therefore, this study aims to quantify the PRSV disease impact in Punjab, Pakistan based on molecular characterization of virus and its management through controlling vector. The samples were collected from the fifteen locations of five districts in Punjab, Pakistan. The highest disease severity 85% was found in district Bahawalnagar whereas the lowest 60% was found in Gujrat. Primers amplified the CP gene region. The generated

sequence was 10,088 bp. BLAST analysis showed 100% similarity to PRSV. Phylogenetic analysis was performed with already available PRSV accessions and found similarity with indian and american strain. This study was also aimed to investigate the efficacy of four insecticides against aphid infestation in orchards. Results showed that Acetamiprid was found highly effective in controlling aphid population. The results of this study indicated that insecticide can be an effective method of controlling aphid populations in orchards.

P1.2-045

PEANUT STUNT VIRUS MOVEMENT PROTEIN HAS A SUBSTANTIAL CONTRIBUTION TO HOST RANGE AND SYMPTOM DETERMINATION

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Text

Cucumber mosaic virus (CMV) is one of the most devastating plant viruses and has an extremely
wide, variable host range. Despite the similar taxonomy and genome structure with CMV, peanut stunt virus (PSV) has a much limited host range, which consists mainly legumes. Since no data is available on the background of the limited host range of PSV, we analyzed it on a common host (Nicotiana benthamiana) and on a selective host (Capsicum annum cv. Bródy). All of the five proteins of cucumoviruses have a role in pathological characteristics, the least information is available on the movement protein (MP). As a result of inoculation with RNA3 reassortant and recombinant viruses, MP was determined responsible for the differences in infections. Since the main function of the MP is to promote cell-to-cell movement of the virus, and plasmodesmata (PD) localization is essential to cell-to-cell movement, intracellular localization and colocalization with PD were compared between CMV MP-eGFP and PSV MP-eGFP. In the case of CMV MP-Egfp a clear colocalization with PD was detected, while the presence of PSV MP-eGFP was divided between the PD and plasma membrane (PM). After plasmolysis of infiltrated cells, CMV MP-eGFP was still colocalized to PD but PSV MP-eGFP was weakly associated with PD. In the present study, we demonstrated that differences in PD localization of CMV and PSV could have consequences on the symptom phenotype (N. benthamiana) and on the host range determination (C. annuum).

P1.2-046

THE EFFECT OF TOMATO SPOTTED WILT VIRUS NSS PROTEIN SELF-INTERACTION ON SILENCING SUPPRESSION AND AVIRULENCE IN PEPPER PLANTS

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Text

Tomato spotted wilt virus (TSWV) is an important plant pathogen causing heavy economic losses worldwide. The NSs protein encoded by the S RNA of the TSWV genome functions as a viral suppressor of RNA silencing (RSS) and also as avirulence determinant in pepper plants. We confirmed in vivo self-interaction of NSs proteins by bimolecular-fluorescence-complementation (BiFC) and yeast-two-hybrid (Y2H) assays. To determine if self-interaction is essential for these functions, three NSs mutants were constructed (NSs/R337A, NSs/H340A, NSs/E344A) in a highly charged alpha-helix structure of the NSs protein that plays a putative role in protein-protein interaction. BiFC, hypersensitive reaction (HR) induction, and RSS assay were carried out with agroinfiltration on Nicotiana benthamiana, and both TSWV-resistant and -susceptible pepper cultivars. BiFC assay demonstrated that the point mutations of the NSs resulted in the loss of self-interaction, thus the alpha-helix structure of the NSs protein plays an important role in oligomer formation. The HR was detected in all the TSWV-resistant pepper cultivars, including the mutant NSs variants. Although the RSS activity was not ceased completely, it was significantly reduced compared to the wild-type NSs. Our results indicated that the monomeric form of NSs is able to induce HR reaction, but RSS is more effective if self-interaction is present.

P1.2-047

GENETIC DIVERSITY ANALYSIS OF BADNAVIRUSES INFECTING BANANA IN BURKINA FASO

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Text

Badnaviruses are serious plant pararetroviruses affecting banana and causes serious economic losses to banana production worldwide. This study aims to examine the variability of BSV and SCBV nature infecting banana in Burkina Faso. Polymerase Chain Reaction (PCR) used the Badna FP/RP specific primers for the RT/RNase H regions present in badnaviruses. The PCR yielded about 579 bp amplicons from banana infected by BSV and SCBV. The 38 BSV isolates recorded low nucleotide identity ranging from 58.9% - 98.1%. Based on percentage nucleotide sequence identity and phylogenetic analyse, BSV isolates were categorized into four groups: 1, 2, 3 and 4. Group 4 shared 76.9% - 100% identity with BSOL species. However, Groups 1 and 3 recorded a low identity ranging, from 76.8% - 79.2%, 68.8% - 79.7% with BSCV, and 72.8% - 79.0% between Group 2 and BSOLV. Groups 1, 2 and 3 were assigned to a potentially new BSV species. The two SCBV isolates recorded a low nucleotide identity of 68% among themselves indicating high diversity. In addition, SCBV_Cd and SCBV_CE showed high nucleotide identity 91.3% and 58.7% with SCBV_C and SCBV, when they were compared to all published SCBV genotypes. In addition, phylogenetic analysis revealed the segregation of SCBV isolates into two

genotypes, SCBV_Cd in C and SCBV_CE segregated in a new genotype namely Z. Recombination analyses showed weak signatures of recombination among some of the BSV and SCBV sequences. Keywords: Banana streak virus, Sugarcan baciliform virus, RT/RNase H, Polymerase chain reaction, diversity

Population genomics of plant pathogens

C2.3-1

A THOUSAND-GENOME PANEL RETRACES THE GLOBAL SPREAD AND ADAPTATION OF A MAJOR FUNGAL CROP PATHOGEN

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Text
Human activity impacts the evolutionary trajectories of many species worldwide. Global trade of agricultural goods contributes to the dispersal of pathogens reshaping their genetic makeup and providing opportunities for virulence gains. Understanding how pathogens surmount control strategies and cope with new climates is crucial to predicting the future impact of crop pathogens. In this project, we address this by assembling a global thousand-genome panel of Zymoseptoria tritici, a major fungal pathogen of wheat reported in all production areas worldwide. We identify the global invasion routes and ongoing genetic exchange of the pathogen among wheat-growing regions. We find that the global expansion was accompanied by increased activity of transposable elements and weakened genomic defenses. Finally, we find significant standing variation for adaptation to new climates encountered during the global spread. Our work shows how large population genomic panels enable deep insights into the evolutionary trajectory of a major crop pathogen.

C2.3-2

THE COEVOLUTIONARY RACE BETWEEN HYALOPERONOSPORA ARABIDOPSIDIS AND ARABIDOPSIS THALIANA AT A TRANSCONTINENTAL SCALE

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Text
Plants and their pathogens are locked in a perpetual coevolutionary battle for survival. We present a transcontinental investigation of coevolution in the *Hyaloperonospora arabidopsidis* – *Arabidopsis thaliana* pathosystem. We generate whole genome sequences of over 400 host-pathogen pairs from natural infections collected throughout both the native eurasian range and the human-commensal colonisation of North America, as well as new near-complete long-read genome assemblies with evidence-based annotation. We investigate the demographic history of both host and pathogen, examine coevolution both generally and of individual gene pairs, and describe variation in the genetic networks of interacting host and pathogen genes. Our results show that the negative-frequency dependent selection on both the pathogen and host genomes leads to the presence of balanced polymorphisms in the wild pathosystem, in contrast to the directional selection generally experienced by pathogens of crop pathosystems.

C2.3-3

ORIGIN AND GENOME DYNAMICS OF FUSARIUM LINEAGES CAUSING GLOBAL EPIDEMICS OF FUSARIUM WILT OF BANANA

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Text
The spatiotemporal origins of plant diseases and the molecular underpinnings of their dissemination remain elusive for many epidemics. Here we discuss our ongoing efforts to study the diversity and spread of the causal agents of Fusarium wilt of banana (FWB) that affect bananas worldwide. We genotyped a global collection of 650 *Fusarium* isolates and traced the origins of FWB to Southeast Asia, the center of origin of banana and a biodiversity hotspot for banana pathogens such as *Fusarium*. While the first FWB epidemic in Gros Michel bananas was caused by a suite of genetically diverse *Fusarium* lineages, we show that the ongoing epidemic that devastates Cavendish banana production is caused by a single clone known as *Fusarium odoratissimum* (TR4). We studied the pangenome of ~70 banana-infecting *Fusarium* isolates and discovered variable genomic regions that range from sub-telomeres to multiple complete chromosomes. These regions are enriched for in planta-expressed effector genes, giving rise to diverse, lineage-specific effector repertoires. Interestingly, ~70% of the genes located in variable regions evolved via gene duplications, and genes associated with segmental duplications occur almost exclusively in these regions, suggesting that these processes drive *Fusarium* evolution. Our study provides novel insights into the origin and genome evolution of banana-infecting *Fusarium* lineages, which provides an evolutionary framework for the molecular underpinnings of pathogenicity.

C2.3-4

COMPARATIVE GENOMICS REVEALS KEY GENETIC POLYMORPHISMS ASSOCIATED WITH INCREASED AGGRESSIVENESS IN AUSTRALIAN ASCOCHYTA RABIEI

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Text

Ascochyta blight of chickpea is a foliar disease that is caused by the necrotrophic ascomycete fungus *Ascochyta rabiei*, causing lesions on leaves, stem and pods and subsequently leading to significant yield and profit losses. In recent years, genomic resources were developed for *A. rabiei*, including the assembly of a near chromosome-level reference genome and transcriptome assemblies. Recent studies by our group revealed that despite a limited genetic diversity of the clonally propagated Australian population (in the absence of a second mating type), *A. rabiei* isolates rapidly adapt and overcome the resistant chickpea cultivars developed and used by the industry. To better understand what features in the *A. rabiei* genome drive this adaptation and pathogenicity potential, we performed an in-depth comparative genomics investigation using whole-genome resequencing data of 230 Australian *A. rabiei* isolates, which were phenotyped and assessed for their aggressiveness against a differential chickpea host set. We identified single nucleotide polymorphism (SNP) variants and loci with variable copy-number (CNV) that are associated with highly-aggressive isolates and annotated them and the genes they impact. These results shed new light on the molecular interactions between *A. rabiei* and chickpea and the underlying genes that govern pathogenicity and will allow us to develop targeted genomic and molecular assays to rapidly identify high-risk isolates.

C2.3-5

GENOME MINING OF PSEUDOMONAS POPULATIONS REVEALS A MECHANISM FOR STRAIN-SPECIFIC KILLING OF PSEUDOMONAS

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Text

The spread of a bacterial pathogen in a host population depends on its ability to overcome host immunity and to defend against surrounding microbes and bacteriophages. Owing to their differential killing activity and capacity to integrate into their host genomes, phages are predicted to influence disease dynamics in plants. To test the influence of phages on a plant bacterial pathogen, we identified phages circulating in a metapopulation of diverse wild Pseudomonas plant pathogens abundant across Europe. We identified viral sequences in over 1500 Pseudomonas genomes and identified a viral sequence conserved across pathogenic isolates of Pseudomonas. Interestingly, through comparative genomics, proteomics and electron microscopy we discovered that this viral sequence was not a phage but was instead a phage-derived element termed a tailocin, a repurposed phage used by the Pseudomonas strains for interbacterial warfare. Different co-occurring Pseudomonas strains

encode different tailocin variants and these variants kill different suites of co-occurring pathogenic Pseudomonas. These results suggest that tailocin variants suppress the dominance of pathogenic lineages. In total, the genome mining of Pseudomonas phage diversity that we perform in this study revealed a different tailocin variants capable of suppressing specific Pseudomonas lineages. Tailocins present a novel and tunable mechanism for suppressing specific Pseudomonas lineages.

C2.3-6

UNDERSTANDING THE DRIVERS OF THE PATHOGEN POPULATION DYNAMICS USING STRAIN-RESOLVED METAGENOMICS

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Text

The agro-ecosystems continue to be threatened by a number of endemic plant diseases. Integrated solutions are suggested to the growers to mitigate the impacts of these diseases, although sporadic severe outbreaks are frequently yield-limiting. Genome sequencing technologies have enabled real-time monitoring of the pathogen population dynamics. However, the real challenge has been to understand how new pathogen variants continue to emerge. Using endemic pathogen, *Xanthomonas*, infecting tomato and pepper as a model pathosystem, we have investigated how intraspecific diversity is structured by environmental parameters, host genotypes and growth dynamics of different pathogen genotypes by conducting metagenome survey and parallel greenhouse experiments. Our strain-resolved metagenomics approach has allowed us to obtain fine-scale resolution into pathogen variation within a single growing season. We documented presence of multiple, often times, more than two pathogen genotypes in a single field, although patterns of seasonal succession vary across different states of the southeastern U.S. Our findings show that strain-level diversity can turnover in response to environmental changes modulating functional traits of communities. Together, increased ecological and evolutionary plasticity can be observed in the endemic pathogen population in the southeastern US in tomato fields despite the absence of obvious host selection pressure in the cultivated tomato varieties.

F2.3-1

MINING HISTORIC HERBARIA TO TRACK PHYTOPHTHORA INFESTANS EFFECTOR AND SOLANUM R GENE DIVERSITY AND EVOLUTION OVER TIME

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Text

Phytophthora infestans caused the Irish Potato Famine of 1845-1852. We performed targeted enrichment sequencing of dried, infected leaf samples from 29 herbarium specimens collected from 1845-1954. Our bait library was designed to enrich both pathogen

effector genes and Solanum host R genes. We leveraged the targeted sequencing approach to generate high coverage of genes responsible for pathogen virulence and host resistance. Many modern well-characterized effector genes have historically been present in *P. infestans*. Both R genes and effectors showed signatures of selection. However, variant calling analysis revealed alternative alleles including avirulent forms compared to the reference genome for many effectors that likely impacted function. The effector *Avr3b* was the only well-described effector not present in Famine era samples but did appear in the mid-1900s. Interestingly, the resistance breaking allele of *Avr1* was present during the famine before the *Solanum R1* gene was deployed by plant breeders. Detailed ploidy analysis of 19 high coverage genomes showed that US-1 lineages appearing in the early 1940s were triploid, in contrast to the FAM-1 lineages from 1845 and thereafter that were diploid. This exploration of historic plant and pathogen genomes will shed light on the past host-pathogen evolutionary relationships of a globally important plant pathogen and could provide insight for future deployment of Solanum R genes.

F2.3-2

HERBARIA IN NATURAL HISTORY COLLECTIONS ILLUMINATE THE EVOLUTIONARY HISTORY AND EMERGENCE OF CITRUS BACTERIAL CANKER

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Text

The field of ancient genomics has triggered considerable progress in the study of pathogens, including those affecting crops. Herbarium collections have been an important source of dated, identified and preserved DNA, whose use in comparative genomics and phylogeography may shed light into the emergence and evolutionary history of plant pathogens. I will present the reconstruction of 13 historical genomes of the bacterial crop pathogen *Xanthomonas citri* pv. *citri* (Xci) from infected citrus herbarium specimens using a shotgun-based deep sequencing strategy. After authentication of the historical genomes based on DNA damage patterns, we compared them to modern genomes to reconstruct their phylogenetic relationships, pathogeny-associated genes content and estimate several evolutionary parameters, using Bayesian tip-dating calibration and phylogeography inferences. Despite a challenging analysis of data, requiring adapted treatment before being compared to modern samples, our results reveal that Xci originated in Southern Asia ~11,500 years ago and diversified during the beginning of the 13th century, after Citrus diversification and before spreading to the rest of the world. This updated scenario links Xci specialization to Neolithic climatic change and the development of agriculture, and its diversification to the human-driven expansion of citriculture through the early East-West trade and later colonization.

P2.3-001

ADVANCES IN GENOMICS OF TILLETIA INDICA CAUSING KARNAL BUNT OF WHEAT

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Text
Karnal bunt of wheat is an internationally quarantined disease affecting trade, quality, and quantity of wheat. The T. indica genome was sequenced using Illumina and Pac Bio platforms with 33.7 Mb and a GC content of 55.0%. The whole genome sequence has been deposited at DDBJ/ENA/GenBank under the accession numbers MBSW00000000. A total of 1737 scaffolds were obtained with an N50 of 58,667 bp. The comparative genome analysis suggested 3751 proteins of T. indica had orthologs in five fungi, whereas 126 proteins were unique to T. indica. Putative pathogenicity-related genes were identified in the genome. Fourteen homologous sequences of putative pathogenicity-related genes were identified in T. indica by in silico analysis. Apart from that, quick diagnostic assays were developed. Genome-wide association mapping was performed using 41,473 SNPs, infection phenotyping data, and population structure, to find single nucleotide polymorphisms (SNPs) linked to virulence genes. Population structure analysis divided the T. indica population in India into three subpopulations with genetic mixing in each subpopulation. The association mapping revealed the presence of 13 SNPs associated with virulence. Using sequence analysis tools, one gene (g4132) near a significant SNP was predicted. Multilocus sequence typing (MLST) revealed that the population of T. indica was highly diverse.

P2.3-002

EXAMINATION OF LARGE CHROMOSOMAL INVERSIONS IN THE GENOME OF ERWINIA AMYLOVORA STRAINS REVEALS WORLDWIDE DISTRIBUTION AND NORTH AMERICA-SPECIFIC TYPES

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Text
Erwinia amylovora is a relatively homogeneous species with low genetic diversity at the nucleotide level. However, phenotypic differences and genomic structural variations among E. amylovora strains have been documented. In this study, we identified 10 large chromosomal inversion (LCI) types in the Spiraeoideae-infecting (SI) E. amylovora strains by combining whole genome sequencing and PCR-based molecular markers. It was found that LCIs were mainly caused by homologous recombination events among seven rRNA operons (rrns) in SI E. amylovora strains and tend to occur between rrns transcribed in the opposite directions and with the same tRNA content (tRNA-Glu or tRNA-Ile/Ala). Based on the LCI types, physical/estimated replichore imbalance (PRI/ERI) was examined and calculated. Among the 117 SI strains evaluated, the LCI types of Ea1189, CFBP1430, and Ea273 were the most common with ERI values at 1.31°, 7.87°, and 4.47°, respectively. These three LCI types had world-wide distribution, whereas the remaining seven LCI types were restricted to North America. Our results indicated on-going chromosomal recombination events in the SI E. amylovora population and showed that LCI events are mostly symmetrical, keeping the

ERI less than 15°. These findings provide first evidence about the prevalence of certain LCI types in E. amylovora strains, how LCI occurs, and its potential evolutionary advantage and history, which might help track the movement of the pathogen.

P2.3-003

POPULATION GENETIC DIVERSITY AND MIGRATION PATTERNS OF EXSEROHILUM TURCICUM FROM MAIZE-BASED CROPPING SYSTEMS IN SOUTH AFRICA

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Text

Exserohilum turcicum causes northern leaf blight of maize and is a major threat to South Africa's maize production. Previous population genetics studies in South Africa revealed a high genetic diversity of the pathogen, being driven in part by cryptic sexual recombination. The population genetic structure of *E. turcicum* in smallholder and emerging commercial farms from the Eastern Cape is, however, unknown, and we address that question in this study. We hypothesized that *E. turcicum* would be highly diverse and with no population differentiation across the province. To test this hypothesis, 203 isolates from three districts, namely Alfred Nzo, Chris Hani and OR Tambo, were genotyped using 12 microsatellite markers and two mating type markers. The results show that *E. turcicum* was highly diverse (Nei's gene diversity = 0.58; Shannon index = 1.29), but that no population differentiation was detected. Both mating types were recovered from all populations, with strains in OR Tambo segregating in accordance with the null hypothesis of random mating, while Alfred Nzo and Chris Hani populations were significantly skewed from the ratio of 1:1 in favor of an abundance of MAT1-2 alleles. Our study confirms the high levels of genetic diversity at local and regional scales in in South Africa, underpinned by high gene flow and sexual recombination. These results are important to consider in future studies on resistance breeding in maize for northern leaf blight.

P2.3-004

GENETIC DIVERSITY AND POPULATION STRUCTURE OF WHEAT STRIPE RUST PATHOGEN (PUCCINIA STRIIFORMIS F. SP. TRITICI) IN TÜRKIYE BASED ON GENOME-WIDE SINGLE NUCLEOTIDE POLYMORPHISMS

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Text

Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is the most devastating disease of wheat both in Türkiye and worldwide. The stripe rust pathogen is highly variable due to its high reproductivity and capability of long-distance dissemination. In this study, we aimed to determine the genetic diversity and population genomic structure of a *Pst* collection collected from six different regions of Türkiye using genome-wide single nucleotide polymorphisms (SNPs) developed by double digest RAD-Seq (ddRADSeq). The gene diversities were higher in isolates from Mediterranean (ME) region and Southeastern Anatolia (SA) region than from other regions. Analysis of molecular variance (AMOVA) revealed that a high gene flow was detected among regions because 62% and 39% of explained variance were from within isolates and among isolates within regions, respectively. A bayesian model-based hierarchical clustering (STRUCTURE) showed the presence of three subpopulations among the *Pst* isolates. The result of principal coordinate analysis (PCoA) also supported these results. The large number of SNPs clearly indicated that a small set of *Pst* is highly diverse. All of these findings may provide a critical information to develop management strategies to wheat stripe rust. The high-quality SNP data generated here can also be used in the development SNP markers for studying the pathogen biology, genetics, and evolution.

P2.3-005

FUSARIUM AND THE EMERGENCE OF BAKANAE DISEASE IN BANGLADESH

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Text

Bakanae is widely distributed in all rice-growing areas in the world, especially in Asian countries. A series of samplings were conducted in 15 districts of Bangladesh for 4 consecutive years from 2019 to 2022. Diseased rice plants from different rice cultivars with typical bakanae symptoms such as root rot, abnormal elongation of stems, wilting, stunting and adventitious roots were collected. Disease Incidence and disease severity were recorded high in the northeast part of Bangladesh. A total of 121 isolates were determined using morphological characteristics, DNA sequences, and phylogenetic analyses of two genes, namely, TEF1-α, and RPB2. The phylogenetic analysis of TEF1-α and RPB2 gene sequences coupled with morphological characterization revealed that the collected isolates belonged to six Fusarium species, viz., Fusarium fujikuroi, F. incarnatum, F. commune, F. verticillioides, F. equiseti and F. proliferatum. Among the isolates, all were found to be pathogenic except F. equiseti under virulence assays. The knowledge on the occurrence of bakanae disease, the association of Fusarium species with bakanae, and their pathogenic nature is still lacking in Bangladesh. Therefore, this study aimed to identify, and characterize the Fusarium species association with bakanae disease, to assess the emergence of the disease and the virulence of the pathogens which will help in formulating effective strategies and policies for better control of bakanae disease in Bangladesh.

P2.3-006

EVOLUTIONARY IMPACT OF CHICKPEA (CICER ARIETNUM) HOST RESISTANCE ON A CLONAL ASCOCHYTA RABIEI POPULATION IN AUSTRALIA

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Text

Ascochyta rabiei is a necrotrophic fungus of chickpea (*Cicer arietnum*), which can lead to complete yield loss under favourable conditions. The Australian population is clonally propagating with low genetic diversity, however, it is still capable of evolving and adapting to overcome host resistance. Evolutionary drivers of the *A. rabiei* population and its adaptation to integrated disease management practices are largely unstudied. This study aims to trace the selection and adaption potential of aggressive *A. rabiei* isolates on varying host genotypes and resistance levels. Six *A. rabiei* genotypes from the Northern NSW chickpea growing region were selected based on their unique allele combination (haplotype) at fifteen SNP loci as determined from whole genome sequencing. These isolates were used to evenly inoculate six chickpea hosts with ranging *A. rabiei* susceptibility under field conditions. *Ascochyta rabiei* was re-isolated from lesions sourced from each chickpea host and genotyped using genetic fingerprinting of the 15 unique SNP loci. This was used to trace changes in isolate distribution between each chickpea genotype indicating host-dependant selection that favours specific isolates. This demonstrates intricate host-isolate interactions and adaptation which will form the basis of a decision-making tool for growers on varietal choice based on the presence of specific *A. rabiei* isolates and provide useful information for informed disease management strategies.

P2.3-007

GENOMIC ANALYSIS OF A NEW VIRUS INVOLVED IN PAPAYA 'STICKY' DISEASE UNVEILS A LINEAGE OF PLANT-INFECTING VIRUSES RELATED BUT DISTINCT FROM TOTIVIRUSES

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Text

Papaya sticky disease (PSD), characterized by the oxidation of spontaneously oozed latex, is a major virus disorder of papaya (Carica papaya) in Brazil. The disease is caused by the co-infection of two viruses, papaya meleira virus (PMeV, a dsRNA toti-like virus) and papaya meleira virus-2 (PMeV-2, an umbra-like virus). Recently, a virus related to but distinct from PMeV was identified in a two-year-old papaya orchard (cv. Passion Red) exhibiting PSD-like

symptoms in Santa Elena province, Ecuador. The virus, referred to as papaya sticky fruit-associated virus (PSFaV), has an 8.8 kbp-long genome sharing 56% nucleotide identity with its counterpart from Brazil. Phylogenetic analyses showed that PMeV, PSFaV, and babaco meleira-like virus (BabMelV) –another toti-like virus identified in Vasconcellea x heilbornii in Ecuador– sharing a most recent ancestor, are the only plant-infecting viruses within the newly proposed 'Fusagraviridae' clade. However, the conserved 'Phytoreo S7' domain is not present in either PMeV, PSFaV or BabMelV. We discuss the genomic and biological features of newly discovered toti-like viruses which delineate distinct evolutionary pathways for those infecting fungi and plants, respectively.

P2.3-008

GENETIC DIVERSITY AND POPULATION STRUCTURE OF 18 TUNISIANS OROBANCHE FOETIDA POPULATIONS USING RADSEQ

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Text

Orobanche foetida Poiret is a holoparasitic plant that lacks chlorophyll and totally depends on its host for its growth. It parasitizes host plant roots and extracts nutrients and water via a haustorium. The fetid broomrape is distributed in the Mediterranean region as a wild plant parasite. However, since 1992, it has become a real threat to faba bean in Tunisia causing serious damages which may reach 90% yield losses. Analysis of the genetic diversity of this parasite is important to better understand its evolution and spread, remaining largely unknown. In this work, we present the first study on genetic diversity and population structure using the robust technique Restriction-site-Associated DNA sequencing (RADseq) for *Orobanche* spp. We collected 244 samples of *O. foetida* from 18 faba bean fields in Tunisia. To overcome the difficulty of SNP discovery in the *O. foetida* genome as a non-model and tetraploid plant, we used three different informatics pipelines, namely UNEAK, pyRAD, and Stacks. This study showed that genetic differentiation occurred in the Tunisian *O. foetida* emphasizing the isolation by distance effect. However, no strong population clustering was detected in this work based on the three data sets and clustering methods used. The present study traces the current real situation of the distribution of *O. foetida* populations in Tunisia and could be a valuable reference for the upcoming research projects focusing on this parasitic plant.

P2.3-009

DIVERSITY AND AGGRESSIVENESS OF FUSARIUM SPP. ASSOCIATED WITH CHICKPEA IN MONTANA

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Text

Root rot caused by Fusarium spp. is a significant problem in chickpea growing regions of Montana. However, it is unclear which species of Fusarium cause the disease and which species are more prevalent. An extensive survey was conducted to identify the Fusarium species associated with chickpea in Montana. Three hundred fifty-seven Fusarium isolates were recovered from symptomatic roots of chickpea plants from 10 counties in 2020 and 2021. Isolates were identified by comparing the sequences of the translation elongation factor 1-α in the Fusarium-ID database. Alignments of the obtained sequences indicated that Fusarium oxysporum was the most abundant species (33%), followed by F. acuminatum (21%), F. avenaceum (15%), F. pseudograminearum (8%) F. culmorum (6%), F. equiseti (2%), F. redolens (14%), F. sporotrichioides (6%), F. solani (6%), F. proliferatum (0.2%), F. torulosum (0.9%), F. tricinctum (0.8%) and F. brachygibbosum (0.1%). The aggressiveness of a subset of 51 isolates representing various Fusarium spp. were tested on the chickpea cv. ‘CDC Frontier’. Non-parametric analysis of variance conducted on ranks of disease severity indicated that F. avenaceum had the most aggressive isolates compared to the other species. This knowledge is helpful for making crop rotation decisions, disease management options, and breeding resistant chickpea varieties against economically important pathogens.

P2.3-010

MATING-TYPE LOCUS ANALYSIS IN GANODERMA BONINENSE, THE BASAL STEM ROT CAUSING PATHOGEN OF OIL PALM.

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Text

Monokaryon genome of G. boninense with haploid genome size estimated at 53.6Mbp (unpublished) were annotated and candidate genes for homeodomain-related genes (HD) and pheromone receptor (PR) were subsequently identified. The in-silico gene-mining results revealed a total of 16 candidate HD genes and 18 candidate PR genes. Validations of in-silico results by amplification of targeted genes were performed on banked G. boninense cultures. Concurrently, a population of G. boninense (basidiocarps and corresponding spores) was sampled from a single oil palm plantation for whole genome resequencing. Alignment of candidate genes, both individually and concatenated sequences, allowed the selection of genes that were capable of distinguishing isolates based on mating type. The cluster consists of four PR genes accurately segregated isolates based on mating type (arbitrarily assigned by mon-mon crossing). HD2 gene could not segregate isolates as intended; hence efforts to investigate the mip gene and other HR candidate genes are still underway. In addition to the testing population sampled from the Southern region of Peninsular Malaysia, three additional populations from Southern, Central and Northern regions will be collected and sequenced by targeted sequencing approach. Surveying of the mating-type diversity at a population level will enable the estimation of mating alleles

available in Peninsular Malaysia and intersections between mating alleles in different localities.

P2.3-011

ADAPTIVE EVOLUTION OF AN EXOTIC FOREST PATHOGEN IS MEDIATED BY INTERSPECIFIC GENIC INTROGRESSION

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Text

Hybridization between the invasive North American fungal plant pathogen *Heterobasidion irregulare* and its Eurasian sister species *H. annosum* is ongoing in Italy. Genome admixing was evaluated in a total of 267 sympatric isolates of the two species using AFLPs. Based on the level of admixing, whole genomes of nine natural hybrids were chosen, sequenced, assembled and compared with those of three "pure" genotypes each of the two parental species. A multi-approach pipeline was used to assign introgressed genomic blocks to each of the two species. Alleles that introgressed from *H. irregulare* to *H. annosum* were associated with pathways related to saprobic processes, while alleles that introgressed from the native to the invasive species were mainly linked to gene regulation. There was no overlap of allele categories introgressed in the two directions. Experiments documented a fitness increase in *H. annosum* genotypes characterized by introgression of alleles from the invasive species, supporting the hypothesis that hybridization results in putatively adaptive introgression. Conversely, introgression from the native into the exotic species appeared to be driven by selection on genes favoring genome stability. Since the introgression of specific alleles from the exotic *H. irregulare* into the native *H. annosum* increased its invasiveness, we propose that two invasions may be co-occurring: the first one by exotic genotypes and the second one by alleles of the exotic species.

P2.3-012

WHO NEEDS A MATE? ADAPTATION IN DEFIANCE OF EXTREMELY LOW GENETIC DIVERSITY IN THE CLONALLY PROPAGATED ASCOCHYTA RABIEI POPULATION IN AUSTRALIA

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Text
Ascochyta rabiei is an economically important pathogen of chickpea, which was introduced to Australia in the 1970s from an unknown source population. Within only a few decades, it successfully established in all chickpea growing regions in Australia, causing substantial losses to the chickpea industry. Previous studies using a small number of microsatellite markers and single nucleotide polymorphisms highlighted the clonality of *A. rabiei* population in Australia, which is in line with presence of only one mating-type (MAT1-2) in Australia. We conducted whole genome sequencing of 230 isolates collected from different agroecological zones between 2013 and 2020 to obtain a higher marker density for accurate marker-phenotype association and to investigate spatial and temporal changes in pathogen population. Low levels of allelic polymorphism further corroborated the highly clonal structure of *A. rabiei* in Australia, with mutations being the only source of variation in the absence of sexual reproduction. Significant population structuring into distinct clonal lineages was detected, which likely resulted from genetic bottlenecks and anthropogenic-driven founder effects in ”new” growing regions. Variation in isolate aggressiveness within the same clonal lineages indicates that evolution of aggressiveness in Australian *A. rabiei* isolates is most likely driven by minor genomic changes at a sequence level or epigenetic factors that require further investigation.

P2.3-013

POPULATION GENOMIC ANALYSES REVEAL EXTENSIVE GENOMIC REGIONS WITHIN SELECTIVE SWEEPS ASSOCIATED WITH ADAPTATION AND DEMOGRAPHIC HISTORY OF A WHEAT FUNGAL PATHOGEN

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Text
Plant pathogens are notorious for their ability to quickly evolve in response to the changing host and environment, resulting in destructive epidemics, particularly in agriculture. The fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), causing wheat stripe rust disease worldwide, is one such pathogen. In the arms race between pathogens and hosts, changes in host populations exerted continuous selective forces on the dynamics of pathogens. However, the footprints of these selection forces and the demography of *Pst* remain poorly explored. In this study, we revealed unprecedented features of worldwide *Pst* populations through population genomic analyses. We detected widespread selective sweep regions across the *Pst* genome. Genes within the selective sweeps were enriched in secreted proteins and effectors and showed functions related to pathogenicity or virulence, temperature tolerance, and fungicide resistance implying that Chinese *Pst* populations suffered positive selection pressures from host and abiotic factors. Moreover, we demographic histories of worldwide *Pst* populations suggested strong bottleneck events for all *Pst* populations during the wheat formation around 10,000 years ago and during modern agriculture around 100 years ago, suggesting crop domestication and breeding programs could continuously contribute to the decline of pathogen-effective population sizes. Our results highlighted the role of modern agriculture on pathogen demography.

P2.3-014

CONTRIBUTION OF HISTORICAL HERBARIUM SMALL RNAS TO THE RECONSTRUCTION OF A CASSAVA MOSAIC GEMINIVIRUS EVOLUTIONARY HISTORY

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Text

Emerging viral diseases of plants are recognised as a growing threat to global food security. However, little is known about the evolutionary processes and ecological factors underlying the emergence and success of viruses that have caused past epidemics. With technological advances in the field of ancient genomics, it is now possible to sequence historical genomes to provide a better understanding of viral plant disease emergence and pathogen evolutionary history. In this context, herbarium specimens represent a valuable source of dated and preserved material. We report here the first historical genome of a crop pathogen DNA virus, a 90-year-old African cassava mosaic virus (ACMV), reconstructed from small RNA sequences bearing hallmarks of small interfering RNAs. Relative to tip-calibrated dating inferences using only modern data, those performed with the historical genome yielded both molecular evolution rate estimates that were significantly lower, and lineage divergence times that were significantly older. Crucially, divergence times estimated without the historical genome appeared in discordance with both historical disease reports and the existence of the historical genome itself. In conclusion, our study reports an updated time-frame for the history and evolution of ACMV and illustrates how the study of crop viral diseases could benefit from natural history collections.

P2.3-015

DETERMINING THE CONTRIBUTION OF ONION TRANSPLANTS TO THE POPULATION GENETICS OF STEMPHYLIUM VESICARIUM IN NEW YORK, USA USING MICROSATELLITE MARKERS.

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Text

Stemphylium leaf blight (SLB), caused by the fungus *Stemphylium vesicarium*, is one of the most important foliar diseases affecting onions (*Allium cepa* L.) in the northeastern United States. Bare root transplants imported from the southwestern United States are used to

establish approximately 25% of the onion production area in NY, with the remainder direct seeded. However, there has been no assessment of the contribution of transplants to SLB epidemics. In 2022, bare root transplants (*n* = 120) from each of eight cultivars were obtained prior to planting, incubated in humid chambers, and observed for *S. vesicarium*. *Stemphylium vesicarium* was present in all eight cultivars, with incidence ranging from 11.2 to 100%. Ten isolates of *S. vesicarium* from each of three cultivars collected prior to planting and ten isolates collected from each field at the end of the season were used to characterize genetic diversity using nine microsatellite (SSR) markers. Populations of *S. vesicarium* were highly diverse based on Evenness, Nei's allelic diversity, Shannon-Wiener, and Stoddart and Taylor's genotypic diversity indices. Multiple alleles were found at each locus independently assorting and resulting in 57 multilocus genotypes (MLGs). Minimum spanning networks (MSN) showed that *S. vesicarium* populations sampled from transplants contributed only slightly to the MLGs found at the end of the season, indicating sources of SLB inoculum other than transplants to be more important.

P2.3-016

GENOMIC DIVERSITY AND VIRULENCE OF SCAB-CAUSING STREPTOMYCES SPP. IN THE PROVINCE OF QUEBEC, CANADA

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Text

Potato common scab is an important bacterial disease afflicting the potato crop in most potato-growing areas around the world. It is caused by numerous *Streptomyces* species and stains that rely on a myriad of virulence determinants. A better understanding of the genomic diversity of this group of microorganisms would help improving control methods, which remain to this day very limited. Over 300 diseased potato tubers from 23 different varieties were provided by 24 Canadian potato growers. 234 *Streptomyces* isolates were retrieved from scab lesions and over 80 % of these isolates were shown to fall into one of the 12 genetic groups established using digital ERIC-PCR genomic fingerprinting. The genomes of 40 of these isolates, including at least one representative strain per genetic group, were sequenced using PacBio SMRT sequencing technology, generating high quality draft genomes. Phylogenetic analysis, pathogenicity assay and genome exploration were carried out. The isolates were shown to belong to multiple species, including *S. scabiei*, *S. stelliscabiei* and *S. acidiscabies*, and to strongly differ in their virulence towards potato tubers. While only half of the sequenced strains were shown to harbor the biosynthetic operon responsible for the production of the thaxtomin A phytotoxin, the main known virulence determinant in scab-causing *Streptomyces* spp., numerous isolates lacking this cluster instead harbor other less characterized virulence determinants.

P2.3-017

DIVERSITY OF MOBILE GENETIC ELEMENTS AND ASSOCIATED FITNESS FACTORS TO ELUCIDATE THE EVOLUTION OF XANTHOMONAS

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Text
Recent advances in computational approaches and the abundance of publicly available genomic data have provided the opportunity to investigate the capabilities of fitness factors to be horizontally transferred among resident plant microflora and pathogens. To understand possible methods of genetic exchange, the program MGEfinder was used with genomes of xanthomonads, including both pathogenic and commensal strains from various geographical locations and spanning across group 1 and group 2, to identify putative mobile genetic elements (MGEs). The putative MGEs were subsequently annotated and screened for genetic indicators of mobility, such as transposases, inverted repeats, phage related genes, and att sites. Additionally, screenings identified cargo genes that could be considered fitness factors, including but not limited to virulence factors and antimicrobial resistance genes. Multiple putative MGEs, including one containing the type III secretion system effector XopAD and another MGE showing potentially novel multi-modal mobility and carrying the copper resistance operon CopLAB with significant similarity to Stenotrophomonas, were identified. The methods in this study can further be applied to expand ecological understanding of pathogen evolution via horizontal gene transfer in addition to identifying the extent to which commensals can act as fitness factor reservoirs.

P2.3-018

HIGH GENETIC DIVERSITY FOUND IN EMERGING EUCALYPTUS PATHOGEN ELSINOE NECATRIX

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Text
Eucalyptus scab and shoot malformation (ESSM) is an emerging disease and a serious threat to the global forestry industry. The disease appeared in North Sumatra, Indonesia in the early 2000s and the causal agent was only recently determined as a novel species, *Elsinoe necatrix*. In this study, we developed 15 polymorphic microsatellite markers and mating type markers using genome sequences for two *E. necatrix* isolates. We characterized 186 isolates of *E. necatrix* collected from different host varieties at four locations in the Toba lake region of North Sumatra using these markers. These populations were found to have a high level of genetic and haplotypic diversity. Discriminant analysis of principal components, haplotype networks and analysis of molecular variance revealed a lack of population structure related to geographic locations and there was high gene flow among sampling regions. Mating type ratios and linkage disequilibrium analyses suggest that sexual recombination is likely, although a sexual state of the fungus has not been found. The results of this study highlight the fact that new genotypes of *E. necatrix*, likely arising from sexual recombination might challenge efforts to manage the disease, thus breeding and selection for tolerance will require substantial host genetic diversity.

P2.3-019

IS A SHIFT IN PATHOGEN STRUCTURE CAUSING THE INCREASED INCIDENCE OF COMMON BUNT CAUSED BY TILLETIA SPP. IN SWEDISH WINTER WHEAT?

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Text

Tilletia tritici (syn. *T. caries*) and *Tilletia laevis* (syn. *T. foetida*) cause the seed-borne disease common bunt (CB) in all wheat growing regions around the world. CB can be effectively controlled by chemical seed treatment. Despite this, the CB incidence in recent years has increased all over Europe. For Sweden, possible reasons for this could be the decreasing number of fungicides available for seed treatment, a larger area of organic production, increased use of farm saved seed or new and more virulent pathogen races. The two species, *T. tritici* and *T. laevis*, are genetically related and biologically similar with identical life cycles. One recent study even suggests a conspecific status of the two species. Traditionally, the species identification has been based on morphological features; the teliospores of *T. tritici* have a reticulate surface, whereas *T. laevis* teliospores are smooth. To explain the increasing problems with this disease, we are analysing CB infected wheat spikes from farmers' fields across Sweden to determine disease incidence, the distribution of the two causal species and their respective virulence spectra. The first objective is to understand the distribution of the species complex based on genotyping-by-sequencing. The second objective is to study the biology and evolutionary history of the pathogen to better understand the epidemiology of the disease.

P2.3-020

UNDERSTANDING THE POPULATION STRUCTURE AND PATHOGENICITY OF BIPOLARIS ORYZAE ON COMMERCIAL AND WILD AUSTRALIAN RICES

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Text

Rice brown spot caused by *Bipolaris oryzae* is an economically important disease of rice worldwide and was associated with the Bengal famine that resulted in the starvation of over two million people in 1943. *Bipolaris oryzae* is the most common leaf-spotting pathogen found on native wild rices in northern Australia. Water shortages in southern Australia have led to attempts to establish a rice industry in the northern wet tropics. However, the presence

of endemic pathogens, such as *B. oryzae*, on native wild rices threaten the viability of this emerging rice industry. There is little known about the genetic diversity of *B. oryzae* in Australia or its virulence on domesticated rice. This study aims to characterise the genetic diversity of *B. oryzae* on wild rice to determine if geographic distribution or host plant species (*Oryza australiensis* and *O. meridionalis*) affect the pathogen population structure. A high-contiguity reference genome of *B. oryzae* using long-read sequence data is currently being developed and will assist in future studies on the pathogen. A spot inoculation method on detached rice leaves is also being developed to allow pathotyping of the different fungal isolates, and to assist with the identification of disease resistance genes in both domesticated and wild rice. This study will help elucidate the epidemiology of brown spot disease in northern Australia, as well as assist with the formulation of novel disease management strategies.

P2.3-022

EMERGENCE AND EVOLUTION OF A NOVEL LINEAGE OF RALSTONIA SOLANACEARUM WITH EXPANDED HOST RANGE

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Text

Members of the Ralstonia solanacearum species complex (RSSC) are historically known to cause brown rot of potato and Moko disease of banana in Central America. A novel lineage detected in the island of Martinique in 1999 exhibited a dramatically different host range and pathogenicity profile. This new lineage, referred to as 4NPB, can infect cucurbits Anthurium and Heliconia spp. and is more aggressive on solanaceous crops than previous variants. We sequenced 500 RSSC strains sampled across Martinique and French Guiana during the emergence of this novel lineage. Pangenome and phylogenetic analyses were performed to identify genomic changes linked with the emergence of the new lineage. This analysis reveals the 4NPB population likely emerged from a population of Moko-causing strains found in association with a tomato host on the mainland, followed by dispersal to Martinique. While 4NPB and Moko-causing strains are closely related, variation in the accessory genome includes the exchange of Type 3 secreted effectors and multiple genes with predicted catalytic activity. Recombination hotspots found between 4NPB and Moko strains include various toxin-antitoxin systems, which are potentially involved in intra-species competition and signaling. These changes may underly the altered pathogenicity and host-range profile of 4NPB, contributing to the dramatic expansion of this lineage across Martinique.

P2.3-024

MORPHOLOGICAL, MOLECULAR AND PATHOGENIC CHARACTERIZATION OF ALTERNARIA SPECIES CAUSING APPLE LEAF AND FRUIT SPOT DISEASE IN CATALONIA (NORTHEASTERN SPAIN)

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Text

Apple tree diseases can cause significant losses in fruit yield and quality. Over the last years, Alternaria leaf and fruit spot disease (ALFS) caused by an *Alternaria* species complex has emerged in Europe.
Since 2013, previously unreported fruit and leaf spot symptoms are being observed in apple tree orchards in Catalonia (Northeastern Spain). Due to its recent detection and particular characteristics, little information is available on the etiology and epidemiology of this disease. This work was aimed at elucidating the etiology of ALFS disease in Catalonia. A morphological, molecular and pathogenic characterization of fungal isolates from apple orchards in Girona province was undertaken.
About one hundred *Alternaria sp*. isolates were recovered from ALFS lesions on apple fruit and leaves. A prevalence of small-spored *Alternaria* species of considerable morphological variation was detected. Isolates from the different *Alternaria* morphotypes were pathogenic on cvs. Golden and Gala apple leaves. Molecular characterization was used to confirm the identity of pathogenic isolates. Results suggest that ALFS disease is present in Northeastern Spain and that a complex of *Alternaria* species belonging to *A. alternata* species group are involved in the disease. Interestingly, *A. mali* was not detected.
This research was supported by Grant PID2021-126505OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe” and 2022 FISDU 00051 (Agaur, Generalitat de Catalunya).

P2.3-025

PHYLOGENOMIC ANALYSES AND COMPARATIVE GENOMIC OF PSEUDOMONAS SYRINGAE ASSOCIATED WITH ALMOND (PRUNUS DULCIS) IN CALIFORNIA

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Text

Bacterial blast and bacterial canker of almond can affect most parts of the almond tree, including flowers, leaves, trunk, and branches. The plant genus *Prunus* comprises the group of hosts associated with the larger number of polyphyletic pathovars and species within the *Pseudomonas syringae* species complex compared to all other known *P. syringae* host plants. In California, the disease has been mainly attributed to *P. syringae* pv. *syringae*, although few studies have attempted to characterize *Pseudomonas* species affecting almond. In this study, whole genome based phylogenomic and comparative genomics were applied to elucidate the

diversity of almond-associated *P. syringae* species and improve pathogen detection. Of the *Pseudomonas* species isolated from almonds, at least three distinct species including *P. syringae* pv. *syringae*, *P. viridiflava*, and *P. cerasi* were found to cause bacterial blast and/or bacterial canker. Furthermore, genome mining indicated the presence of antibiotic resistance genes, including resistance to copper, tetracycline, and aminoglycoside, the antibiotics commonly used to control these pathogens. *Pseudomonas syringae* pv. *syringae* and *P. cerasi* genomes contained the ice nucleation protein which correlated with their verified ice nucleation ability. Finally, we used bioinformatics tools to design species specific primers for the identified pathogenic species. The general structure of other fluorescent pseudomonads obtained from almonds is also discussed.

P2.3-026

POPULATION DYNAMICS AND ADAPTIVE ARCHITECTURE OF THE FUNGUS PSEUDOCERCOSPORA FIJIENSIS IN RESPONSE TO RESISTANCE IN BANANA

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Text

The deployment of durable resistances in crops will be decisive to control epidemics of plant pathogens while reducing pesticides use. The quantitative resistance of plants is supposed more durable than the qualitative resistance, but an erosion of this type of resistance has been frequently observed through adaptation of pathogens. It has been the case of new banana hybrids resistant to *Pseudocercospora fijiensis*, a major fungal pathogen of this crop. A more durable deployment strategy would be to combine resistances with specific and antagonistic interactions in order to constrain and limit the adaptation of pathogen populations. To test this hypothesis, we are developing in the pathosystem banana/*P. fijiensis* the following approach. (i) Quantitative traits of interaction between *P. fijiensis* and banana genitors (used for genetic improvement of banana) will be evaluated. (ii) Adaptive architecture of *P. fijiensis* populations to resistance of these genitors will be compare using genome scan approach. (iii) The genotype x genotype interactions between *P. fijiensis* isolates and genitors will be characterized from a cross-inoculation experiment under control conditions. (iv) Then all the data obtained will be used to parameterized a demo-genetic model to find efficient and durable combinations and deployment strategies of resistance. The first results obtained following such an approach will be presented.

P2.3-027

COMPARATIVE GENOMIC ANALYSIS OF PSEUDOMONAS SAVASTANOI PV. FRAXINII ISOLATED FROM ASH TREES IN THE UK

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Text

Pseudomonas savastanoi pv. fraxinii (*Psf*) causes ash canker and is widespread in the UK. The genetic diversity and variation in virulence of this species is understudied. With the widespread infection of ash dieback, the health of the ash tree population is decreasing. This may alter the *Psf*-ash interaction, leading to loss of the bacterial disease or changes in severity due to a weakened tree immune system. This study aims to establish the genetic diversity of *Psf* in the UK, the impact of disease on the ash microbiome, and the potential for increase in virulence due to progressive tree stress caused by ash dieback. Epiphytic and endophytic populations of *Psf* were isolated from healthy and diseased trees from a range of woodlands in the UK. REP-PCR was used to assess the genetic diversity of collected isolates. A subset was selected for comparative genomics to investigate phylogeny and population structure, using Psf NCPPB 1006 as a reference. Phylogenetic analysis was conducted based on core genome single nucleotide polymorphisms (SNPs), after removal of recombinant regions. The pan-genome will then be characterised, and functionally significant genomic features will be annotated to identify genes with putative involvement in virulence. This information will give insight into the molecular basis of this disease and how the pathogen has evolved within the UK.

P2.3-028

PERILOUS COEXISTENCE: CHILLI LEAF CURL VIRUS AND CANDIDATUS PHYTOPLASMA TRIFOLII INFECTING CAPSICUM ANNUUM, INDIA

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Text

Molecular computing was used to investigate the possible causal agents of chilli crop samples showing mixed symptoms of yellow leaf curl and little leaf type diseases in the Uttar Pradesh, India. Total genomic DNA was extracted from twenty-five samples and amplified by uniplex and duplex PCR using a universal primer pair for begomovirus and phytoplasma. Mixed infection samples show positive amplified products for begomovirus (DNA-A and betasatellite) and phytoplasma (16S rRNA and Sec A). The identified begomovirus was identified as a strain isolate of the previously described Chilli Leaf Curl Virus, which is known to infect *Solanum lycopersicon*, in Oman, whereas the 16S rRNA was identified from the source *Candidatus Phytoplasma trifoli*i, which is known to infect Helichrysum flowering plants in India. Subsequently, molecular computing research based on phylogenetic interweaves, putative recombination, amino acid selection, and genetic diversity were investigated, revealing divergent evolutionary patterns with significant variation and recombination events. The majority of the sequence variations observed in begomovirus and phytoplasma were caused via inter- and intra-specific recombination. These findings could be the first i*n silico* combined infection analysis of ChiLCV and *Ca.P.trifolii* in a chilli crop in India, revealing the potential adaption and evolution of begomovirus and phytoplasma to a new geographic range and crop.

P2.3-029

DIVERSITY OF PERONOSCLEROSPORA PHILIPPINENSIS (W. WESTON) C.G. SHAW CAUSING DOWNY MILDEW IN SUGARCANE AND CORN IN THE PHILIPPINES

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Text

There are four species of Peronosclerospora that cause downy mildew of sugarcane and corn in Asia but Peronosclerospora philippinensis is the most widespread. The pathogen has long been infecting corn and sugarcane in the country but the information regarding its genetic structure and variability is still lacking. In this study, a total of 63 pure isolates were identified and subjected to multi-locus analysis using the partial sequences of three barcoding genes cox2, 28S rRNA, and rps10. Genetic diversity analysis based on concatenated DNA sequences of the three genes (1.3 kbp; n=40) showed a low nucleotide diversity (?) of 0.013, a low average number of nucleotide differences (k) of 1.695, and a moderate haplotype diversity (hd) of 0.604. In the haplotype network and phylogenetic analyses, P. philippinensis population from Cotabato largely formed a separate group from the rest of the PH populations. However, two isolates within the Cotabato populations, and another two isolates from Negros Occidental population formed two independent groups. Overall, the local isolates of P. philippinensis exhibited a low genetic diversity and a rudimentary genetic structure. Furthermore, this study reports the occurrence of two major groups of downy mildew in the country which can have a relevant implication in the management of downy mildew.

P2.3-030

IMPACT OF FUNGICIDE APPLICATIONS ON THE POPULATION STRUCTURE OF ZYMOSEPTORIA TRITICI IN EUROPE

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Text

Septoria tritici blotch (STB) caused by the hemibiotrophic fungus *Zymoseptoria tritici* is one of the most important foliar diseases of wheat worldwide with yield losses reaching 50% under conducive climate conditions. STB disease control is highly reliant on fungicide application which increases the risk of development of fungicide resistance. Knowledge of pathogen's population structure is important to understand the evolutionary potential of fungicide resistance in *Z. tritici*. To study the influence of fungicide applications on the population structure of *Z. tritici*, 377 pathogen isolates were collected in Denmark, Estonia, France,

Germany, Ireland, Lithuania, Norway, Slovenia, and Sweden in 2019. These isolates were analysed using amplicon based sequenced of housekeeping genes (*actin, BTUB, cal, cyp, EF1, GAPDH, hsp80-1, PKC, TFC1*) supplemented with genes associated with fungicide targets including DMI (*CYP51*), SDHI (*Sdh B*, *C*, and *D*), and QoI (cytochrome *b*). A total of 308 unique genotypes were detected. 274 genotypes were detected only once and were unique to each of the nine populations, while 34 genotypes were reported 2-10 times within the populations from Germany, Denmark, Estonia, Lithuania, Norway, and Ireland. Results of the population structure from the housekeeping genes will be compared to the fungicide resistance gene dataset.

P2.3-031

SPATIAL-TEMPORAL GENETIC DIVERSITY OF NEOSCYTALIDIUM DIMIDIATUM POPULATIONS IN TAIWAN

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Text

Neoscytalidium dimidiatum, which causes diseases on several economically important crops, is an emerging plant pathogen worldwide. In this study, 11 simple sequence repeat (SSR) markers were developed to investigate the spatial-temporal genetic structure of *N. dimidiatum* populations in Taiwan. A total of 216 isolates were collected from dragon fruit (*Hylocereus* spp.; n= 205) and Cattleya orchid (n= 11) in 15 Counties/Cities between 2011 and 2021. Eight alien isolates obtained from various hosts were also included, making a total of 224 isolates in the analyses. Among the *N. dimidiatum* populations obtained from dragon fruit (DF) and Cattleya (Ca), 8 and 11 SSR loci were fixed, respectively, and only 2 to 3 alleles were observed in the rest loci. On contrary, only one locus was fixed and 2 to 7 alleles were observed in 10 SSR loci from the alien isolates, suggesting a higher genetic diversity of *N. dimidiatum* populations at the global scale. In addition, 4 and 1 multilocus genotypes (MLG) were identified respectively from the DF and Ca populations whereas all the alien isolates belong to different MLG, again supporting aforementioned observations. Based on the spatial-temporal analyses, a single dominant MLG was identified across space and time in DF populations, suggesting that *N. dimidiatum* may have sustained in Taiwan primarily via asexual reproduction and the DF population may have experienced founder effect, bottleneck effect, or genetic drift in the past.

P2.3-032

SEQUENCING AND RECOMBINATION ANALYSIS OF WATERMELON MOSAIC ISOLATES FROM THE CZECH REPUBLIC

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Text

Watermelon mosaic virus (WMV) is a virus from the genus potyvirus. It is one of the most economically important viral pathogen infecting cucurbits in the world. Several molecular subgroups were reported by Desbiez et al. (2007). The occurrence of WMV in the Czech Republic was first reported by Svoboda (2011) based on ELISA testing. However, no further molecular analyses were done. Currently, and while attempting to update the viral species frequency infecting cucurbits in the Czech Republic, five full-length coding region of WMV isolates were obtained by high throughput sequencing and also by Sanger sequencing (MW188031; OP585149-OP585152) together with 26 more isolates sequenced in the CP coding region. The screening for recombination events in the complete dataset revealed the presence of eighty independent recombination events. The frequency of recombinant WMV isolates reached 96.24%. Therefore, the watermelon mosaic virus showed to have the highest number of recombinants compared to any other potyviruses. Molecular dating analysis suggests that watermelon mosaic virus was originated from northern China at least two thousand years ago, and that the virus moved from non-cucurbit hosts to watermelon one thousand years ago.

P2.3-033

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF PHYTOPHTHORA INFESTANS ISOLATES FROM KENYA AND NIGERIA

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Text

In Sub-Saharan Africa (SSA), despite increased potato production, yields have not reached their full potential mainly due to pests and diseases. Late blight caused by *Phytophthora infestans* is a major constraint to profitable potato production in SSA. The genetic structure of *P. infestans* populations is dynamic and new genotypes with different epidemiological characteristics are constantly emerging. To address this challenge, the Feed the Future Global Biotech Potato Partnership has developed biotech potatoes with 3 resistant (*R*) genes to late blight. To evaluate the durability of the 3 R-gene potatoes in SSA, it is important to understand the genetic diversity of *P. infestans* strains in SSA countries. In East Africa, previous work revealed that genotype EU_2A1 dominated *P. infestans* populations. However, little is known about the genetic composition of *P. infestans* populations in West Africa. In this study, samples were collected from infected potato leaves on FTA cards from locations in the main potato growing regions in Kenya and Nigeria in 2022. Nucleic acids (DNA) were extracted from the FTA cards. Genotyping was carried out using standardized multiplex markers (12 SSR markers) and cleaved amplified polymorphisms markers were used to determine mating type. Fungicide sensitivity to metalaxyl-M was carried out on

isolates collected in Kenya. Results from these studies will be presented and will serve as a baseline to inform future deployment of biotech potatoes in SSA.

P2.3-034

ASPEN MOSAIC-ASSOCIATED VIRUS POPULATIONS IN FINLAND AND SWEDEN

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Text

A new *Emaravirus*, aspen mosaic-associated virus (AsMaV), was identified as the causal agent of the mosaic disease in *Populus tremula*. The genome of AsMaV consists of five negative-sense single-stranded RNA (-ssRNA) molecules. The RNA1 (7.1 kb) encodes for the viral RNA-dependent RNA polymerase (RdRP, 268.2 kDa), RNA2 (2.3 kb), RNA3 (1.6 kb), RNA4 (1.6kb) and RNA5 (1.3kb) encode for the glycoprotein precursor (GPP, 73.5 kDa), viral nucleocapsid protein (N, 35.6 kDa), a putative movement protein (MP, 41.0 kDa) and a protein of unknown function (P28, 28.1 kDa), respectively. Different regions of the virus genome were used to investigate genetic diversity and population genetic parameters. Full-length AsMaV-RNA3, -RNA4, and partial -RNA1 were amplified via RT-PCR with specific primer pairs. Subsequently, the amplicons were investigated by RT-PCR-RFLP and selected variants were subjected to sequencing. The results showed that AsMaV has a conserved genome. However, isolates from Finland and Sweden are belonging to two different phylogenic groups and probably have to be assigned to different populations. AsMaV variants were discriminated better based on RNA3 rather than RNA4 or partial RNA1.

P2.3-035

POPULATION GENOMIC ANALYSES UNCOVER DIVERSITY, DIFFERENTIATION OF SUBPOPULATIONS AND MECHANISMS OF EVOLUTION OF FUSARIUM ASIATICUM IN A HISTORIC CONTEXT

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Text

Fusarium head blight (FHB) is an economically important disease on wheat that can result in severe yield losses. In addition, infected grains can accumulate mycotoxins such as the trichothecenes DON, 3ADON, 15ADON and NIV that pose a threat to human and livestock health. F. asiaticum is a major etiological agent of FHB and dominant in Eastern Asian regions including southern China. In order to understand the diversity, population structure

and evolutionary history of F. asiaticum in China, a collection of over 2000 isolates from 127 geographic sites was assembled. Subsequently, we generated a pangenome of F. asiaticum incorporating the 245 high quality F. asiaticum genomes sequences and their phenotypes from selected strains. Population genomic analysis revealed the presence of three distinct opulations. POP1 mainly comprises NIV producing strains from all regions, POP2 is dominated by 3ADON strains from the Yangtze river and Hunan, and POP3 were NIV strains from Sichuan. Ancestral population sizes and populations split time of three populations analyses revealed the evolution of F. asiaticum populations over the last 10,000 years and its correlation with historical documented changes in the crop-rotation system. And POP1(NIV) was the ancestral population. In addition combined analyses of chemotype composition, population structure, spread of spore trajectory and population genomics revealed long-distance (~300 km) migration that was never reported for Fusarium.

P2.3-036

GENOMIC AND PHENOTYPIC CHARACTERIZATION OF EPIDEMIOLOGICALLY MOST SUCESSFUL HAPLOTYPES OF THE FIRE-BLIGHT PATHOGEN, ERWINIA AMYLOVORA

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Text
Fireblight, a disease of many plant species in the *Rosaceae* family, is caused by *Erwinia amylovora* (*Ea*). Epidemiologically, colonization and infection of blossoms is a crucial stage of the disease. Analysis of the phylogeography and population structure of the species based on minisatellites confirmed presence of high number of diverse haplotypes but also high representation of certain haplotypes during outbreaks (Bühlmann *et al*., 2014). With the aim to ellucidate the basis of the highly abundant *Ea* haplotypes we have selected a number of isolates of both epidemiologically successfull and unscuccessful haplotypes for further characterization. Whole genome sequencing, as well as phenotypic characterization is underway. The preliminary results will be presented

P2.3-037

IDENTIFICATION OF FOUR NOVEL SPECIES OF PSEUDOMONAS ASSOCIATED WITH BACTERIAL LEAF SPOT OF CUCURBITS

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Text
Bacterial leaf spot of cucurbits (BLS) is an emerging disease in the southeastern United States and has also been documented in Serbia. Severe BLS outbreaks, such as were seen in southern Georgia and Florida in the spring of 2013-14, may lead to foliar blighting, reduced yields, delayed fruit maturity, and transplant death, causing significant economic losses for cucurbit growers. Previously believed to be solely caused by Pseudomonas syringae sensu stricto, recent characterization of forty isolates from symptomatic cucurbit foliage from the southeastern U.S. and Serbia has revealed BLS to be associated with multiple Pseudomonas species. Whole genome sequencing, 16S rRNA analysis, average nucleotide identity, and multi-locus sequence analysis were useful in identifying nine Pseudomonas species, spanning three P. syringae phylogroups, as well as four undescribed species outside of the P. syringae species complex. Twelve of forty isolates were identified as P. syringae, twelve as P. alliivorans, nine as P. capsici, one as P. viridiflava, and one as P. lijiangensis. New species were most closely related to P. parafulva, P. corrugata, P. asiatica, and P. cichorii. The discovery and characterization of new causal agents of BLS as well as of new Pseudomonas species may lead to changes in management strategies as well as a greater understanding of the epidemiology of the disease.

P2.3-038

GENETIC DIVERSITY AND VIRULENCE IN A WORLDWIDE COLLECTION OF SUNFLOWER BROOMRAPE (OROBANCHE CUMANA WALLR.) POPULATIONS

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Text
Orobanche cumana Wallr. is a holoparasitic weed that infects roots of sunflower in large areas of Europe and Asia causing serious damages which may reach 90% yield losses. The parasitic interaction between sunflower and *O. cumana* generally follows a gene for gene model, with resistance in sunflower and avirulence in *O. cumana* controlled by dominant alleles at single loci. However, more complex genetic control of resistance has been also reported in some resistance sources. Analysis of genetic diversity of the parasite is important to better understand its evolution and spread. This work presents a study on genetic diversity and population structure, and virulence performed with 78 broomrape populations from Mediterranean and Black Sea areas. Molecular diversity was performed at inter- and intra-population level by genotyping 162 SNP markers on 1700 broomrape individuals. Virulence was evaluated by artificial inoculations in control conditions on eight race differential sunflower genotypes. The present study shed the light on the current distribution, genetic and pathogenic variation in *O. cumana* showing that genetic clusters are associated mostly with the geographical origin of the populations. There are some clusters with relatively high diversity while others present little molecular divergence. Virulence assays showed the presence of races E, F, G and G+ distributed all along the clusters. These results will help to improve resistance breeding and management strategies.

P2.3-039

GENETIC DIVERSITY ANALYSIS OF BROOMRAPE (OROBANCHE CUMANA) POPULATIONS IN SUNFLOWER GROWING AREAS IN EUROPE.

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Text

Orobanche cumana or broomrape is an obligate root parasite of sunflower (*Helianthus annuus*) that strongly impacts yield in southern and eastern Europe. The host-parasite system of *O. cumana* and sunflower is characterized by a typical gene-for-gene interaction. The extensive use of sunflower varieties carrying monogenic resistance genes enhanced the selection pressure on the parasite, leading to the emergence of new races. The two most recent races of *Orobanche* that were officially described are referred to as race F and G. This work reviews the results of monitoring broomrape populations in 8 different European countries during the past 10 years. Seeds of *O. cumana* collected in sunflower fields were tested for their virulence on a differential set of sunflower varieties carrying different resistance genes. Race F is still the most predominant in most regions, but in east European countries a wider diversity of races and an increased incidence of the more aggressive race G was observed. The genetic diversity of the isolates was studied using a set of 180 SNPs that allowed to classify them according to their geographic origin and showing higher levels of heterozygosity in eastern Europe populations. These results will be corroborated by more recent GbS data that were obtained for a subset of the collection. All in all, this study provides an overview of the pathogenicity profiles and the molecular diversity of *O. cumana* populations across the major sunflower markets in Europe.

P2.3-040

POPULATION GENOMIC ANALYSES REVEAL POSSIBLE HYBRIDIZATION AND DEEP GENETIC STRUCTURE IN THE BARLEY PATHOGEN PYRENOPHORA TERES

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Text

Pyrenophora teres (Pt) f. *teres* (Ptt) and *Pyrenophora teres* f. *maculata* (Ptm) are important fungal pathogens in barley crops, causing substantial economic losses worldwide. France is a major barley producer, but there is a lack of knowledge about the population biology of Pt, making its control challenging. Thus, this work aimed to provide information useful to guide management strategies through the study of Pt population structure. A total of 183 isolates were obtained from nurseries in different barley-producing regions of France. Isolates were characterized using genotyping-by-sequencing, and the analysis of these data showed a

clear differentiation between the two forms of Pt. The most abundant was Ptt (n=177 isolates), the scarcest Ptm (n=3), and three isolates were identified as possible hybrids. The most relevant factor shaping the structure of Ptt was the type of barley, i.e. winter or spring, over geography and barley variety. Furthermore, an analysis using a dataset with samples from Europe, Asia, Africa, and America showed that most of the French isolates cluster independently, but the ones that come from spring barley group with samples from Europe, Asia, and Africa. As a recommendation, special attention should be given to the areas where hybrids are found, as they might have higher virulence. Furthermore, the exchange of barley material with America should be carried out carefully to avoid the introduction of new genotypes.

P2.3-041

POPULATION GENOMIC ANALYSES REVEAL PATTERNS OF HOST SPECIALIZATION AND HYBRIDIZATION IN CERATOCYSTIS FIMBRIATA, C. EUCALYPTICOLA AND C. MANGINECANS

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Text

Species of Ceratocystis are important pathogens of forestry and agricultural crops. *C. fimbriata*, the causal agent of black rot of sweet potato, was the first species described in the genus. Two cryptic species closely related to *C. fimbriata*, *C. eucalypticola* and *C. manginecans*, have recently been described causing wilt disease in *Acacia*, *Eucalyptus*, *Mangifera* and *Punica*. Their species boundaries have not been well defined, which has been a source of taxonomic confusion. We sequenced genomes of a large number of isolates of *C. fimbriata*, *C. eucalypticola* and *C. manginecans* from five hosts and 11 counties across five continents. Population genomic analyses revealed three lineages, defined by their patterns of host association. The first lineage included isolates only from sweet potato (member of the Solanales), which represents *C. fimbrita*. The second lineage included isolates infecting *Eucalyptus* and *Punica* (members of the Myrtales), known as *C. eucalytpicola*. The last lineage consisted of isolates infecting *Acacia* and *Mangifera* (members of the Fabales and Sapindales), known as *C. manginecans*. Hybrids of *C. eucalypticola* and *C. manginecans* were identified from regions where they co-occur, but there were no hybrids between *C. fimbriata* and other two species. Overall, our results reveal signatures of host specialization in these fungi. They also provide the basis for our current investigations to identify genomic regions underlining their pathogenicity and host specificity.

P2.3-042

DIVERSITY OF XANTHOMONAS NASTURTII, THE CAUSE OF BLACK ROT OF WATERCRESS

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Text

Watercress (*Nasturtium officinale*) is a rapidly growing member of the *Brassicaceae* family that is mainly grown in water beds and is one of the oldest known leaf vegetables. *Xanthomonas nasturtii* causes black rot of watercress, producing lesions around the hydathodes, yellowing and wilting of leaves. This disease was first described in 2017 in samples from Florida, USA, although it has probably been present in crops for much longer. Subsequently, the disease was also found in Hawaii and in Europe, in Spain and Portugal. Isolates were obtained from leaf samples from farms, shop-bought bagged leaves and from seed. Characterisation of isolates was completed using fatty acid profiling, *gyrB* partial gene sequencing and pathogenicity tests. Three watercress accessions were susceptible to all tested isolates, but no symptoms were observed in savoy cabbage.
Whole-genome sequence comparisons showed genetic diversity amongst the isolates, with isolates from Spain being the most diverse. There is some evidence for the presence of genes linked to heavy metal tolerance in some isolates that could be linked to the extensive application of copper based treatments in some farms. Using the genome sequences obtained in this study, we are developing new markers for diagnostics of *X. nasturtii*. Control of the disease is difficult as the bacteria can probably be easily transmitted through water in the water beds. Seed testing should be used to select clean seed lots for each new crop.

P2.3-043

PHYLOGENETIC IDENTIFICATION OF ALTERNARIA SPP. BELONGING TO SECTIONS ALTERNARIA AND INFECTORIAE ISOLATED FROM WHEAT SEEDS WITH BLACK POINT SYMPTOM

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Text

Wheat (Triticum spp.) is the most industrially important crop worldwide. In Mexico, the main wheat-producing areas are located in the northwest and center of the country. In these regions, blighted spikes have been observed, causing a loss of commercial quality of the seed due to a typical black lesion at one end of the kernel. Therefore, this study aimed to

identify the causative agents of this symptom through molecular analysis. From 2015 to 2018, 48 spike samples exhibiting this symptomatology were collected on wheat fields in three states of Mexico. From the seeds, 244 isolates were obtained, which were identified via multilocus phylogenetic reconstruction using the entire ITS rDNA region and partial sequences of the lsu, gapdh, and alt a-1 genes. Additionally, the phylogenetic genealogical concordance test calculating the PHI Homoplasia Index (>0.05) recognized new species in the section Alternaria: A. angustiovoidea, A. brassicinae, Alternaria sp., and Alternaria sp. nov. were identified; in the section Infectoriae three new species were described. Additionally, two haplotypes of A. brassicinae and two haplotypes were identified in Alternaria nov. sp. belongs to section Infectoriae. Pathogenicity tests confirmed the disease-causing capacity of all evaluated isolates in the varieties Junco, Cirno, and Borlaug. These results can be used in wheat breeding programs to incorporate resistance into new varieties for Mexico's central wheat-producing regions.

P2.3-044

DIFFERENCES IN F. MUSAE GENOMES

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Text
Fusarium musae has recently been described as a cross-kingdom pathogen causing crown rot of banana, a post-harvest disease, keratitis and skin infections as well as systemic infections in immunocompromised patients. To better understand the diversity within strains of the species, the entire genome of 19 *Fusarium musae* strains was obtained through Illumina and Nanopore sequencing assembling short and long reads. The length of the assembled nuclear DNA ranged from 43.04 Mbp to 45.54 Mbp. A similar divergence is shown in mitochondrial DNA ranging from 56493 bp to 59256 bp. Comparative analysis revealed differences in number of secondary metabolites gene clusters (from 41 to 47). A special focus on Enniatin-Beauvericin cluster in *F. musae* identified evolutionary divergences of NPRS gene within the species. Our results are a fundamental step to better investigate phylogenetic relationship within *F. musae* strains with diverse origin and will provide essential knowledge for functional studies of genes involved in the environmental adaptation and in the infection process on humans and banana fruit.

P2.3-045

COMPARATIVE GENOMICS AND ASSOCIATION WITH PATHOGENIC TRAITS IN XANTHOMONAS

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Text
The sudden appearance and rapid spread of pathogen populations can be associated to changes in environmental conditions or to exposures to susceptible hosts (as when introduced to a new geographical region). However, often, these spreads are also associated to genetic changes in the population: mutation or acquisition of new genetic features, that confer the population a fitness advantage. Currently bacterial genomic data is being generated at exponential proportions, and much of it is underexploited. We are developing strategies to integrate methods for genomic analyses in a way that given a set of bacterial genomes and any trait of interest, a user can obtain possible genes or genomic regions associated to said trait. I will show how we have used these startegies to find genes associated to emerging or aggressive bacterial populations. Including how we identifed clusters of genes associated with the emerging *Xanthomonas vasicola* pv. *vasicola* populations infecting corn in South America and the U.S; as well as how we identified candidate genes in *Xanthomonas oryzae* pv. *oryzae* associated to resistance breakdown in field trials.

P2.3-046

POPULATION GENETICS OF TWO INVASIVE FUNGI (CRYPHONECTRIA PARASITICA AND FUSARIUM CIRCINATUM) IN SPAIN

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Text
Invasive species are known to cause economic as well as ecological damage. Fungal species can invade other territories where they meet naïve hosts and cause diseases or even epidemics. However, why some fungi are successful invaders remains largely unknown, i.e., they become established in a new area while others cannot. One hypothesis is that multiple introductions and sexual reproduction help establish invasive fungi. *Cryphonectria parasitica* and *Fusarium circinatum* are textbook examples of the fungal invasions that were introduced to Europe by accident. These introductions were separated temporally, i.e., *C. parasitica* was introduced almost a century ago, whereas *F. circinatum* is a recently introduced species. We used molecular genetic tools to compare both fungal populations in Spain, considering vegetative compatibility types, mating types and DNA sequencing. We found multiple introduction events of *C. parasitica*, whereas *F. circinatum* was clonal and indicated only one introduction event to Spain. However, we did not find evidence of sexual reproduction in our study area, and it looked like both fungi were still reproducing asexually. Our study also found that quarantine measures are essential to prevent the introduction of new genotypes to an area and to avoid the establishment of invasive species.

Keywords: Population genetics, invasive pathogens, *Fusarium circinatum, Cryphonectria parasitica*

P2.3-047

ANASTOMOSIS GROUP TYPING OF RHIZOCTONIA SOLANI KÜHN INFECTING SOLANACEOUS VEGETABLE CROPS

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Text

Present study determines the occurrence of anastomosis groups of Rhizoctonia solani on Potato, Tomato, and Chilli with their morpho-molecular characterization. At least 63, 67, and 58 fungal isolates were recovered from potato, tomato, and chilli and identified as R. solani based on nuclear number and morphological characteristics. Restriction analysis of PCR-amplified ribosomal DNA with four discriminant enzymes (MseI, AvaII, HincII, and MunI) revealed recovered isolates belong to; AG-2-1, AG-2-2, AG-3 PT, AG-4 HG I, AG-5, and AG-6. Isolates were further paired with tester strains of R. solani AGs which confirmed the results of AG composition revealed by RFLP analysis. Amplification of ITS region of rDNA with primers ITS1/ITS4 and sequence analysis exhibited 99-100% identity with already reported AGs. Isolates recovered from potato belong to AG-3 PT (76.5%), AG-5 (8.5%), AG-4 HG I (4.2%), AG-2-1 (6.3%), and AG-2-2 (4.2%). AG-3 PT was widely distributed to major potato growing areas while others were confined to distinct locations. Isolates recovered from tomato belong to AG-3 PT (64.2%), AG-2-1 (14.2%), AG-2-2 (9.5%), AG-5 (7.1%), and AG-4-HGI (4.7%). AG-3 PT was widely distributed to major tomato growing areas followed by AG-2-1 while other groups were confined to distinct locations. Similarly, AG-4 HGI (59.4%) was also widely distributed to chilli growing areas. Other AGs recovered from chilli belong to AG-2-1 (16.2%), AG-6 (10.8%), AG-3 PT (8.1%), and AG-5 (5.4%).

P2.3-048

BLUEBERRY VIRUS L: A NOVEL AND WIDESPREAD BLUEBERRY VIRUS IN THE UNITED STATES

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Text

There are not many newly discovered viruses infecting agricultural crops in 2023 that are found in ~ 80% of the samples tested; collected across a continent. This is the case of

Blueberry virus L (BlVL), the subject of this presentation. While screening material using high throughput sequencing novel virus-like sequence were identified. Genome sequencing and virus characterization places BlVL in the genus Luteovirus. Luteoviruses encode six or seven proteins including two that are involved in movement. Yet, a small number do not have readily identifiable movement proteins and BlVL belongs to this group, not only based on its genome structure but also its phylogenetic placement. Over 600 samples collected across five US states from both coasts of the continent were screened for BlVL and 79% was found infected. About 300 samples were sequenced and the virus diversity is >18% at the nucleotide level. This is the first luteovirus present in blueberry and its high incidence makes BlVL the most widespread blueberry virus in the USA.

P2.3-049

COMPARATIVE GENOMIC ANALYSIS AND PHENOTYPIC STUDIES OF PECTOBACTERIUM CACTICIDUM AND PROPOSAL OF RECLASSIFICATION TO A NEW GENUS ALCORNIA

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Text

Pectobacterium cacticidum strains can infect cacti and agave. Contrary to the other Pectobacteria, its optimum growth and virulence temperature is much higher (37oC versus 28oC) and exhibits an increased resilience to drought. Since *Pectobacteriaceae* show high potential for environmental spread and adaptation to different hosts, there is a high possibility that a global transmission of this species in an era of climate warming could cause substantial damage to the horticultural and crop industry. It is, therefore, essential to better assess its virulence potential.

We performed whole genome sequencing of five *P. cacticidum* strains. The average genome size was 4.16 MBp, and the GC content of 51%. Genome comparisons with other *Pectobacterium* species yielded 516 unique proteins, most of which were involved in signalling and cellular processes, including quorum sensing mechanism, urea and iron hydroxamate transport, synthesis of rhamnolipids and siderophores. We tested 93 phenotypic characteristics with the BIOLOG system to confirm the activity of the genes present in the genome that can be important contributors to the pathogenicity of this species. We also performed pathogenicity tests to affirm the virulence of the strains. Most of them were virulent towards Opuntia and potato. Based on the genomic data showing that these species differ markedly from other Pectobacteria, we suggest reclassifying *P. cacticidum* into a new genus *Alcornia.*

Funding: OPUS18-2019/35/B/NZ9/01973

P2.3-050

INTER-CONTINENTAL POPULATION DIFFERENTIATION SPOT FORM OF NET BLOTCH ISOLATES

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Text
Spot form of net blotch, caused by *P. teres* f. *maculata* (*Ptm*), is a significant necrotrophic fungal disease of barley that spread globally in the 20th century. Genetic relationships were analysed among a geographically diverse collection of 338 isolates from Australia, Southern Africa, North America, Asia Minor and Europe. The results, based on three independent genetic differentiation methods and genome-wide DArTseq data, indicated divergence of *Ptm* populations between continents, although some admixturing was evident. Highest genetic diversity was present among Turkish isolates together with regional sub-structuring. The Australian population was defined by a low genetic diversity; however, genotypic grouping and haplotype data from mutants of *Cyp51A*, which confers insensitivity to triazole fungicides, provided evidence for a recent incursion into Australia, likely involving isolates originating in South Africa.

POST-HARVEST - Part 1: Interactions of postharvest pathogens with the host and its microbiome

C2.5-1

MOLECULAR MECHANISM OF FRUIT CELL MEMBRANE RESISTANCE RESPONSE TO POSTHARVEST FUNGAL PATHOGENS

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Text
Fruit, as an important part of people’s diet, provides rich nutrition for human health. However, fungal pathogens frequently cause diseases in fruit during postharvest periods, resulting in huge economic losses. Outcomes of fungal diseases depend on both plant defense responses and fungal pathogenicity. Plant defense responses are highly sophisticated and are generally divided into preformed and induced defense responses. Cell membrane is the first barrier of plant against pathogen infection. Plasma membrane-resided receptor like kinases has also a pivotal role in perceiving and transducing signals into cells to accomplish

defense responses. Our research demonstrate the important roles of cell membrane proteins in disease resistance, immune response, and PCD regulation. Overexpression of tomato SlREM1 increased susceptibility of tomato to Botrytis cinerea, indicating that SlREM1 is a positive regulator of plant cell death and provide clues for understanding the PCD molecular regulatory network in plants. Moreover, we reveal a tomato FERONIA homolog SlFERL involved in immune responses to B. cinerea via a new pattern-triggered immune pathway, that SlFERL recognizes BcPG1 and precisely tuning MAPK signaling. These results provide a new perspective for understanding the molecular mechanism of cell membrane resistance response to fungal pathogens, and are beneficial to giving theoretical guidance for the creation of new technologies of postharvest disease control.

C2.5-2

INSIGHTS INTO THE EFFECTS OF AGRONOMICAL MANAGEMENT PRACTICES IN ASPERGILLUS INCIDENCE AND CARPOSPHERE'S MICROBIAL COMMUNITIES OF GRAPEVINE (CV. SYRAH)

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Text

Aspergillus bunch rot is considered one of the most important diseases of grapevines resulting in severe yield losses and, major qualitative deterioration of grape products due to the production of mycotoxins. We investigated, in a two-year field study, the impact of agronomic practices like defoliation to enhance grape microclimate (DF), pruning method to reduce grape bunch density (LBD), and irrigation cut-off (NIR), at three developmental stages of grapevine (Pea size berry, Veraison, and Harvest), on (i) grape composition (TA, pH, and TSS), (ii) on the frequency of occurrence of *Aspergillus* on grape berries and (iii) on the overall composition of grape carposphere microbiome. The density of *Aspergillus* on grape berries was significantly reduced by the applied management practices (DF, LBD, NIR). Amplicon sequencing analysis showed that both the phenological stage and the agronomic practices employed (particularly NIR and DF) imposed significant changes in the α-diversity and β-diversity of the grape carposphere bacterial and fungal communities. The NIR, LBD, and DF treatments which supported lower *Aspergillus* populations, network analysis revealed negative co-occurrence patterns between *Aspergillus* and several bacterial genera (*Streptococcus, Rhodococcus, Melitangium*) reported to have antifungal properties suggesting potential natural attenuation mechanisms for the control of *Aspergillus*.

C2.5-3

THE ASSEMBLY AND DYNAMICS OF THE FRUIT MICROBIOME: WHAT CAN IT TELL US ABOUT BIOLOGICAL CONTROL OF POSTHARVEST DISEASES

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Text

The microbial ecology of fruit associated microbes lags behind that of other plant-associated microbial communities. Determining the host factors that influence fruit surface microbiome assembly and dynamics is crucial to unravel the basic ecological processes involved in the disease process of postharvest pathogens. In our recent studies, we found a greater host effect of fruit stage over genotype in shaping the fruit microbiome structure, characterized by strong community succession. We also see a set of core members that persists throughout the fruit stages and identified some of these members exhibiting differential abundances at specific stages. We demonstrate that the turnover pattern dominates over its antithetic nestedness pattern in driving community succession during the fruit developmental stages and lead to taxonomic homogenization after harvest during storage. We report for the first time, the existence of an underlying universal dynamic model in fruit-associated microbiome assemblies. We provide evidence of microbial cross-domain interactions by showing strong correlations in diversities and community composition and detect some microbe-microbe co-occurrences. Additionally, we show that disease process on harvested fruit is complex and may involve, besides the pathogens, other components of the microbiome.

C2.5-4

IDENTIFICATION OF PUTATIVE NECROTROPHIC EFFECTORS OF MONILINIA SPP. USING A MODIFIED TRV-EXPRESSION VECTOR AS A HIGH-THROUGHPUT INFILTRATION METHODOLOGY

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Text

Nowadays, brown rot, caused by the genus *Monilinia* is one of the most important fungal diseases affecting stone fruit. *Monilinia* spp. are necrotrophic plant pathogens, whose life cycle is completed with the gain of nutrients from dead cells through the production of necrotrophic effectors. The recognition of these effectors, which depends on the host genotype, leads to the development of the disease. In this study, we tested the cell death-inducing capacity of 14 putative effector genes, selected by their high expression during plant infection. To test their necrotrophic effect, we used modified *Tobacco Rattle Virus* (TRV) constructs, to express their proteins in host plant tissue by agroinfiltration. Based in our outcomes, this methodology resulted efficient in producing the effector proteins transiently in both *Nicotiana benthamiana* and the non-model plant *Prunus* spp. Our results showed that the effector gene codified as MFRU_030g00190 produced necrosis after infiltration in all *Prunus* genotypes tested, but not in *N. benthamiana*, and two other effectors caused necrosis in both species. Moreover, the *Prunus* individuals codified as 37p15-14 and *Cadaman* seem to be more tolerant to the infiltration; in contrast the 37p15-16 seems to be

quite sensitive to all tested effectors. This procedure could be the basis for genetic analysis of the response generated during the infection and to identify loci in regions that confer susceptibility to the disease in breeding programmes.

C2.5-5

MICROBIOMES: AN IMPORTANT TOOL TO ELUCIDATE THE EPIDEMIOLOGY OF POSTHARVEST PATHOGENS

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Text

Postharvest diseases can be caused by latent pathogens infecting at different phenological phases in orchard. It is of great importance to understand the infection time, in order to intervene with preventive treatments in orchard. Microbiome analysis is a flexible tool to elucidate the epidemiology of plant pathogens. With the introduction of new apple varieties, dry lenticel rot, caused by Ramularia mali, and white haze, associated to Entylomatales and Golubeviales, are emerging. The epidemiology of these pathogens remains unclear. By using metabarcoding, we characterized both epiphytic and endophytic microbiomes of two apple cultivars from early fruit development up to the end of shelf life. R. mali developed in both cultivars as an endophyte at second fruit fall, then white haze symptoms appeared on fruit ripe for picking. This was confirmed in endophytic samples through qPCR specific for R. mali. Among the genera associated to white haze, Golubevia was the most abundant epiphyte from beginning of ripening to the end of shelf life. The analysis of the airborne mycobiome present in the orchard, by using a spore trap in orchard and metabarcoding, permitted to evaluate when fungal pathogen spores are released in orchard: Enylomatales started to occur at the end of the fruit development, whereas R. mali spores were released since flowering. Metabarcoding helps to elucidate the epidemiology of fungal pathogens and to design a targeted crop protection strategy.

C2.5-6

ETHYLENE SENSING VIA GPCRS AND MAPK PATHWAY IN COLLETOTRICHUM GLOEOSPORIOIDES IS VITAL FOR HOST INFECTION AND REPRESENTS POTENTIAL TARGETS FOR DISEASE MANAGEMENT

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Text

Ethylene is a plant hormone that plays important roles in regulating the ripening processes of

fruits. Meanwhile, competitive binding inhibitors, 1-methylcyclopropene (1-MCP), is commonly applied to prevent ethylene perception in the plant for the purpose of delaying or inhibiting the ripening processes, and thus achieving fruit quality preservation. It's noteworthy that ethylene can be also sensed by plant pathogenic fungi to accelerate their infection and disease. However, the molecular mechanisms of responses to ethylene in fungi remain largely unclear. It is also obscure whether 1-MCP can interfere with the recognition of ethylene by pathogenic fungi. In this study, the anthracnose disease pathogen, Colletotrichum gloeosporioides, was investigated via transcriptomic and reverse genetic analysis to reveal that ethylene can cause transcription changes of a large set of genes, which are mainly responsible for appressorium development and virulence expression, and these processes are likely coordinated via GPCRs and MAPK signaling pathways. 1-MCP treatment could inhibit the promoting effect of ethylene on the appressorium development and gene expression. From the perspective of phytopathogenic fungi, this study explores the molecular mechanisms of response to ethylene and the antifungal mechanism of 1-MCP, and provides new ideas for further improving the ethylene-related handlings on harvested fruit crops to reduce the losses caused by fungal diseases.

P2.5-001

CITRUS FRUIT POST-HARVEST FUNGAL PATHOGENS AT PAKISTAN'S KHANPUR ORCHARDS

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Text

Losses associated with fungal diseases of citrus fruits have been reported in all citrus-growing regions of Pakistan. However, this issue has not been addressed in the Khanpur region. Khanpur is a significant citrus-growing region in Pakistan and significant losses have occurred in this region. Citrus fruits contribute to the income of citrus growers in Pakistan. It is necessary to develop crop management practices to reduce the extent of postharvest losses in citrus fruits. In this study, the fungal pathogens linked to citrus fruit diseases in the orchards of Khanpur were identified, and their pathogenicity was assessed. Fungal isolates were collected from symptomatic citrus fruits from randomly selected orchards within the Khanpur area of production. The fungal isolates, recovered from orchards that were identified morphologically and further confirmed by Internal transcribed spacer (ITS), belong to the genera of Colletotrichum, Alternaria, Aspergillus, Botryosphaeria, Lasiodiplodia, Penicillium, and Fusarium. Research was needed to fully understand the disease development. This knowledge improved the development of management practices to develop cost-effective spray programs. It would also be wise to investigate biological and physical post-harvest management interventions to increase storage and marketability.

P2.5-002

USING GENOMICS TO STUDY THE FUNCTION OF THE MICROBIOME IN THE DISEASE PROCESS IN APPLE FRUIT AFTER HARVEST

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Text

The 'pathobiome' concept is highly relevant to the study of postharvest diseases. A shift from native microbiome to a pathobiome occurs during the disease and usually involves transitions in microbial composition leading to pathogen proliferation and disease development. Specific wound-colonizing bacteria taxa may have direct or indirect effects on the development of the disease through positive interactions with the pathogen and taxa that are antagonistic to its function. Genomic data is likely to be the key to deciphering the current "black box" of the trophic exchanges in wound sites. Here, we are representing a set of genome-scale metabolic models of the microbiome based on the recovery of metagenome-assembled genomes to delineate the complex microbial interactions associated with disease progression. 'Golden Delicious' apple fruit-P. expansum pathosystems are using as a model for necrotrophic postharvest disease development. The experimental is based on profiling two wound microbiomes: i. microbiome of uninfected surface wounds; ii. pathobiome of fruits wounds infected with P. expansum. This project aims to construct an in-silico representation of key members of these communities and conduct simulations of the interactions within the two communities. The simulations will predict pathogen-suppressing/enhancing microorganisms and identify the community's metabolic stimulants of positive/negative elements. Experimental systems will validate predictions in-vivo.

P2.5-004

IMPACT OF MICROBIAL INTERVENTION ON THE ASSEMBLY AND DYNAMICS OF THE APPLE FRUIT MICROBIOME

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Text

Microbes residing on the fruit surface are integral components of the fruit and may play an important role in determining its quality and shelf life. Factors involved in the assemblage of fruit-associated microbial community are just beginning to be studied. In this regard, it has been shown that besides the main drivers like fruit genotype, geographical location and management practices, the microbial assembly and dynamics of the domesticated apple varies greatly between different stages of its development in the field and cold storage after harvest. Additionally, a core taxa was identified persisting across all stages and genotypes tested. This work was designed to explore the effect of application of Aureobasidium sp., one of the core member of the apple microbiome and a known biocontrol agent, applied either as preharvest sprays at different stages starting from the flowering stage or as postharvest

treatment, or both. We present results showing the implications of microbiome disruption on the microbial community structure, assembly and dynamics using culture dependent and high throughput sequencing approaches, consequences on fruit physiological characters and disease status of the fruit at the end of storage. Significant effect on the microbial richness, diversity, temporal dynamics and associations between different taxa were observed in treated fruit in congruent with changes observed in the fruit physiological characters and disease incidence.

P2.5-005

IDENTIFICATION OF FUNGI ASSOCIATED WITH MOULD OF POME FRUIT STEMS AND CALYX SEPALS AFTER CA STORAGE

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Text

Calyx and stem mould is a superficial mould restricted to the pome fruit pedicles and calyx sepals after long-term controlled atmosphere (CA) storage. Markets have reported these moulds on fruit arriving at export destinations. resulting in costly repacking or consignment rejections. This study aims to identify the fungi causing these moulds on stems and calyx sepals of pome fruit. Isolates were collected from symptomatic apple and pear fruit from commercial packhouses in the Western Cape of South Africa. Molecular identification of 100 fungal isolates causing calyx and stem end mould were done using gene areas Alt a 1, OPA1-3 using PCR- RFLP, TEF-1 and RPB2. PCR-RFLP amplification i31 apple isolates classified as A. arborescens and 27 isolates as A. alternata/A. tenuissima. From the 13 pear isolates, five isolates were grouped as A. aborescens and the remaining eight isolates as A. alternata/A. tenuissima. PCR using TEF-1 and RPB2 amplification identified nine isolates as C. cladosporiodes and nine isolates as either Epicoccum nigrum/ layuense. Other fungal genera such as Diplodia, Aureobasidium and Fusarium were also identified, albeit in lower frequencies. In conclusion, a number of fungi were identified as causing stem and calyx mould on pome fruit from CA storage, with the potential for these fungi to occur in complexes, rather than individually. Understanding these complexes and the epidemiology of the members will help in designing appropriate management strategies.

P2.5-006

THE IMPORTANCE OF A GOOD RECOVERY OF THE APPLE MICROBIOTA FOR THE ANALYSIS OF ITS MICROBIOME

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Text

It is essential to know microbiome of apples, one of the most eaten fruits worldwide, in order to understand the interactions of its microbial communities and their effect on pre and postharvest fruit quality. However, the recovery and isolation of these microbial communities from the fruit's surface presents certain challenges due to its low abundance, specially if we compare it with other parts of the plant, such as the rhizosphere. The objective of this study was to identify and establish a reproducible method able to recover the highest possible number of microorganisms from the surface microbiome attached on apple peel. Three different buffer solutions (Tris-EDTA, Tris-EDTA with 2% Tween 80, and Phosphate Buffered Saline (PBS)), three different recovery methodologies (10 minutes of shaking, 20 minutes of shaking, and 20 minutes of shaking plus 5 minutes of sonication) as well as the number of washings needed to extract the largest number of microorganisms possible were evaluated. The results of the investigation showed that the method that recovered the largest number of cultivable microorganisms was the use of two washings with buffer Tris-EDTA with 2% Tween 80 with agitation during 20 minutes at 180 rpm. The present study shows the importance of choosing a good washing method to be able to recover the maximum concentration of microorganisms from the whole surface of apples eliminating possible biases in the results. Financing: PID2020-117607RR-I00

P2.5-007

FUNGAL COMMUNITY DIVERSITY IN CITRUS FRUIT AT DIFFERENT RIPENING STAGE

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Text

Citrus is the most popular fruit around the world. Post harvest decays are often encountered and have great impact on citrus industry. There are diverse fungal communities in citrus fruit under natural conditions. By decoding the sequences of fungal ITS through Illumina MiSeq technologies. the diversity of fungal communities in the rind and flesh of Wanmi No 1 (Citrus reticulata Blanco cv. Ponkan) at the growth, ripening and storage stages was analyzed. A higher degree of fungal diversity was observed in the rind than in the flesh at both the growth and storage stage, on the contrary, the opposite results were found at the ripening stage of citrus fruit. The dominant genera were different at different stage, with Medicopsis and Collectotrichum in the rind at the growth stage, Collectotrichum at the ripening stage, Botrytis, Erythrobasidium and Strelitziana at the storage stage; while in the flesh, Penicillium and Cladosporium accounted for 1/4 of the whole community at the growth stage, Botrytis with more than 50% of the total at the ripening stage, Penicillium and Alternaria accounted for 90% at the storage stage. The population of plant pathogenic fungi Cladosporium, Botrytis, Erysiphe, Penicillium, Alternaria and Fusarium in the rind was larger than in the flesh. The large population of fungi and the change pattern in the flesh suggest that the postharvest fruit decay should be a result of the interaction of fungi.

P2.5-008

THE WHITE-COLLAR COMPONENT BCWCL2 REGULATES CITRIC ACID SECRETION TO MAINTAIN REDOX HOMEOSTASIS AND FULL PATHOGENICITY IN BOTRYTIS CINEREA

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Text

Botrytis cinerea, a well-known phytopathogenic fungus, causes gray mold disease in a variety of crops. The pathogenicity of *B. cinerea* depends on a complex network of molecular interactions, which are modulated by various factors such as oxidative stress, metabolic pathways, and morphological changes. In this study, we aimed to investigate the role of the white-collar component BcWCL2 in regulating citric acid secretion to maintain redox homeostasis and full pathogenicity in *B. cinerea*. The results showed that the disruption of BcWCL2 significantly decreased the secretion of citric acid, leading to a reduction in oxidative stress tolerance and pathogenicity. Furthermore, the transcript levels of BcVEL1, BcPacC, transporter Bcin02g07440, pyruvate carboxylase Bcin09g02790, and citrate synthase Bcin02g02750 involved in citric acid metabolism were downregulated in the Δ*bcwcl2* knockout strain. *B. cinerea* produces multicellular appressoria dedicated to plant penetration, named infection cushions (IC). Redox homeostasis regulated by BcWCL2 is critical for IC formation and the early stages of disease. Specifically, These results provide new insights into the molecular mechanisms underlying the pathogenicity of *B. cinerea* and the regulation of citric acid secretion. The discovery of the role of BcWCL2 in maintaining redox homeostasis and full pathogenicity highlights the importance of citric acid metabolism in the pathogenesis of phytopathogenic fungi.

P2.5-010

SYMPTOMATIC AND PATHOGENIC CHARACTERIZATION OF ALTERNARIA SPECIES ASSOCIATED WITH CITRUS POSTHARVEST DISEASES IN MOROCCO

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Text

Alternaria postharvest diseases of citrus caused mainly by Alternaria alternata (Fr.) Keissl are a serious worldwide problem leading to significant economic losses. The aim of the present study is to determine the symptomatic, cultural, and pathogenic variability among forty-five Moroccan Alternaria spp. strains based on their hosts. Results showed several disease symptoms on the rind of the infected citrus fruits. Gray and dark-brown to black lesions were produced and developed around the peduncle, end-stem zone, and the surface of the oranges and mandarin fruits whereas other types of lesions spread on lemons surface fruits as dry brown lesions. Symptoms caused by the natural infection of the Alternaria pathogens on mandarins were almost similar, as well as on the lemons fruits. The colony and growth rate characterization of each group of Alternaria spp. on PDA medium revealed clear

and considerable differentiation among isolates. Pathogenicity assays of each isolate were realized on wounded fruits of three commercial varieties, ‘Salustiana’ orange, ‘Ortanique’, and ‘Nadorcott’ mandarins. Findings demonstrate also high significant variations among the isolates in terms of lesion diameter produced on inoculated fruits. The disease severity on ‘Ortanique’ fruits was slightly higher than that observed on fruits of ‘Nadorcott’ and ‘Salustiana’ varieties. In fact, no correlation was found between cultural, pathogenic characters and hosts of the studied Alternaria isolates.

P2.5-011

REDUCE THE POST-HARVEST LOSSES IN ORGANIC BEETROOT PRODUCTION

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Text
The market for organic agriculture is rapidly growing. In Switzerland, the production of organic Beetroot is particularly renowned. However, their storage until spring has become increasingly difficult in recent years, and losses due to post-harvest rots can lead to over 50 % by March. The causes for the various storage rots in beetroot are currently unclear, and therefore there are few measures to prevent them in organic production. Pathogen infections causing storage rots in beetroot can occur via the seed, in the field, or post-harvest. Understanding the process of infection is, therefore, critical to find preventive solutions. Here, we present the results of a two-year project that aim to reduce post-harvest losses and elucidate the causes of storage rots in organic beetroot production. Analysis of stored beetroot revealed Fusarium and Phoma as predominant pathogens, while Botrytis, Rhizoctonia, and Pythium as additional causative agents of storage rots. Field trials in cooperation with four producers of organic beetroot were performed, where the production from sowing to storage was monitored. Different measures, such as steam sterilization of the seed, the use of biocontrol products in the field and before storage, or processing and cooling methods after harvest, as well as cultivar differences were investigated. The various measures were found to affect seed health, seedling emergence, leaf health, and the quality of beetroot after storage.

POST-HARVEST - Part 2: Sustainable managements of postharvest diseases: new technologies and approaches

C3.3-1

MINICELL-ENCAPSULATED DSRNA (ME-DSRNA): A PROMISING AND SCALABLE PLATFORM FOR TARGETED BIOCONTROL OF PHYTOPATHOGENIC FUNGI

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Text

Gray mold, also referred to as botrytis fruit rot, is a prevalent postharvest disease that presents a significant threat to a diverse array of fruits and vegetables. The disease is caused by Botrytis cinerea, a fungal pathogen that can infect fruit either during growth or after harvest, ultimately leading to consequential losses in storage, transportation, and marketing. One potential method for managing gray mold is spray-induced gene silencing (SIGS), which utilizes topical dsRNA applications. While SIGS displays considerable potential as an environmentally-friendly and target-specific disease management approach against phytopathogenic fungi, its broad application is hindered by issues regarding the stability, efficacy, and scalability of dsRNA. In the present study, we demonstrated that Escherichia coli-derived anucleated minicells could serve as a scalable and cost-effective platform for dsRNA production and encapsulation. Our research showed that the use of minicell-encapsulated dsRNA (ME-dsRNA) substantially enhanced the stability of dsRNA. ME-dsRNA remained safeguarded from RNase degradation and was stable on strawberry surfaces, making it capable of persisting under field-like conditions. In addition, we found that ME-dsRNAs that targeted chitin synthase class III (Chs3a, Chs3b) and DICER-like proteins (DCL1 and DCL2) genes of Botrytis cinerea selectively silenced the target genes and resulted in substantial fungal growth inhibition in vitro.

C3.3-2

EFFICACY OF ANTAGONISTIC YEASTS IN THE CONTROL OF BROWN ROT OF NECTARINES AND EFFECT ON FRUIT MICROBIOME

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Text

Monilinia fructicola, the causal agent of brown rot, is one of the most important pathogens affecting nectarines during storage. Current management strategies include preventive treatments with synthetic fungicides, posing technical, environmental and toxicity issues. We aimed to evaluate the efficacy of treatments with antagonistic yeasts to control brown rot of nectarines. A screening trial was set up by treating inoculated fruits with 14 yeast strains. The most effective strains (MS, *Metschnikowia* sp., AP47, *M. fructicola*, FR4A, *Aureobasidium* sp.) were tested in semi-commercial conditions. Fruits were maintained in storage rooms at 1 °C for 28 days, followed by 4 days of shelf-life at 25 °C. After storage, all treatments showed a significant rot reduction compared to the control. The efficacy of MS strain was comparable to

the chemical control treatment, making the antagonist as competitive as fungicides. All strains maintained a significative rot reduction at the end of shelf-life. The evaluation of postharvest quality parameters, including firmness, total soluble solids and titratable acidity showed that none of the three tested yeasts affected nectarine quality. A metabarcoding analysis was conducted to evaluate the effect of the treatments on the microbial population of the nectarines. Results proved that treatments with antagonistic yeasts represents a promising tool for reducing postharvest losses preserving the fruit quality.

C3.3-3

POSTHARVEST DECAY MANAGEMENT OF CITRUS IN THE UNITED STATES WITH CYPROCONAZOLE AND NATAMYCIN, NEW HIGHLY EFFECTIVE CONVENTIONAL AND ORGANIC FUNGICIDES, RESPECTIVELY

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Text

Citrus postharvest decay management is challenging because Penicillium spp., the main postharvest pathogens, are at high risk for fungicide resistance development, few fungicides are effective against sour rot caused by Geotrichum citri-aurantii, and the crop is very susceptible to decay development. The demethylation inhibitor (DMI) cyproconazole was found to have incomplete cross resistance to the DMIs imazalil and propiconazole that are currently registered on citrus in the United States. It was significantly more effective than propiconazole in managing sour rot and more efficacious than imazalil in managing green mold caused by imazalil-resistant isolates of P. digitatum. Mixtures of cyproconazole and propiconazole at half rates were also effective against sour rot and more effective than imazalil to manage imazalil-resistant isolates. Registration of cyproconazole is pending. Packinghouse applications with the polyene natamycin reduced the incidence of sour rot and Penicillium decays to low levels, but for highest decay control, it will be best used in combination with other modes of action. Because the risk for resistance development against natamycin is considered low, its use in mixtures represents an effective anti-resistance measure. With the next-generation DMI cyproconazole and with several formulations of natamycin approved as organic treatments in the United States, a new era in postharvest decay management of citrus is on the horizon.

C3.3-4

REDUCING BROWN ROT AND MAINTAINING PLUM QUALITY DURING COLD STORAGE WITH COMPOSITE EDIBLE COATINGS CONTAINING AVOCADO SEED EXTRACT

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Text

Reduction of brown rot, caused by *Monilinia fructicola*, is a major challenge in the postharvest storage of fresh Japanese plums (*Prunus salicina* Lindl.), which are highly perishable. The use of plant extracts with antifungal properties could be a sustainable natural alternative to polluting chemical fungicides for brown rot control. An extract obtained from avocado seeds (AVS) was found to completely inhibit the in vitro fungal growth of *M. fructicola*. This extract was then incorporated into composite edible coating matrixes based on hydroxypropyl methylcellulose (HPMC) or Arabic gum (AG) as hydrocolloids and beeswax as lipid. Coated fruits were stored for 5 weeks at 1 °C, followed by 3 days at 7 °C and 5 days of shelf life at 20 °C, simulating cold storage, transportation, and shelf life, respectively. After cold storage, the HPMC-AVS and AG-AVS coatings reduced disease incidence by 30% with respect to uncoated control fruit and disease severity by 50 and 62%, respectively. After shelf life, AG-AVS significantly reduced disease incidence and severity by 13 and 42%, respectively. The coatings also reduced the fruit respiration rate, preserved fruit firmness and alleviated chilling injury symptoms. Additionally, the coatings had no impact on the fruit physicochemical and sensory quality, and AG-AVS improved fruit gloss. These findings show the potential of composite edible coatings incorporating AVS extract to reduce brown rot and preserve plum postharvest quality.

C3.3-5

MANAGEMENT OF BROWN ROT INFECTIONS ON STONE FRUIT USING EPIDEMIOLOGICAL KNOWLEDGE

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Text

Monilinia spp. is the main pathogen that affects stone fruit causing significant production losses, especially in seasons with favourable climatic conditions for disease development. In the last years, our research has been focused on epidemiological studies of Monilinia spp. in the ‘Valle del Ebro’ (Spain). The epidemiology of this pathogen has been deeply investigated in both under field and postharvest conditions. Studies conducted in the field provided the knowledge to develop a prediction model that define the brown rot epidemic pattern in this area. Likewise, the epidemiology in the packinghouse has shown the importance of the main postharvest handling operations and the influence of temperature and RH on conidia survival to develop the disease. Currently, the standard practices for controlling this disease are conducted by means of spray programs of synthetic fungicides in the field. From the epidemiological model, a practical warning system for fungicide applications in the field has been developed that includes parameters such as: i) fruit susceptibility, ii) the presence of inoculum in the field, and iii) climatological factors. This warning system has been validated for six seasons conducting a total of 38 trials on peach and nectarine crops in the ‘Valle del Ebro’. Our results suggest that the use of the proposed warning system will be an effective tool to control Monilinia spp. in stone fruit and allow a reduction of chemical treatments applied in the field.

C3.3-6

EPIGENETIC CONTROL OF LONG-LASTING DEFENCE PRIMING FOR THE PROTECTION OF FRUIT AGAINST POSTHARVEST DISEASES

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Text

Tomato is a major crop world-wide however its production is heavily limited by Botrytis cinerea. Due to the toxicity of post-harvest pesticide application, alternative control methods such as priming are being investigated. Our work has shown that priming seedlings (2 weeks old) with the chemical β-aminobutyric (BABA) results in long-lasting resistance against B. cinerea in the fruit as well as transgenerational resistance. This work explores how changes in DNA methylation mark long lasting priming in fruit and explores the hypothesis that young plants display a greater epigenetic imprinting capacity. Using whole genome bisulphite sequencing analysis (WGBS), differentially methylated regions (DMRs) specific to the phenotype of long-lasting resistance have been identified. Our results illustrate that BABA treatment impacts CHH context methylation depending on timepoint of application, confirming our hypothesis. Interestingly however, changes in CHH methylation after BABA treatment are not maintained throughout the life of the plant. A transcriptomic analysis on B. cinerea infected fruit BABA treated plants identified differentially expressed genes (DEGs) associated with resistance against B. cinerea. By overlapping our DEGs of interest with our resistance associated DMRs we have identified markers of long-lasting priming in tomato fruit which could serve for targets of durable resistance in other crops.

F3.3-1

COLD AND WILD ENVIRONMENTS: SOURCE OF POTENTIAL BIOCONTROL AGENTS FOR POSTHARVEST FUNGAL DISEASES MANAGEMENT

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Text

New BCAs (Biocontrol Agents) are urgently needed to reach the ambitious target of reduction of 50% of the pesticide use by 2030. The diversity of the required BCA application promoted the search for strains that best perform in specific environments and conditions, such as the fungal postharvest diseases during the cold storage.
An *Aureobasidium* strain (UC14) isolated from wild environments and sampled during the cold season is under evaluation as an antagonist for postharvest diseases management of stored fruits. According to sequence analysis of ITS, EF1, and ELO, the strain resulted taxonomically distinct from the several concurrently isolated black yeasts that were all identified as *Aurebasidium pullulans*. To verify the effectiveness of the UC14 strain as new BCA to apply during postharvest storage, it was evaluated by *in vitro* (volatile and non-volatile metabolites) and *in vivo* assays against different fungal pathogens (*Monilinia* spp., *Penicillium* spp), and

with several different fruits and storage conditions. Interesting results were obtained by qPCR analysis, that detected the reduction in pathogen abundance on fruit supporting the promise of strain UC14 for postharvest applications.

F3.3-2

CONTROL OF POMEGRANATE POSTHARVEST DISEASES BY IN THE FIELD AND AFTER HARVEST TREATMENTS

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Text

In Italy, the demand of fresh/processed pomegranates has recently grown due to the nutraceutical properties. Fruit can be cold stored no more than few months due to fruit rots caused by *Botrytis*, *Alternaria*, *Coniella*, and *Penicillium* fungal genera. The first three genera infect pomegranates during the blooming stage leading to latent infections, instead species belonging to *Penicillium sensu lato* are "wound" pathogens exploiting macro- and microinjuries occurring during harvest and postharvest handling. The scarcity of conventional and alternative fungicides for this minor crop has enhanced the need to find new solutions/strategies for disease control. In the blooming phase, 'Wonderful' plants were treated with low environmental impact compounds: a bio-stimulant made of red algae, a chitosan solution, and two formulations based on *Aureobasidium pullulans* and *Bacillus amyloliquefaciens*. Furthermore, harvested pomegranates with cracking were dipped in ozonated water or in neutral electrolyzed water (NEW) to evaluate the activity of these physical control means against “wound” pathogens. The research disclosed the effectiveness of *B. amyloliquefaciens* and of NEW in reducing postharvest rots due to latent and wound infections, respectively. In pomegranates, the integrated use of low environmental impact products and physical means can represent an ecofriendly alternative to fungicides, fitting into the concept of sustainable agriculture also in organic farming.

P3.3-001

PRE AND POST HARVEST DISEASES OF ONION IS A MAJOR THREAT IN ONION PRODUCTION AND FOOD SECURITY IN BANGLADESH

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Text

In Bangladesh, onion (Allium cepa L.) is generally used as spices and sometimes as vegetables. Area under onion cultivation in the country is 185,000 ha with an annual production of 1954,000 mt and an average yield of 12.34 t/ha. The yield per hectare is very low as

compared to the yield of other onion growing countries. Incidence of pre-harvest diseases like seedling mortality, purple blotch, stemphylium blight, downy mildew and bulb rot is responsible for such low yield. Post-harvest disease may also incur about 30-40% losses in stored bulb. With a view to control both pre and post-harvest diseases a comprehensive study was undertaken, where a series of experiments was conducted for testing some systemic and non-systemic fungicides, botanicals, chemicals and bio-control agents under field conditions and to increase the yield and keeping quality of bulb. Pre-storage bulb curing and improvement of storage methods were tested to control post-harvest diseases. Rovral(Iprodione) and Luna sensation (Fluopyram + Trifloxystrobin) were effective against purple blotch and stemphylium blight, Autostin(Carbendazim) against black mould and Cupravit (Copper) against soft rot. Trichoderma viride and Safeda (botanical) were also effective against black mould. Hydrogen per oxide and low storage temperature (AC room) reduced incidence of black mould and soft rot. Pre-storage bulb curing improved their storage life and keeping quality.

P3.3-002

IMPACT OF UV-C IRRADIATION ON ALTERNARIA LEAF SPOT DEVELOPMENT IN BLUEBERRIES

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Text

Blueberries are regarded as a super food and a crop with the most promising production and commercial prospects worldwide. However, the productivity of blueberry plants is hampered by *Alternaria* spp. causing Alternaria leafspot and fruit rot at the pre-and postharvest stages, respectively. Meanwhile, shortwave ultraviolet (UV-C) light has been successfully used as an alternative treatment to control several pre- and postharvest diseases of fruit. Therefore, this study aimed to investigate the effect of UV-C irradiation as an alternative treatment to prevent Alternaria leaf spot development on artificially inoculated 'Biloxi' and 'Legacy' blueberry leaves. The results of this study indicated that the germination of *A. alternata* spores was inhibited at UV-C dosages between 3.55 and 5.94 kJ/m^2. Furthermore, the findings showed that the UV-C dose of 5.94 kJ/m^2 as a preventative treatment significantly reduced disease severity (lesion diameter) and incidence in artificially inoculated blueberry leaves. This study furnishes evidence that UV-C irradiation at a dosage of 5.94 kJ/m^2 can provide a suitable alternative to the currently adopted commercial Nufarm Azoxy 250 SC applications in preventing Alternaria leaf spot development in ('Biloxi' and 'Legacy') blueberries.

Keywords: Super Food, Alternative Disease Control, Preventative

P3.3-003

PHYTOPATHOGENS AND POSTHARVEST DISEASE MANAGEMENT: A SUSTAINABLE ALTERNATIVE ANTAGONISTIC YEASTS AS BIOCONTROL AGENTS IN FRUIT

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Text
Fruit crop cultivation is a significant component of farmers' agri-horticultural economic activities. Fruit losses from pests and illnesses in the field, storage, transit, and market can account for up to 50% of total production due to a lack of sufficient storage facilities. One reported alternative to the use of synthetic chemical fungicides for controlling postharvest fruit deterioration is biological management employing microbial agents, particularly yeasts. Twenty-nine yeasts were isolated from various sources, and of those, YZ1, YZ7, and YZ27 displayed a wide range of antagonistic activity (mycelial growth inhibition) against the test pathogens in vitro. These isolates' identities as Candida tropicalis YZ1 (CtYZ1), Saccharomyces cerevisiae YZ7 (ScYZ7), and C. tropicalis YZ27 were determined by molecular methods (CtYZ27). When CtYZ1, ScYZ7, and CtYZ27 (1-4108 CFUml1) were applied, the mean lesion diameter of wounds on bananas that had been artificially inoculated with C. musae decreased by 88.7%, 89.3%, and 94.2%, respectively, as opposed to 74.6% in the fungicide-treated fruits (Carbendazim 1.0 g l1) over the control at 4 days. All three yeasts also considerably decreased the latent natural decays brought on by fungi on strawberries, litchis, and bananas. As an alternative to synthetic fungicides, the study's findings could be expanded upon and investigated in the management of post-harvest diseases of fruits.

P3.3-004

POSTHARVEST USE OF NATAMYCIN, A BIOFUNGICIDE TO CONTROL POSTHARVEST DISEASES OF FRESH FRUITS

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Text
Natamycin is a naturally occurring antimycotic compound. It has been used as an additive in the food industry to reduce the growth of yeasts and molds on certain foods. Natamycin is classified as a biofungicide by the U.S. EPA and has been registered in the U.S. to control postharvest diseases on certain fresh fruits. It has also been certified in 2022 for use on organic fruits. In a series of studies, we evaluated different application technologies to apply natamycin to control postharvest diseases of blueberries, mandarins and table grapes. Natamycin applied either as a spraying or dipping treatment was effective to control gray mold caused by *Botrytis cinerea* and Alternaria rot caused by *Alternaria* spp. on blueberries. On mandarins, natamycin was effective to control *Alternaria* spp. and *B. cinerea*, including those strains that are resistant to other citrus postharvest fungicides as there was no cross resistance between natamycin and other fungicides. Natamycin was effective to control Mucor rot on mandarins caused by *M. piriformis* which is naturally tolerant to most other postharvest fungicides. Natamycin applied as a fog treatment showed as a promising tool to control postharvest diseases of table grapes. Our studies showed that natamycin is an effective tool to control major postharvest diseases of certain fresh fruits and manage fungicide resistance in pathogens such as *B. cinerea* and that it also provides a tool to control postharvest diseases on organic fruits.

P3.3-005

IN VITRO INHIBITION OF FUNGI CAUSING POSTHARVEST GRAY AND BLUE MOLDS ON FRESH HORTICULTURAL PRODUCE BY AGRICULTURAL BY-PRODUCT EXTRACTS

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Text

Botrytis cinerea (BC) and *Penicillium expansum* (PE), the causal agents of gray and blue molds on several fresh fruits and vegetables, are among the most important postharvest pathogens worldwide. Agricultural by-products can be rich in bioactive compounds, many with antifungal properties. They could be a sustainable alternative to chemical pesticides used to control postharvest fungal diseases. The extraction of value-added compounds from agricultural by-products contributes to circular economy and the EU Green Deal as well. Almond skin (AMS) and avocado seed (AVS) extracts were obtained using ultrasound-assisted extraction and their total phenolic content and total antioxidant capacity were determined. The capacity of extracts to inhibit BC and PE was investigated using a microtiter assay. AVS showed the highest inhibition capacity, with 99% inhibition of both BC and PE, while AMS inhibited BC and PE by 65 and 99%, respectively. The results suggest that the presence of phenols and antioxidants in the extracts may be responsible for the antifungal activity and that these by-product extracts have potential as novel eco-friendly antifungal agents for the management of postharvest diseases. Further in vivo studies are needed to validate these findings.

P3.3-006

GROWTH INHIBITION OF COLLETOTRICHUM MUSAE USING PLANT ESSENTIAL OILS ENCAPSULATED IN METAL ORGANIC FRAMEWORKS NANOPOROUS MATERIALS

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Text

Being highly perishable, ‘Cavendish’ banana fruits are affected by economically important postharvest diseases, like crown rot and anthracnose, which are caused by *Colletotrichum musae*. Fungicide treatment is considered as an effective management strategy however, its repeated applications pose risk to consumers’ health, environment, and even fungal population. The use of plant essential oils (EOs), like thymol and limonene, are extensively studied as an alternative control method due to its antimicrobial properties. In this proof-of-concept study, thymol and limonene were encapsulated in metal organic frameworks (MOFs) nanoporous materials (ZIF-8 and UiO-66) for sustained release that shall limit fungal diseases. An optimized protocol was developed to achieve a high encapsulation efficiency of EOs in

MOFs (EO@MOFs). *In vitro* assays using several concentrations of EO@MOFs were conducted at 14°C and under controlled atmosphere (CA) storage to determine the inhibition capacity against *C. musae*. Encapsulated thymol reduced the growth of *C. musae* better than limonene. Higher concentration of EO@MOFs favorably slowed down the growth of *C. musae* for 11 days at 14°C and CA storage. The data suggest that volatile plant EOs when released from MOFs have the potential to slow down the growth of *C. musae* and may have some utility in banana postharvest disease control.

P3.3-007

POTENTIAL OF ANTAGONISTIC YEASTS, BOTANICALS AND CHEMICALS FOR THE MANAGEMENT OF GREEN MOULD ROT OF KINNOW MANDARIN CAUSED BY PENICILLIUM DIGITATUM

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Text

Post-harvest green mould rot caused by *Penicillium digitatum* is a serious disease in Kinnow mandarin in the northern parts of India. Disease incidence of the post-harvest green mould rot ranged between 14 -27.5 % during 2021-22. Potential of antagonistic yeasts, botanicals and GRAS (Generally Regarded as Safe) chemicals was evaluated against the pathogen first under in vitro conditions and then the effective ones were evaluated for the management of the disease. Different strains of *Saccharomyces cerevisiae* yeast inhibited the mycelial growth of the pathogen by 48.1- 66.6 per cent. Among botanicals, aqueous extracts of *Roylea elegans* (84.81%) was found to be most effective in inhibiting the mycelial growth of the pathogen. In GRAS chemicals, Salicylic acid (0.25 %), resulted in complete inhibition of mycelial growth of the pathogen. Fruit dip with Salicylic acid (0.25 %) was found to be the most effective with complete reduction in disease severity. Fruit dip in yeast isolate *S. cerevisiae* (R) and aqueous extract of *R. elegans* reduced the disease severity by 98.7 and 80.0 %, respectively. However, fruits kept in impregnated wraps with salicylic acid (0.25 %) and aqueous extract of *R. elegans* (10%) reduced the disease severity by 50.0 and 33.3 %, respectively. Yeast treated fruits significantly improved the quality of Kinnow fruits over botanicals and GRAS chemicals with increased levels of titratable acidity, reducing sugar, total solid sugars and ascorbic acid.

P3.3-008

HOT WATER TREATMENT IMPROVES PEACH FRUIT COLD RESISTANCE THROUGH PPHSFA4C-MEDIATED HSF-HSP AND ROS PATHWAYS

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Text

Peach fruit is cold-sensitive and susceptible to chilling injury (CI), and hot water (HW) treatment can reduce their CI. However, the mechanism of HW treatment reduced CI has not been characterized. In this study, HW treatment suppressed the increases in CI index, electrolyte leakage, and malonaldehyde (MDA) content in peach fruit during cold storage. It also decreased reactive oxygen species (ROS) accumulation and increased the activity of ROS-scavenging enzymes. Transcriptome analysis indicated that HW treatment might contribute to the crosstalk between the heat shock factor-heat shock protein (HSF-HSP) and ROS pathways during cold storage. PpHSFA4c was a key transcription factor and could up-regulate defense genes to alleviate the CI of peach fruit during storage. Furthermore, PpHSFA4c could activate the expressions of PpHSP18.5, PpHSP70, PpHSP83, PpAPX1, and PpAPX3 by interacting with their promoters. These studies indicated that HW treatment alleviated the CI, increased antioxidant activity, and maintained ROS homeostasis of peach fruit through the PpHSFA4c-mediated HSF-HSP and ROS pathways. This study provides novel insights into the regulatory mechanisms of HW treatment alleviating CI in postharvest peach fruit, and expands the theoretical basis for commercial application of HW treatment technology to maintain quality and reduce CI in cold-sensitive fruit.

P3.3-009

SHRIMP WASTE EXTRACTS: A VIRTUOUS EXAMPLE OF REUSE OF WASTES TO ACHIEVE THE SUSTAINABLE MANAGEMENT OF PAST-HARVEST DISEASES

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Text

Plant pathogenic fungi are responsible for various diseases affecting agricultural productions both in pre- and post-harvest. With the perspective of reducing environmental pollution and related consequences for human health, nowadays, the research is strongly focused on valorizing waste, especially those largely generated by processing industries. Among these, shrimp waste stands out for their composition, reach in proteins, chitin, calcium and phosphorus, and other substances.

In order to valorize the reuse of waste, this study investigated the in vitro and in vivo antifungal activities of extracts (water-extract, EtOAc-extract, MetOH-extract and nitric-extract) obtained by minimal processing of shrimp waste. In vitro tests were carried out on fungi and oomycetes of the genera *Alternaria*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Plenodomus* and *Phytophthora*, while in vivo tests were conducted on citrus and apple fruit inoculated with *Penicillium* species. The four shrimp extracts were also analyzed by HPLC-ESI-MS-TOF.

Results from the in vitro tests highlighted that the nitric-extract determined, in all the pathogens tested, values of MIC and MFC ranging from 2 to 3.5%; it was also strongly

effective in preventing citrus and apple fruit molds by *Penicillium*. This study could highly contribute to the identification of natural and ecofriendly substances for the control of pre- and post-harvest plant pathogens.

P3.3-010

EFFECT OF SOWING TIME ON DISEASE MANAGEMENT TO INCREASE QUALITY, QUANTITY AND SHELF LIFE OF TOMATO

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Text

Tomato is one of the important vegetable crop in Bangladesh. Farmers from northern part of Bangladesh identified some economically profitable local varieties for late planting of Tomato. Seedlings were planted at December-January and harvested at April-May. Price of Tomato is higher in May. The optimum sowing and harvesting time of tomato is November and March respectively. This experiment was conducted with three varieties (BARI Tomato 2, BARI Tomato 15 and Dinajpur local) for three sowing time (November, December and January). Incidence of late blight is significantly highest in November sowing for BARI Tomato 15, but no significant difference between varieties in December and January sowing. Incidence of TYLCV is significantly highest for Dinajpur local in January sowing, but no significant difference between varieties in November and December sowing. The harvesting time was March-April for November sowing, April only for December sowing, and April to May for January sowing. Significantly highest fruit yield was obtained in December sowing for all varieties followed by November and January sowing. The Dinajpur local variety showed highest shelf life, firmness, Vitamin C content and soft rot disease tolerance for all sowing dates. Therefore, late sowing of local varieties is a profitable farmer's practice for disease management, quality, quantity and long shelf life of Tomato, and could be a good criterion for varietal development program.

P3.3-011

VOLATILE ORGANIC COMPOUNDS ASSOCIATED WITH NEONECTRIA DITISSIMA INFECTION IN APPLES (MALUS PUMILA CV GALA)

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Text

Post-harvest diseases in apples during long-term storage result in significant amounts (3-10%) of loss. This is mainly caused by fungal pathogens or physiological disorders. The ingress of fungal disease through fruit changes the volatile organic compounds (VOC) profile emitted. This study aimed to identify unique VOCs that can be used as identifiers for fungal infection in

stored apples. Disease-free gala apples were inoculated with *Neonectria ditissima* and sampled weekly. Apples were placed in glass jars, sealed, and incubated at 20°C for 1 hour after which a charcoal-filtered airflow of 1 Lmin-1 was maintained for 1 hour through a Volatile Capture Trap with volatile emissions captured on a porapak-Q absorbent filter. Volatiles were eluted using 1mL of dichloromethane into an Agilent 1.5mL HPLC vial and analysed using Gas Chromatography/Mass Spectrometry. Dodecyl hexanoate and 9-decen-1-yl hexanoate were detected in the early stages of the infection while Styrene, terpinen-4-ol, and 2-methylpentyl formate were detected during decay. The concentration of apple-related volatiles were significantly reduced in diseased apples. Controlled Atmosphere conditions restrict detailed surveys on the incidence of disease spread during storage. Identifying changes in the volatile profile of stored apples in early and later infections may help to provide greater granularity in calibrating the disease progression and help growers make more informed decisions on store management practices.

P3.3-013

CAN ONION STORAGE DISEASES BE DETECTED BY SMELL?

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Text

Bulb onion is a vegetable crop that is well suited for post-harvest storage and is often stored up to 8 months. However, a variety of bacterial and fungal diseases can occur during the storage period, causing severe losses. The early development of these diseases may be difficult to detect in storage facilities where large quantities of onions are stored in bins. But onions developing storage diseases give off odors that differ from those of uninfected onions. Deployment of electronic gas sensor arrays, also known as e-noses, could aid in detection of diseases. E-noses for various purposes are commercially available, and can detect the changes in composition of odorant compounds in the air as they develop. To ensure accurate training of these tools, the key odorants that mark the difference between healthy and diseased onions should be identified. To this end, we used solid phase microextraction (SPME) to sample the headspace of uninfected (control) and artificially infected onion bulbs at different time points post infection and analyzed the samples using gas chromatography-mass spectrometry (GC-MS). Both bacterial and fungal pathogens were used. We found differences in the amount of volatiles released between control bulbs and bulbs at different stages of infection. We also found that certain compounds were released only by infected onions.

P3.3-014

BIOCONTROL EFFICACY OF WICKERHAMOMYCES ANOMALUS ON TOMATO FIELD DISEASES AND STUDY OF THE RELEVANT MECHANISMS VIA MICROBIOME ANALYSIS

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Text

Tomatoes are vulnerable to infections by pathogens found in complex field environments. Most infections are triggered by an imbalance between plant growth-promoting microorganisms and pathogens, leading to various tomato rots and high economic losses. This study aimed to test the biocontrol ability of an antagonistic yeast, *Wickerhamomyces anomalus*, towards tomato gray mold disease in the field and explore its relevant mechanisms through microbiome analysis on tomato surface microbial community. The results showed that *W. anomalus* decreased the tomato disease index during the plant growth period, along with changes in the microorganism community composition. *W. anomalus* treatment increased the abundance of some plant growth promotion bacteria, such as *Pantoea* sp. and *Pseudomonas* sp. and biocontrol agents, such as *Golubevia* sp. and *Papiliotrema* sp., and decreased the abundance of some potential pathogens, such as species of *Alternaria*. All these results suggest that *W. anomalus* could control tomato field diseases, and regulating microorganism composition might be a possible mechanism of the yeast.

P3.3-015

BIOCONTROL ABILITY AND ACTION MECHANISM OF AUREOBASIDIUM PULLULANS S2 AGAINST BOTRYTIS CINEREA IN TOMATO FRUIT

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Text

Gray mold is destructive to the production, transportation, and storage of tomatoes. The application of antagonistic microorganisms to control fruit diseases is a potential alternative to fungicides. This study investigated the effect of *Aureobasidium pullulans* S2 on controlling gray mold in tomatoes and explored the possible mechanisms. The results demonstrated that *A. pullulans* S2 effectively controlled the incidence of gray mold in tomatoes. The response of *Botrytis cinerea* to different components of the *A. pullulans* S2 culture showed that live yeast cells had *B. cinerea* inhibition ability. Host resistance induction, biofilm formation and production of volatile organic compounds (VOCs) with antimicrobial effects could also be important mechanisms of action of *A. pullulans* S2. We identified twelve VOCs; among them, phenylethanol could play a vital role in *B. cinerea* inhibition. These results indicate that *A. pullulans* S2 may be an adequate substitute for fungicides in managing tomato fruit post-harvest diseases.

P3.3-016

NATIVE VINEYARD NON-SACCHAROMYCES YEAST USED FOR BIOLOGICAL CONTROL OF FUNGAL ROT IN STORED TABLE GRAPE.

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Text

The most common strategy to avoid post-harvest table grape decay, caused by spoilage fungi such as *B. cinerea* and *A. niger*, is the use of fungicides (preharvest) and of SO_2 generator pads (postharvest). Excessive doses of SO_2 can damage table grapes, causing early browning, berry cracking and fruit injury. Furthermore, SO_2 and fungicide residues on fruit can cause allergies in consumers. Because health authorities also require reducing human and environmental exposure to chemicals, great attention has been recently focused on identifying new Biological Control Agents (BCAs). At CREA-Viticulture and Enology of Turi, 31 different non-*Saccharomyces* yeast were isolated from new table grape genotypes, showing different degrees of tolerance to grey mold. By performing two consecutive in vivo trials, five yeast strains resulted effective to control the grey mold, both when applied at high (10^7 CFU ml-1) and low concentrations (10^5 CFU ml-1). These results we allowed to select them as possible BCAs. The same isolates, applied at low concentrations, were also effective in reducing grape black rot, caused by *A. niger*. In our conditions, the production of soluble enzymes and Volatile Organic Compounds represent the main mechanisms of the antagonistic activity of these yeast strains. Moreover, their inability to lyse red blood cells, make them excellent candidates for the development of new commercial products, which can be used both in organic agriculture and post-harvesting process.

P3.3-017

PHYSICOCHEMICAL PROPERTIES OF PEACH FRUIT ASSESSED BY NON-DESTRUCTIVE METHODOLOGY RELATED TO THE DEVELOPMENT OF MONILINIA FRUCTICOLA DURING POST-HARVEST

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Text

The concomitant evaluation of physicochemical properties and development of important diseases, such as brown rot in peaches during post-harvest, is hampered using destructive methods for the physicochemical analysis. This study aimed to evaluate the physicochemical characteristics in wounded and unwounded ‘Chimarrita’ and ‘Maciel’ peaches inoculated with *Monilinia fructicola* assessed by non-destructive methods. Colorimeter, near infrared spectroscopy and portable spectrophotometer DA-meter® were used to evaluate the physicochemical changes that naturally occurred in peaches during post-harvest and due to pathogen development on the same fruit simultaneously. Incidence and severity of brown rot were evaluated at 24, 48 and 60 h post- inoculation. Also, the sporulation of *M. fructicola* on fruit surface was evaluated. Scanning electron microscopy was performed at the 10th d after inoculation. The results indicated that differences between wounded and unwounded fruit

related to soluble solids content (SSC), pulp firmness, titratable acidity, dry matter and IAD index did not affect the incidence of *M. fructicola* in both cultivars. However, *M. fructicola* colonization and consequent lesion size in inoculated wounded 'Chimarrita' fruit was influenced by the interaction between dry matter and color in the inoculation moment. Finally, the pathogen colonization caused the increase of SSC, the reduction of dry matter and titratable acidity and reduction followed by increase of firmness.

P3.3-018

OPTIMIZATION OF TRAGACANTH GUM NANOCAPSULES IMPREGNATED WITH PEPPERMINT ESSENTIAL OIL FOR THE EXTENSION OF SHELF LIFE OF CHILLI PEPPER

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Text
Chilli pepper fruits' short shelf life necessitates using natural antimicrobials to retain their freshness and extend their shelf life. The impact of peppermint oil-integrated Tragacanth gum nanocapsules on fresh chili pepper fruits was investigated. In this work, the oil sample was analyzed using GC/GC-MS. The nanoencapsulated oil was characterized using selected physicochemical parametres. In addition to the oil's characterization, an in-vitro kinetics study was conducted. The pathogenicity of the fungi isolated from rotten chili pepper fruit was also determined. In addition, the nanoencapsulated oil was employed to evaluate the in-vitro and in-vivo control of anthracnose in the fruits. Fresh chilli pepper fruits were evaluated for quality (pH, electrical conductivity, ascorbic acid, water activity, total phenolic content, antioxidant activity, total soluble solids, respiration, colour, ethylene and browning index) using response surface methodology. The GC/GC-MS analysis revealed that eucalyptol (90.70%) was the most abundant constituent of the peppermint oil. Trichoderrma harzianum, Colletotrichum gloeosporioides, and Aspergillus flavus were isolated from rotten chilli pepper; C. gloeosporioides was proved to be the source of rot in chilli pepper fruits. The nanoencapsulated oil shows that it could be used to control anthracnose disease in chilli pepper fruits, and disparity in the quality assessment indicates the bioactivity of nanoencapsulated peppermint oil.

P3.3-019

EFFICACY OF THE GRAS SALT SODIUM METABISULFITE TO CONTROL CURATIVELY POSTHARVEST FRUIT DECAY

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Text
The present study evaluate the effectiveness of the GRAS salts, sodium metabisulfite (SMB), ammonium bicarbonate, sodium bicarbonate and potassium dihydrogen orthophosphate

firstly in vitro against the main fungal species of postharvest fruit decay, Alternaria alternata, Botrytis cinerea, Penicillium italicum and Penicillium digitatum. Results showed that SMB at 0.2% inhibited completely mycelium growth of the fungal specie. Ammonium bicarbonate and sodium bicarbonate were less efficient at 0.2 %. The least efficient was potassium dihydrogen orthophosphate with mycelial growth inhibition ranging between 26% to -23% indicating mycelial growth promotion to up 23 %. Experiments were also conducted in in vivo using SMB at concentrations 0.2, 0.5 and 1% preventively and curatively against the most destructive fungus B. cinerea on postharvest apple fruit. Results based on decay length showed that SMB when used as preventive treatment was inefficacy even with the highest concentration. In curative treatment, this salt was highly efficient at 0.5%. Meanwhile, the dose of 1% induced the onset of phytotoxicity observed around the wound in the form of cellular disintegration on the epidermis. To conclude, the appropriate concentration of SMB retained for postharvest treatment is 0.5% used as curative treatment of dipped fruit. Further experiments in semi-commercial trials should be conducted during storage to confirm the effectiveness of SMB. This work was conducted within the framework of the PRIMA StopMedWaste project, which is funded by PRIMA, a programme supported by the European Union.

P3.3-020

PLANT VOLATILE ORGANIC COMPOUNDS IN FRUIT PRESERVATION: MICROBIAL INHIBITION, INDUCTIVE DEFENSE AND INNOVATIVE APPLICATIONS

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Text

The postharvest fruit rot caused by diverse pathogens brings severe economic loss to the fresh fruit industry. Conventional chemical preservatives or fungicides are gradually fading away from the interests of both researchers and consumers. Thus, natural products are considered promising substitutes, especially botanical volatile organic compounds (VOCs) with bio-compatibility, accessibility and practicability, which are found versatile in defending against postharvest rot. Based on precise quantification of plant volatiles, we discovered that the plant volatile organic compound (E)-2-hexenal and linalool was the key VOC induced by *Botrytis cinerea* in strawberry and tomato. It is found that VOCs not only directly act on the pathogen but also regulate the defense of fruit with a dose-dependent action pattern. To control the volatility and reactivity of VOC, we created several VOC release control productions. With a cost mentality, we use the cyclodextrin to embed the VOC molecules. We also invented a new kind of sustained VOC release agent by Michael's addition reaction among glutathione and (E)-2-hexenal. This production showed a good environmental responding VOC releasing capability. All of the innovations had sound effects on fruit preservation to control the postharvest disease. These technology innovations in the sustained release of VOCs provided new feasibility in their application in fruit preservation.

P3.3-021

EVALUATION OF CHITOSAN ALONE OR MIXED WITH SODIUM METABISULFITE IN CONTROLLING POSTHARVEST FRUIT DECAY

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Text

Fruit decay during postharvest storage is a critical issue that have required efficient biological treatments to reduce waste. In this study, fruit of apple (var. Golden) and of citrus (vars. Maltaise, Thompson and Clementine) were separately inoculated with each of the fungal species Botrytis cinerea, Penicillium italicum and Penicillium digitatum. Results showed that 'Thompson' and to a lesser extend 'Maltaise' were the most sensitive to rots of Penicillium digitatum and Botrytis cinerea. Apple fruit were the least susceptible mainly to Penicillium digitatum. The least pathogenic fungal species was Penicillium italicum whatever the kind of fruit. To reduce decay incidence, aqueous solution of Chitosan (chitoplant at 1%), sodium metabisulfite (at 0.5%) and mixture of both compounds (1% and 0.5% respectively) were applied on 'Maltaise' and 'Golden' inoculated respectively with Penicillium digitatum and Botrytis cinerea. Fruit were disinfected, injured and inoculated with the target pathogen. After 2 h incubation, fruit were dipped for 1 min in the solution already prepared and incubated at room temperature. Results showed that chitosane was slightly effective compared to sodium metabisulfite in decreasing fruit rot diameter. Mixture of both compounds showed a depressive effect compared to each single product.
This work was conducted within the framework of the PRIMA StopMedWaste project, which is funded by PRIMA, a programme supported by the European Union.

P3.3-022

POSTHARVEST FUNGAL DISEASES OF POMEGRANATES IN SOUTHERN ITALY

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Text

High-value trade and favorable climate have encouraged pomegranate cultivation and processing in Italy, where Akko and Wonderful are the most widespread cultivars. In this agro-industrial chain, fungal pathogens are chiefly responsible for product losses. Most of the infections happen in the field during blooming, remaining latent till storage and sale; important losses can be caused also by pathogens getting entrance during harvest and postharvest due to wounds created by "wound" fungi, pests, and abiotic damages. Being a minor crop, conventional and alternative fungicides are scarce, making control of fungal pathogens very difficult. To reduce disease incidence, description of mold symptoms and characterization of fungal etiological agents represent a key-step. Disease incidence of fungal species from symptomatic fruit was assessed according to morphological and molecular features. Main fungal diseases were gray mold, blue mold, black heart, black spot, anthracnose, and dry rot. Results showed latent pathogens as the main cause of rots, being

the most abundant *Alternaria alternata*, *Coniella granati*, and *Botrytis cinerea*. Furthermore, among wound pathogens different species within *Penicillium* and *Talaromyces* genera were recorded. Other genera involved in minor postharvest diseases were *Aspergillus*, *Colletotrichum*, and *Cytospora*. To develop effective control strategies, knowledge of pomegranate fungal pathogens is needed facilitating decision systems to play a leading role.

P3.3-023

EXPLORING THE POTENTIAL OF NATURAL AND SYNTHETIC PHOTOSENSITIZING COMPOUNDS FOR ECO-FRIENDLY MANAGEMENT OF GRAY MOLD IN STRAWBERRIES

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Text

Gray mold is a major menace in fruit crops including cultivable strawberries, caused by cryptic species of fungal pathogen *Botrytis*. Quiescent infection during flowering and fruit ripening resulting in postharvest losses. Persisting usage of fungicides in management of this disease possesses threat to the environment. Photodynamic inactivation of fungi using certain light-absorbing compounds could be an alternative approach. Photosensitizer candidates ranging from compounds of plant origin, food-grade additives, and commercial dyes were screened. These compounds were initially categorized based on its absorption spectra ranging from 250-800 nm wavelengths. The mixture of conidia-photosensitizer candidates was then irradiated with light wavelengths ranges between UV-B (8 µmol/m2/s for 10min) to green, blue, and red (≈120 µmol/m2/s for 30min). Treated samples were inoculated on the surface of potato dextrose agar media and incubated with 18h photoperiod to observe the colony growth, morphology, and intensity over a period of 4 days. Similar experiment was repeated with successful candidates, and germination assays were carried out 6h post-treatment followed by ROS measurement and radical scavenging assays. Preliminary results showed that curcumin, new methylene blue and rose bengal dyes has strong photosensitizing ability in suppressing *B. cinerea* under *in vitro* conditions with blue, red, and green light respectively. The outcomes will be validated further by *in planta* studies.

P3.3-024

EFFICACY OF BIOFUMIGATION WITH ESSENTIAL OILS IN THE CONTROL OF POSTHARVEST ROTS OF NECTARINES

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Text

Nectarines can be affected by many postharvest diseases, leading to production losses. Natural are promising alternatives to pesticides to control storage rots. In this work, the efficacy of biofumigation of essential oils (EOs) through slow-release diffusors was assessed to control postharvest rots of nectarines. A screening test was set up by treating fruits inoculated with *Monilinia fructicola* with EOs of red thyme, fennel, basil, oregano and lemon. Fennel, basil and lemon EOs exhibited the greatest inhibition activity at the end of storage and were selected for setting up a semi-commercial trial, where nectarines were not inoculated. At the end of the storage, all treatments showed a significant rot incidence reduction compared to the control. Quality analyses showed that biofumigation with EOs did non affect nectarine firmness, total soluble solids and titratable acidity. Metabarcoding was used to evaluate the effect of treatments on the nectarine microbiome. The abundance of some fungal genera were modified. Treatments were able to reduce the abundance of *Monilinia* spp. at the epiphytic level, especially in shelf-life. However, basil EO seems to favour the presence of *Penicillium* spp. during shelf-life. Results obtained provide new insights for the development of sustainable strategies for the management of postharvest diseases and the reduction of production losses.

P3.3-025

INHIBITORY ACTIVITY OF COMMERCIAL ESSENTIAL OILS IN VOLATILE PHASE AGAINST BOTRYTIS CINEREA AND MONILINIA LAXA

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Text

Gray mold and brown rot, caused by *Botrytis cinerea* and *Monilinia* spp., are postharvest diseases that produce significant losses during fruit and vegetables storage. Natural alternatives to traditional fungicides are being assessed as part of sustainable strategies for postharvest disease control, including the use of volatile organic compounds (VOCs) form plants and microorganisms. In this study, VOC chambers were used to evaluate the antifungal effect of VOCs from commercial essential oils (EOs) of *Melaleuca alternifolia*, *Origanum vulgare*, *Thymus vulgaris*, *Thymus serpyllum*, *Melaleuca alternifolia*, *Lavandula officinalis*, *Lavandula hybrida*, *Citrus bergamia*, *Rosmarinus officinalis*, and *Cinnamomum zeylanicum* against *B. cinerea*, and *M. laxa*. The tested concentration ranged from 2.82 µL/L to 363,64 µL/L, and mycelial growth was measured. Minimum Inhibitory Concentrations (MICs) against *B. cinerea* were 22.73 µL/L for *O. vulgare*, *T. vulgaris*, and *T. serpyllum*; 181.82 µL/L for *M. alternifolia* and *L. officinalis*; and 363.64 µL/L for the rest of EOs. *M. laxa* MICs were 11.36 µL/L for *O. vulgare* and *T. vulgaris*; 22.73 µL/L for *T. serpyllum*; and 181.82 for the other EOs. Overall, *B. cinerea* was less susceptible to the tested EOs than *M. laxa*. Further in vivo research should be conducted to evaluate the potential use of VOCs from these EOs in the control of gray mold and brown rot.

This work was conducted within the framework of the PRIMA StopMedWaste Project

P3.3-026

INTEGRATION OF TRANSCRIPTOMICS AND PROTEOMICS REVEALS THE INHIBITORY EFFECT OF CARVACROL ON POLYSACCHARIDES METABOLISM OF THE CELL WALL IN ALTERNARIA ALTERNATA CAUSING GOJI FRUIT ROT

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Text

Previous studies showed that carvacrol (CVR) significantly inhibited the mycelial growth of *A. alternata* in vitro and reduced Alternaria rot in goji fruits in vivo. However, the antifungal mechanism of CVR against *A. alternata* has not been elucidated. The present study aimed to explore the antifungal mechanism of CVR against *A. alternata* using RNA-seq and 4D-DIA protein quantification methods. The results showed that most genes and proteins of differential expression were largely matched to carbohydrate metabolites. Further investigation found that the contents of cell wall polysaccharides containing chitin and β-1,3-glucan and the activities of the enzymes related to the biosynthesis of these polysaccharides were significantly decreased by CVR treatment, while the activities of chitinase and the β-1,3-glucanase of degrading the two polysaccharides were increased by CVR treatment. Proteomics and transcriptomics together indicated that changes in cell wall polysaccharides metabolism were associated with the expressions of genes and proteins related to β-1,3-glucan, chitin, mannose, and trehalose. Meanwhile, CVR fumigation accelerates the conversion of ethanol to acetaldehyde by up-regulation of the expression of alcohol dehydrogenase, resulting in the accumulation of acetaldehyde which caused toxicity to the pathogenic fungi. In summary, this research explained the general molecular mechanism of the changes of the cell wall polysaccharides in *A.alterntata* in response to CVR.

P3.3-027

STRATEGIES TO OVERCOME STEM END ROT DISEASE IN PAKISTAN MANGOES, A MAJOR THREAT IN EXPORT MARKETS.

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Text

Stem end rot (SER) a post-harvest disease poses a major threat to mango industry in Pakistan. Efforts were made to understand the infection process of the pathogens involved under Pakistani growing conditions. Fully matured mango fruit cultivar Sindhri were stored in cold storage (12 oC) and at ambient storage (32 oC). After removal of fruits different fungal pathogens were (Lasiodiplodia theobromae, Alternaria alternata, Phomopsis mangifera, Colletotrichum gloeosporioides) were recovered from rotted and decayed mango fruit. On inoculation on healthy fruit L. theobromae produce the largest lesion (63.6 mm) in ambient storage whereas in cold storage again produce largest lesion (44.5 mm). The pathogens are

present as endophytic in developed stems and colonize fruit endophytically through extension of hyphae. Pakistani mango industry is facing a great challenge in post-harvest disease management of mango fruit. For the management of SER fungal pathogens six different fungicides Prochloraz, native, Scholar, Cabrio Top, Tecto®, and Amistar® were evaluated at five different concentrations. Maximum disease inhibition was observed at higher concentrations. When healthy mango fruit was treated with different concentration of Nativo® and cabio Top®, significant disease reduction was observed. In addition to fungicides, plant extracts like Syzygium aromaticum and Moringa oleifera were applied at different concentration of mature healthy mango fruits.

P3.3-028

GENOME-SCALE PHYLOGENETIC AND SYNTENIC ANALYSES REVEAL RELATIONSHIP AMONG MONILINIA FRUCTICOLA, MONILINIA LAXA AND MONILINIA FRUCTIGENA WITHIN SCLEROTINIACEAE

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Text

The most important fungi causing brown rot and blossom blight on fruit trees are *Monilinia laxa*, *Monilinia fructigena* and *Monilinia fructicola*. They are Ascomycetes included in the *Sclerotiniaceae* family, causing worldwide severe losses on stone and pome fruits production in pre and postharvest. The complete draft genomes of *M. fructicola* strain Mfrc123, *M. laxa* strain Mlax316, and *M. fructigena* strain Mfrg269 have been investigated to clarify the evolutionary history of the *Monilinia* genus within *Sclerotiniaceae*. Phylogenomic analyses suggest *M. fructicola* genetically distant from *M. laxa* and *M. fructigena*, although the three species likely share a common ancestor. *Botrytis* and *Sclerotinia* were the closest related taxa to the *Monilinia* genus in the *Junctoriae* section, and all were in a monophyletic lineage strictly related to *Rustroemiaceae*. The syntenic studies confirm the close relationship among the three *Monilinia* genomes and, even though minor, with *Botrytis cinerea* and *Sclerotinia sclerotiorum* species. The coding sequence divergence (measured by Ks values) confirmed that *M. laxa* and *M. fructigena* are phylogenetically closely related each other, while *M. fructicola* is somewhat divergent. The three *Monilinia* genomes were genetically closer to those of *Sclerotinia sclerotiorum* than *Botrytis cinerea*, and *M. laxa* was the closest to the other tested fungi of the *Sclerotiniaceae* family.

This work was partially conducted within the framework of the PRIMA StopMedWaste Project

P3.3-029

CHITOSAN AND OTHER EDIBLE COATINGS TO EXTEND SHELF LIFE, MANAGE POSTHARVEST DECAY, AND REDUCE LOSS AND WASTE OF FRESH FRUITS AND VEGETABLES

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Text
Fresh fruits and vegetables contain high percentage of water and continue metabolic activity after being harvested, resulting in ripening, increased sensitivity to decay-causing fungi, and consequent loss and waste. Edible coatings are prepared from naturally occurring renewable sources and can contribute to reducing waste, respecting environment, and consumer health. Chitosan and other edible coatings (such as shellac, carboxymethyl cellulose, hydroxypropyl methylcellulose, bee wax, and glycerol) form a thin layer surrounding fresh produce that acts as a protective agent, extending shelf life, and have the potential to control their ripening process and maintain nutritional properties of the coated product. Chitosan and other edible coatings can have antimicrobial, film-forming and eliciting activities, that additively or synergistically prevent fungal decay, keep the quality, and reduce fresh product waste.

This work was conducted within the framework of the PRIMA StopMedWaste Project

P3.3-030

ANTIFUNGAL ACTIVITY OF NATURAL EXTRACTS AND ESSENTIAL OILS AGAINST MONILINIA FRUCTICOLA IN VITRO AND AS INGREDIENTS OF PECTIN-BASED EDIBLE COATINGS FOR POSTHARVEST PRESERVATION OF COLD-STORED NECTARINES

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Text
The in vitro antifungal activity of different natural extracts and essential oils against Monilinia fructicola, the causal agent of brown rot of stone fruits, was evaluated as mycelial growth reduction on amended PDA plates. The most effective agents [lemongrass (LG), geraniol (GE), and Commiphora myrrha (MY)] were selected as antifungal ingredients of composite edible coatings (ECs) formulated with citrus pectin and beeswax. ECs were applied in in vivo curative experiments to 'Lucibella' nectarines artificially inoculated about 24 h before with M. fructicola. The EC with 0.2% GE was the most effective, with disease incidence reductions of 80 and 55% after 3 and 4 weeks of storage at 1 °C and 90% RH, respectively. Furthermore, this GE-EC reduced brown rot severity by up to 93% after 3 weeks. The LG-EC (0.4%) also reduced disease severity by 77% after 3 weeks. Regarding fruit quality, all tested ECs

significantly reduced fruit weight loss and maintained higher firmness than control nectarines after 4 weeks at 1 °C plus 3 days at 20 °C, without adversely affecting the fruit physicochemical (titratable acidity, soluble solids content, and volatiles content) and sensory (overall flavor, off-flavors, firmness, and external aspect) quality. Moreover, the MY-EC provided higher gloss than the rest of ECs. These results can contribute to the development of new safe and eco-friendly commercial antifungal ECs to control major diseases and preserve postharvest quality of stone fruits.

P3.3-031

MANAGEMENT OF GUAVA ANTHRACNOSE THROUGH SYNTHETIC FUNGICIDES AND MEDICINAL PLANT EXTRACTS

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Text

Guava (Psidium guajava) is a tropical fruit that is widely cultivated in many parts of the world, particularly in India, Brazil, Mexico, and Southeast Asia. It is rich source of nutrients with various health benefits. Guava is susceptible to range of pathogen that can affect its growth, yield and quality. One of the most common and devastating diseases of guava is anthracnose cause by colletotrichum gloeosporioides. The goal of the current research was to check the efficacy of synthetic fungicides and medicinal plants extract against Guava anthracnose. For this purpose five synthetic fungicides (Chlorothalonil, Mancozeb, Thiophanate-methyl, Azoxystrobin and Difenoconazole) at three concentrations (100, 200, 300 ppm) and five medicinal plants extract (Ocimum sanctum, Datura stramonium, Curcuma longa L, Piper nigrum and Azadirachta indica) at 5, 10, 15% concentrations were evaluated under in vitro conditions using poisoned food technique. Results revealed that among synthetic fungicides difenoconazole was found highly effective with least mycelial growth (8.75mm) followed by Mancozeb, Azoxystrobin, Thiophanate-methyl and Chlorothalonil, while among medicinal plants maximum growth inhibition was recorded by Piper nigrum (13.45mm) at highest 15% concentration followed by Curcuma longa L, Azadirachta indica, Datura stramonium, Ocimum sanctum. The findings of our study suggested that botanical extracts and fungicides could be efficiently used against anthracnose of guava.

P3.3-032

INNOVATIVE SUSTAINABLE TECHNOLOGIES TO EXTEND THE SHELF LIFE OF PERISHABLE MEDITERRANEAN FRESH FRUIT, VEGETABLES, AND AROMATIC PLANTS AND TO REDUCE WASTE: THE EXPERIENCE OF PRIMA STOPMEDWASTE PROJECT

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Text
Postharvest losses of fruit, vegetables, and aromatic plants have high economic impact in the Mediterranean area and contribute to food waste. One of the United Nations Priorities, the ZeroHunger Challenge, consists of cutting food waste by half by 2030. StopMedWaste Project (2020-2024) see the interaction of 8 Research Units (UNIVPM, CUT, UNIBA, INRAT, UNITO, UE, IVIA, IKACHEM and DECCO) to join efforts to extend the shelf life of fresh fruit, vegetables, and aromatic plants by applying physical means, natural compounds and biocontrol agents. These treatments are being applied in the laboratory, under semi-commercial conditions, and in the packinghouses. The effects of these treatments on fruit quality, decay, and development of foodborne pathogens are under monitoring during storage, transportation and shelf life, to define their impact on food waste. Results achieved till now showed the beneficial effects of treatment with physical means (ozone, electrolysed water, UVc), natural compounds (chitosan, essential oils, bicarbonates and other antifungal edible coatings), and biocontrol agents in improving the quality of fresh fruit (citrus, pomegranates, peaches, nectarines, apricots, plums, sweet cherries, strawberries, table grapes), vegetables (tomatoes, cucumbers) and aromatic plants (spearmint, basil), that allowed to keep quality and reduce decay, and then waste.
This work was conducted within the framework of the PRIMA StopMedWaste Project

P3.3-033

MANAGEMENT OF POSTHARVEST BROWN ROT OF PEACHES AND NECTARINES BY NATURAL COMPOUNDS AND BIOCONTROL AGENTS

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Text
Stone fruits are susceptible to postharvest diseases caused by fungal pathogens able to cause losses and waste. The main pathogens of these crops are *Monilinia* spp., which cause brown rot. The effectiveness of different formulations based on natural compounds and biocontrol agents to manage brown rot on peach fruits (cvs +5Tardibelle and Extreme 486) and nectarines (cv Carene) were evaluated. Commercial formulations of chitosan, sweet orange essential oil, *Bacillus subtilis*, *Bacillus amyloliquefacens*, *Metschnikowia fructicola*, *Aureobasidium pullulans*, COS-OGA, a mixture of thymol, geraniol and eugenol, *Swinglea glutinosa* extract and nettle extract were applied by dipping fruit, using as a reference a synthetic fungicide (fludioxonil) and an untreated control. Fruits were immersed for 30

seconds in the solutions, stored at 2°C for 7-14 days and exposed to shelf life for 10 days. A significant reduction of disease incidence was observed in fruits treated with fludioxonil in all trials. Among alternative compounds, tendency toward disease reduction on +5Tardibelle was observed with application of chitosan and of *B. subtilis*, and on Extreme 486 with application of *Aureobasidium pullulans*. These results suggest that the use of alternative compounds requires a careful evaluation of applicative conditions for different cultivars, and proper application protocols need to be evaluated.

This work was conducted within the framework of the PRIMA StopMedWaste Project

P3.3-034

IN VITRO ANTIMICROBIAL ACTIVITY OF CHITOSAN HYDROCHLORIDE AND COS (CHITO-OLIGOSACCHARIDES)-OGA (OLIGO-GALACTURONIDES) ON FIELD AND POSTHARVEST FUNGAL PATHOGENS

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Text

Postharvest infections of fresh produce can cause major economic losses and food insecurity. Synthetic pesticides used in the management of fungi can have detrimental impacts on the environment, then sustainable innovative strategies are required. The use of natural compounds such as chitosan, a natural biodegradable biopolymer, in plant protection is promoted. In this study, in vitro antifungal activity of chitosan hydrochloride (CH; 100%) and COS (chito-oligosaccharides)-OGA (oligo-galacturonides (COS-OGA; 1.25%) was evaluated by monitoring mycelial growth of *Alternaria alternata*, *A. brassicicola*, *Botrytis cinerea*, *Monilinia laxa*, *M. fructigena*, and *M. fructicola*. A list of concentrations of CH and COS-OGA was prepared in PDA. The inhibition increased with the concentration for both formulations, and a different degree of sensitivity was observed. The most sensitive pathogen was *M. fructigena*, that was completely inhibited in the growth at the 0.5%. A continuous decreasing sensitivity to both chitosan formulations was observed by *M. laxa*, *M. fructicola*, *A. alternata*, *B. cinerea* and *A. brassicicola*. At the same concentrations, COS-OGA showed an antimicrobial activity slightly lower than CH. Our results demonstrated antimicrobial efficacy of these compounds and their potential use as innovative sustainable compounds in plant protection. In vivo studies are required to confirm in vitro results.

This work was conducted within the framework of the PRIMA StopMedWaste Project

P3.3-035

PHYSIOLOGICAL CHANGES INDUCED AFTER OZONE TREATMENT ON PEACH FRUITS

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Text
The conservation of high-quality standards for prolonging the shelf life of fruit is a priority for horticultural products. Ozone has been suggested to be an environmentally-friendly method for postharvest storage of fruits, and the effects of ozone on their physiology need to be clarified. This study investigated the effects of postharvest treatment with ozone on peach fruits (*Prunus persica* L.), cultivars 'Summer Royal' and 'Extreme 486' grown in orchards in Marche region (Central-Eastern Italy) in 2020 and 2021. The peach fruits after harvest were stored at 4 °C with or without ozone treatment (45 ppb continuously in 2020 year, and in alternating between 50 and 200 during the day and night in 2021 year) for 10 and 20 days. After treatments the peach samples were collected after 0, 24, 48, 72, 96 and 168 hours of shelf life. The expression of selected target genes involved in signalling pathways regulating plant defence, pathogenesis-related protein, cell wall-degrading enzymes, oxidative and abiotic stress, phenylpropanoid pathway and fruits ripening were assessed by real-time quantitative polymerase chain reaction (RT-qPCR). The result suggests the ozone treatments affects the fruit physiology mainly involved on ripening process and oxidative stress. This study represents a useful approach to understanding molecular basis of peach physiology change induced by ozone treatment.
This work was conducted within the framework of the PRIMA StopMedWaste Project

P3.3-036

EFFECTS OF OZONE EXPOSURE ON BROWN ROT OF PEACH FRUITS IN COLD STORAGE

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Text
Pre and postharvest losses caused by fungal decay affect fresh fruit production globally. Stone fruits are susceptible to brown rot caused by *Monilinia* spp. We evaluated the effects of two sprayers (traditional and innovative with flow control) for preharvest treatments, and postharvest application of gaseous ozone in storage temperature for the control of brown rot. The experiment was carried out in Marche Region, Central-Eastern Italy, on peach cv Royal Summer and Extreme 486, sprayed with fungicides (tebuconazole in 2019 and 2020 and boscalid+pyraclostrobin in 2021) in the field. Immediately after harvest, fruits were stored at 4 °C with or without ozone treatment (45-50 ppb and 200 ppb during the day and night, respectively) for 10, 20 and 25 days. Fruits were removed from cold storage, transferred at 20 °C and decay was measured daily during 10 days shelf life. Concentration of 200 ppm used during the night on cv Extreme 486 induced slight phytotoxic effects on fruits. There were no significant differences between the two sprayers tested for preharvest treatment. Ozone treatment at 50 ppb day/200 ppb night was effective in controlling *Monilinia* spp. The application of ozone can contribute to the management of postharvest diseases of peaches. Further research on different cultivars and ripening stage should be conducted to determine an appropriate concentration of ozone for storage of peaches.
This work was conducted within the framework of the PRIMA StopMedWaste Project

P3.3-037

IN VITRO AND IN VIVO EFFECT OF NATURAL SALTS TO CONTROL POSTHARVEST CITRUS GREEN MOLD DISEASE CAUSED BY PENICILLIUM DIGITATUM IN MOROCCO

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Text

The green mold caused by the wound pathogen *Penicillium digitatum* causes more severe post-harvest citrus damage with significant economic impact.The present study aimed to evaluate the *in vitro* and *in vivo* antifungal activity of certain salts, including potassium sorbate, sodium benzoate, sodium tetraborate and sodium bicarbonate against P. digitatum. In vitro, the PDA medium was supplemented with salt solutions to obtain the final concentrations of 0, 500, 1000, 1500 and 2000ppm before inoculation. The in vivo tests were carried out by inoculating the fruits before or after treatment with salts. The fruits were therefore immersed in saline solutions of 0,2% or 4% for 2min. *In vitro* results showed that potassium sorbate reduced mycelial growth of *P. digitatum* by 71% at 500 ppm, with an IC50 = 2.75 ppm. Complete inhibition of *P. digitatum* was observed with sodium bicarbonate and sodium tetraborate at 1000ppm. The *in vivo* curative treatment showed significant antifungal activity of all the salts tested. However, potassium sorbate and sodium benzoate completely inhibited the development of green mold on ‘Valencia late’ oranges at 2% concentration. This is an efficacy similar to that obtained by the conventional fungicide "Imazalil" used as a positive control. For preventive treatment, sodium tetraborate and sodium benzoate inhibited the development of *P. digitatum* by 67 and 79% respectively at the concentration of 4%.

P3.3-038

DEVELOPMENT OF CASSAVA STARCH-BASED COATINGS FUNCTIONALIZED WITH ANTIFUNGAL AGENTS AS AN ALTERNATIVE POSTHARVEST TREATMENT TO IMPROVE QUALITY AND EXTEND SHELF LIFE OF CAVENDISH BANANAS

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Text

Tropical fruits are traded worldwide with Latin America as a major global exporting force and Europe and Asia as giant and growing import markets. However, postharvest diseases caused by fungi (e.g. crown rot in bananas) represent important losses for exporters along

the commercialization chain. At present, these problems are mainly treated by the postharvest use of traditional (rather toxic) fungicides that are increasingly restricted in major international markets. We have developed starch-based water-soluble coatings that generate a systemic response in fruits by regulating fruit's respiration process and the incorporation of antifungal agents. Formulations with a reduction of traditional fungicides and with bio-based antifungal agents were tested in vitro and in vivo with the two main strains causing banana crown rot: Fusarium sp and Colletotrichum sp. The in vitro evaluation for the coatings with 60% reduction of traditional fungicide resulted in an antifungal effect equal to the traditional treatment; while bio-based agent formulation achieved a colony-forming units inhibition around 70% for each strain. For the in vivo evaluation, bananas were stored for 20 days at 15°C and 90% RH, matching with typical shipping conditions. Afterward, destructive and non-destructive tests were applied to banana clusters. In all formulations, a reduction of crown rot was observed, and the ripening process was slowed.

POST-HARVEST - Part 3: Eco-epidemiological perspectives generating new concepts on postharvest diseases and mycotoxins

C4.6-1

ONE SMALL MOLECULE WITH BIG BIOLOGICAL IMPACTS: NEW ROLES FOR PATULIN IN HOST-MICROBEPLANT INTERACTIONS DURING BLUE MOLD DECAY OF APPLE FRUIT

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Text

Patulin, a mycotoxin produced by Penicillium spp. during postharvest pome fruit decay, causes acute and chronic effects in humans, withstands pasteurization, and is not eliminated by fermentation. While much is known about the impact of patulin on human health, there are significant knowledge gaps concerning the effect of patulin in postharvest fruit-pathogen-microbe interactions. Inoculation of six apple cultivars with purified patulin reproduced some blue mold symptoms which were cultivar-independent and dose-dependent. Identical symptoms were also observed in pears, Arabidopsis, and mandarins showing the toxin is non-host specific. Six Penicillium spp. isolates exposed to exogenous patulin exhibited delayed germination after 24 hours, yet all produced viable colonies after 7 days. However, four common postharvest fungal pathogens were completely inhibited by patulin, suggesting its role to allow the blue mold fungus to dominate the postharvest infection court. Using clorgyline, a broad-spectrum pump inhibitor, it was demonstrated that active efflux plays a role in Penicillium auto-resistance to patulin. The work presented contributes new knowledge concerning patulin auto-resistance in Penicillium, its function in the decay process, and the toxins exclusionary role in fungal-fungal interactions. Our findings provide seminal work to

explore the mechanisms of patulin action with the long term aim of developing toxin and decay mitigation strategies.

C4.6-2

PREHARVEST FACTORS ASSOCIATED WITH GRAY MOLD DEVELOPMENT IN EUROPEAN PEARS

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Text

Postharvest gray mold rot in European pears is caused by *Botrytis cinerea*. The infection by *B. cinerea* in storage leads to huge economic losses to pear packers. To understand pear fruit developmental stages and their association with *B. cinerea* colonization, we designed this study in a commercial orchard in southern Oregon. In addition, we investigated the impact of weather conditions and fungicide sprays on pear infection timing(s). Thirty blossom and fruit samples were collected each during petal fall, fruit set, fruitlet, mid-summer and commercial harvest. The samples were plated on half strength PDA media and data on *B. cinerea* isolation was recorded. In year 1 and year 2, the highest isolation of *B. cinerea* was observed during petal fall and fruit set stages (40% - 73%). The samples collected during fruitlet through commercial harvest had the least *B. cinerea* colonization in both years (4% - 26%). In first year, three precipitation events during the early season resulted in three applications of fungicides and in second year, there were four applications. Relative humidity during early season ranged from 40 to 92%, and during summer months, it ranged from 32 to 65%. The combination of fungicides application during earlier stages of fruit development and drier environment conditions during later stages resulted in least *B. cinerea* colonization during commercial harvest. This subsequently resulted in no gray mold rot incidence in the storage in both years.

C4.6-3

EPIDEMIOLOGY OF GRAIN CONTAMINATION WITH ZEARALENONE AND DEOXYNIVALENOL

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Text

Zearalenone (ZEA) and deoxynivalenol (DON), produced by the fungus Fusarium graminearum and related species, are two common mycotoxins that affect the quality, market value, and utilization of cereal crops such as corn and wheat. DON is a type B trichothecene that causes vomiting and feed refusal, among other illnesses, whereas ZEA, which causes various types of cancers and reproductive disorders in mammals, is a class III carcinogen that belongs to the xenoestrogen group of compounds. In wheat, grain contamination with ZEA and DON occurs in the field in association with F. graminearum infection and Fusarium head blight

(FHB), a disease that affects the spikes. DON and ZEA levels may increase in storage and are influenced by complex interactions among host-, pathogen-, and environment-related factors. Efforts to predict grain contamination with, and minimize postharvest losses due to, ZEA and DON must begin in the field, and require a thorough understanding of factors affecting contamination. Results from control-environment and field experiments will be presented and discussed on FHB-DON and FHB-ZEA relationships, the influence of temperature, relative humidity, and rainfall on these relationships, and factors associated with the conversion DON to DON-3-glucoside, a masked form of the toxin that often goes undetected while maintain its toxic effects. In-field, harvest, and postharvest strategies for mitigating DON and ZEA contamination of grain will also be discussed.

C4.6-4

INOCULUM DYNAMICS AND ENVIRONMENTAL FACTORS ASSOCIATED WITH THE PREHARVEST CONTAMINATION OF CITRUS FRUITS BY GEOTRICHUM CITRI-AURANTII

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Text

The aim of this study was to establish the methodology and determine the relationships between climatic factors and contamination of citrus fruit by the soil-inhabitant pathogenic fungus Geotrichum citri-aurantii. This pathogen causes sour rot decay of citrus. The field trial was set up in a ‘Murcott’ mandarin experimental orchard in Salto, Uruguay. Fruit sampling was conducted weekly from March to June 2022. Six fruits from 2-3 trees per experimental unit were arbitrarily selected within 50 cm from the orchard floor. Rainfall, air temperature, wetness and relative humidity sensors were placed in the center of the orchard. Wind sensors were placed at each sampling location. The mean number of epiphytic spores of G. citri-aurantii per fruit (abundance) and the proportion of fruits positive for G. citri-aurantii (incidence) were determined in the laboratory by washing and plating serial dilutions. Climatic factors were related to G. citri-aurantii abundance or incidence through generalized linear models specifying the normal or binomial error structure, respectively. For both incidence and abundance, a significant negative relationship was observed with rainfall and wind speed. In contrast, the relationship was significantly positive for wind gusts. The variables wetness and maximum temperature were negatively and significantly related only to abundance. Further field trials are necessary to validate the models and confirm the results obtained in this first year of the study.

C4.6-5

ASSOCIATED TRADE BARRIERS WHILE MANAGING POSTHARVEST DISEASES IN SWEETPOTATO

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Text

Over the last decade, exports of US grown sweetpotatoes have steadily increased to meet the growing demand in Europe and other countries. Persistence and re-emergence of soilborne pathogens threatens sweetpotato success as a profitable export for US producers, who supply a large share of the global sweetpotato market. Diseases lead to severe yield loss without cultural and chemical management. However, recent changes in fungicide residue tolerances for imported crops by the European Union have reduced chemical control options to manage pathogens. Ceratocystis fimbriata is a fungal pathogen that causes black rot, one of the most devastating diseases afflicting sweetpotatoes. The immense infection potential is due to C. fimbriata being transmissible to sweetpotatoes anytime in its production cycle, from seed roots to postharvest storage, which can result in annual losses as high as $150 million in North Carolina sweetpotato production alone. In this session we will discuss trade barriers associated with sweetpotato production using a black rot epidemic as a case study, and ongoing trans-disciplinary research and extension efforts to ensure the long-term sustainability of US sweetpotato exports.

C4.6-6

RE-EMERGING DISEASE: THE SOUR ROT IRRUPTION ON PEACH PRODUCTION

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Text

In the recent years, stone fruit producing areas in Spain have experienced an outbreak of sour rot (SR) disease affection probably related to climate change, while prior to 2016 SR incidence was low or absent. The main microorganism responsible for SR is *Geotrichum candidum*, and symptoms on fruit are described as a white layer of mycelial growth on fruit with sour smell. Moreover, currently, there are no effective treatments for SR control in stone fruit. In this study we investigated: 1) Potential sources of inoculum in 4 stone fruit orchards; 2) Dispersion of G. candidum artificially inoculated in soil at different conditions of wind, agricultural crop management, or both; and 3) Effect of *Trichoderma asperellum* (strain T34) to reduce the concentration of *G*. *candidum* inoculum in the soil. Our results indicated that *G*. *candidum* was present in all field samples evaluated, and soil presented the highest concentration. Furthermore, we observed that the inoculum of *G*. *candidum* was dispersed at all conditions evaluated, pointing out the dust as an important vehicle for dispersion. Finally, we found that T34 reduced the level of *G*. *candidum* inoculum in the soil in 76% within the two days after inoculation. After 7 days, the reduction of *G*. *candidum* was 47%, and this reduction remained stable for one month. Our study has provided a potential crop management tool to control SR by reducing the main source of inoculum and *G. candidum* dispersion .
Funding:Operació 16.01.01.PDR2014-2022

P4.6-001

SUSCEPTIBILITY TO SOUR ROT (GEOTRICHUM CITRI-AURANTII) IN MANDARIN AND HYBRIDS IN ASSOCIATION WITH FRUIT QUALITY PARAMETERS

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Text

The objective of the work was to evaluate the susceptibility to sour rot (Geotrichum citri-aurantii) in mandarins and hybrids and to explore the association with fruit quality parameters. Disease severity (mean lesion size in 12 fruit) was analysed in 30 samples from 11 cultivars distributed in 7 experiments. Each cultivar was tested at least twice. For each experiment, an analysis of variance between cultivars was performed and the difference between means was evaluated by Tukey's test. Fruit firmness, colour, juice content (%), acidity, and total soluble solids were analysed for all samples. The association between severity and quality parameters, variety, and sample origin (grove) were analysed using a multiple linear model. Significant differences between cultivars were found in each experiment. Cultivars were classified into three groups using an arbitrary scale (more, moderately, and less susceptible). According to our result, Clementine cv "Clemenules" was the most susceptible cultivar, Mor cv. "Moria", Montenegrina, and Ellendalle were moderately susceptible, and Orri, Nankou, and Clementine cv. "Clemendor" were less susceptible. In Satsuma cv. "Owari", and hybrids Tango, Nova, and Afourer mean lesion size varied by experiment and susceptibility could not be established. A total of 58.41% of the variability was predicted by de model. The best quality parameters for predicting aggressiveness were de variety and firmness.

PROGRESS IN DISEASE CONTROL - Part 2

F6.1-1

INTEGRATING DIAGNOSTIC TOOLS TO PREDICT DISEASE PRESSURES IN WINTER WHEAT AND REFINE THE APPLICATION OF BIOLOGICAL FUNGICIDES

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Text
Approximately 70% of fungicide applied on European wheat fields is primarily aimed towards the management of the fungal disease Septoria tritici blotch (STB), although the uptake of biological fungicides (biofungicides) in field-based cropping systems remains poor. The aim of this study was to integrate diagnostic tools into the fungicide decision process to improve disease prediction and refine biofungicide application. Another objective of the study was to investigate the mechanisms of a biofungicide in STB control.
A winter wheat field trial was conducted using traditional agronomy-based risk prediction and a novel method of disease forecasting- spore trapping and weather-based risk modelling- to influence the application rates of synthetic and biological treatments. The effects on STB control were assessed using visual disease assessments, while the data from molecular analyses (qPCR) of spores and symptomless leaves was used to retrospectively forecast disease severity in the crop.
In synthetic fungicide treatments, the diagnostics-based programme showed similar results to the agronomist-lead programme. However, the biofungicides did not effectively control STB, with significantly lower control in the diagnostics programme, prompting an investigation into the biological mechanisms for disease control, or lack thereof. This study will explore the effect of the biofungicide at various stages of STB ingress on the wheat phylloplane and STB control under greenhouse conditions.

F6.1-2

SITE-SPECIFIC SOIL PEST MANAGEMENT IN CALIFORNIA STRAWBERRY & VEGETABLE CROPPING SYSTEMS

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Text
Effective management of soilborne pathogens is essential for commercial strawberry production in California. To minimize the impact of these pathogens a site-specific management program is under study that tracks pathogen load, disease incidence, and crop productivity for strawberry and vegetable crops grown in rotation to determine fumigant application rates within a field. Disease risk across the field is assessed by TaqMan soil quantification assays for three of the major lethal pathogens of strawberry (*V. dahliae*, *M. phaseolina* and *F. oxysporum* f. sp. *fragariae*) coupled with prior season disease incidence.

Management decisions on the type of fumigant, a risk-based assessment of rates for different areas of a field, and application methods are based on this information. Plant vigor and disease incidence is monitored during the growing season on a field wide and individual plant basis by drone flights every 1-2 weeks and a tractor mounted sensor system. Two GPS-enabled systems are used to collect precision yield data with cumulative yield maps generated on a regular basis. Correlations between drone/tractor sensor data and yield are explored to evaluate efficacy of the fumigation treatment and develop yield prediction models. An economic analysis of precision pathogen management provides information to support grower decision making. Results from field trials indicate that variable rate fumigant application based on risk can effectively manage disease and maintain yield.

PROGRESS IN DISEASE CONTROL - Part1

C4.1-1

VINE DISEASES AND INTEGRATION OF BIOSOLUTIONS IN CONTROL STRATEGIES

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Text

Downy mildew and powdery mildew are the main diseases affecting grapevines, in terms of impact on production and number of applications during the season. French viticulture is evolving to develop new production models based on agroecological approach for the future. This innovative approach is based on different pillars: resistant varieties, soil management and biosolutions. Biosolutions may include biocontrol (registration as for conventional pesticides) as well as biostimulants. Future strategies may need to combine both categories to achieve complete IPM. Recent work from ITV, the French technical institute of vine, has mostly explored the implementation of biocontrol in vineyards. The relatively low and variable level of efficacy of available products first implied to combine them with lower dose rate of conventional fungicides, which is only a first step to sustainable and resilient IPM strategies. Further work aims at developing strategies based on decision rules (by using DSS) and characteristics of spray products. Such approach strongly relies on spraying quality which has to be perfect (face by face application at all vine stages). Based on the most recent results achieved from trials distributed throughout the French vineyard, this presentation will demonstrate that some of these strategies are highly promising to achieve IPM and identify determinants of its efficacy.

C4.1-2

OPTIMIZING FIELD BASED CONTROL OF PHYTOPHTHORA CAPSICI: USE OF MULTIPLEX DROPLET DIGITAL PCR TO QUANTIFY BIOLOGICAL AND PATHOGEN POPULATIONS

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Text

Phytophthora capsici is an oomycete pathogen of vegetable crops with worldwide distribution and serious economic impact. The pesticides currently in use to control P. capsici have limited efficacy, safety concerns and public perception issues. Foretryx®, with active ingredients Trichoderma asperellum and Trichoderma gamsii, has been reported to effectively reduce the severity of phytophthora root and crown rot. The objective of this study was to control phytophthora root and crown rot in pepper by developing integrated pest management practices that incorporate the biofungicide, Foretryx®. Field based experiments in 2021 and 2022 studied the effect of biofungicide, bed type, mulches, and frequency of irrigation on root and crown rot of pepper. Results showed a significant effect of bed type and biofungicide applications in both years. However, the application of Fortryx® alone significantly reduced the incidence of the disease, as compared to the mock-treated plants, but only in 2021. A novel multiplex droplet digital PCR (ddPCR) assay was developed to analyze populations dynamic of T. asperellum, T. gamsii and P. capsici, associated with each treatment. The ddPCR analyses provided population numbers for the pathogen and biologicals in each field season, providing an indication on the apparent result discrepancy between years 2021 and 2022. We expect that this novel technology will have great value in biocontrol management practices of plant pathogens.

C4.1-3

DEVELOPMENT OF MYOSIN INHIBITOR TO CONTROL RICE BLAST AND FALSE SMUT

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Text

Rice blast and false smut caused by Magnaporthe oryzae(M. oryzae) and Aspergillus oryzae(A. oryzae) respectively cause the devastating loss of rice yield worldwide. At present, two diseases mainly rely on chemical agents for control. Phenamacril is a self-developed fungicide in china and it is proved to be a myosin-5 inhibitor. However, phenamacril only control some species of Fusarium and it has no obvious inhibitory activity against other pathogens. This study is aim to develop new myosin-5 inhibitors to control rice blast and false smut. Firstly, we found that, among the pathogens sensitive and insensitive to phenamacril, the type of residue 375 is only the difference in myosin-5 drug sensitivity site. Second, phenamacril had no inhibitory activity on ATPase of myosin-5 from M. oryzae(MoMyo5), conversely, the ATPase activity of K375M that comprised mutagenesis of K375 to M in MoMyo5 was dramatically inhibited. Next, the residue 375 was the only

difference in crystal structure of MoMyo5 and myosin-5 from F. graminearum. In the sight of K375 in MoMyo5, we designed and synthesized a series of compounds. Ultimately, YJY-22 showed the best activity and its median effective concentration on M. oryzae was 0.5μg/mL, more relevantly, its control effect was equivalent to tricyclazole in the field. YJY-22 also inhibited false smut with high activity. Our discoveries laid a theoretical foundation for the creation of targeted fungicides based on amino acid differentiation in a target.

C4.1-4

FUNGICIDE SENSITIVITY OF COLLETOTRICHUM SPECIES CAUSING BITTER ROT ON APPLE IN THE MID-ATLANTIC UNITED STATES

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Text

Bitter rot of apple has triggered losses for apple growers in the Mid-Atlantic United States. We sampled > 500 apples with bitter rot obtained from 38 orchards across PA, VA, MD, DE, and OH. The causal species listed in decreasing abundance were identified as *Colletotrichum fiorninae*, *C. chrysophilum*, *C. noveboracense*, *C. siamense*, *C. fructicola*, *C. henanense*, and *C. gloeosporioides* sensu stricto. A sample of 220 isolates plus 32 reference isolates were tested in an initial fungicide sensitivity screen. A subsample of isolates was tested to obtain EC50 and EC25 values for 22 fungicide active ingredients from FRAC groups 1, 3, 7, 9, 11, 12, and 29. These fungicides varied widely in efficacy both within and between FRAC groups, with our *Colletotrichum* isolates largely insensitive to many of the active ingredients. Comparisons of our *in vitro* results with field trial results conducted in several Eastern U.S. locations suggested that EC25 values (concentrations that reduce growth by 25%) are better predictors of fungicide efficacy in normal field conditions than EC50 values. Benzovindiflupyr (FRAC 7), pyraclostrobin (FRAC 11), trifloxystrobin (FRAC 11), fludioxonil (FRAC 12), and fluazanim (FRAC 29) controlled disease most effectively in the field. Little resistance was found; however, a few *C. siamense* isolates were resistant to FRAC groups 1 and 11 with confirmed β-tubulin E198A and cytochrome-b G143A mutations, respectively.

C4.1-5

CAN OLIGONUCLEOTIDES BECOME AN ALTERNATIVE TO CONVENTIONAL FUNGICIDES FOR PLANT DISEASE CONTROL?

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Text
Botrytis cinerea and Podosphaera xanthii, the causal agents of the gray mold and the cucurbit powdery mildew diseases, respectively, are one of the main limiting factors of horticultural crops production worldwide, consuming up to 40% of fungicides in its control. However, these fungi have been categorized by the Fungicide Resistance Action Committee as phytopathogens with a high risk for fungicide resistance development, a fact that has been demonstrated in our country. In addition, and according to the "farm to fork" strategy of the recent European Green Deal, the diversity of fungicides available to growers will be reduced by 50% in 2030. For this reason, alternative control tools and molecules with fungicide activity are needed. In our research group, we intend to check if the efficacy of the emerging RNA interference (RNAi) strategy, called "spray-induced gene silencing" (SIGS), could be a valid sustainable solution and an alternative to the use of conventional fungicides for the control of B. cinerea and P. xanthii. For this purpose, several double-stranded RNA (dsRNAs) have been designed against targets genes involved in the virulence/pathogenicity of both pathogens. To improve the application of these oligonucleotides in the field, their encapsulation to create nanoparticles is being carried out. If we succeed, new molecules with fungicidal action, could be included to obtain a sustainable plant protection control programs in the field.

C4.1-6

IDENTIFICATION AND UTILIZATION OF ANTIFUNGAL AND DEFENSE-STIMULATING MOLECULES FOR ASIAN SOYBEAN RUST CONTROL

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Text
Soybean is one of the most important crops worldwide. Its major foliar disease is Asian soybean rust (SBR) that is caused by the fungus Phakopsora pachyrhizi. Because elite varieties with resistance to all isolates of P. pachyrhizi are not available, current management strategies almost exclusively rely on the utilization of synthetic pesticides. To reduce their application while preserving crop productivity, alternative SBR control measures are needed. Here, we show that nonhost resistance-associated coumarins and phylloplane proteins suppress rust spore germination and illustrate strategies for their utilization. We provide evidence that foliar application of coumarins and combinatorial overexpression of specific coumarin biosynthesis genes in transgenic soybean plants reduces their susceptibility to SBR. Furthermore, screening for molecules which prime the plant immune system for augmented stress responses disclosed natural and near-natural compounds that boost soybean’s resistance to SBR. Combining strategies with differing, but complementary mode of action may provide sustained protection of soybean from SBR.

F4.1-1

COMBATING ONION BACTERIAL DISEASES WITH PATHOGENOMICS TOOLS AND ENHANCED MANAGEMENT STRATEGIES

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Text

The ‘Stop the Rot’ project (https://alliumnet.com/stop-the-rot/), funded by the USDA National Institute of Food & Agriculture Specialty Crops Research Initiative, aims to help onion growers reduce losses to bacterial diseases through understanding interactions of the host, pathogens, and environment. Outcomes include improved diagnostic tools and enhanced bacterial disease management strategies. Surveys of >130 locations in 2020-2021 generated isolates of 116 bacterial genera from onion foliage and bulbs in seven regions of the USA, with distribution and pathogenicity varying across regions. Very few strains caused symptoms in onion bulb scale pathogenicity tests. Pantoea, Pseudomonas, Burkholderia, and Enterobacter were the most prevalent genera. Microbiome analyses revealed different complex bacterial communities in asymptomatic vs. symptomatic bulbs. Genomic analyses of Pantoea agglomerans strains showed some carry the HiVir gene cluster associated with virulence to onion. Copper tolerance genes found in ~50% of sequenced P. agglomerans isolates might explain the poor efficacy of copper bactericides in many trials. Trials of bactericides and irrigation, fertility, cultural, and post-harvest practices, evaluated across the USA, demonstrate that irrigation timing and method, and late-season cultural practices can be optimized to reduce losses to bacterial diseases. Economic evaluations of results ensure recommendations are practical, viable, and financially sustainable.

F4.1-2

TEN YEAR OF PSTS10: A PERSPECTIVE ON RECENT EVOLUTIONS OF FRENCH POPULATIONS OF PUCCINIA STRIIFORMIS F.SP. TRITICI (CAUSE OF WHEAT YELLOW RUST)

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Text

Wheat yellow rust, caused by *Puccinia striiformis* f.sp. *tritici* (*Pst*), is an important disease in France and in Europe. Ten years ago, in 2013, the PstS10 genetic group (Warrior (-)) was detected in France for the first time and became in a short time the dominant genetic group in this area. Its emergence has been associated with the breakdown of different resistant cultivars. In this work, we propose a summary of main evolutions observed in the past decade considering different datasets. We first illustrate the evolution of *Pst* population (based on 1626 pathotyped samples), with 4 variants of PstS10 detected in France since 2013 and the evolution of their frequencies. We then analyse the behaviour of French cultivars exposed to *Pst* at seedling stage under controlled conditions (340 cultivars) and at adult stage in the field (355 cultivars). We first analyse datasets individually, illustrating major tendencies. We then show how combining these different datasets can provide information on possible sources of resistances and their durability. For example, comparing seedling and field behaviour could help detecting adult resistance. Associations between variations in variant frequencies and variations of cultivar resistance level in the field could suggest the presence of specific resistances that could be further investigated. Finally, we expose key perspectives of further investigations aiming at improving the resistance of French cultivars towards recent populations of *Pst*.

P4.1-001

NANOPARTICLES OF CHITOSAN, ANTIOXIDANTIS AND ORGANIC ACID AND THEIR NATIVE ON FUNGI CAUSING ROOT ROT OF GROWING CUCUMBER PLANT IN GREEN HOUSES

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Text

Abstract

Root rot disease of cucumber, is the common epidemic fungal diseases causing significantly losses of cucumber plants and their productivity during growing winter season under plastic houses are wilt and root rot diseases. Several nanoemulsions formula of nanoparticles of chitosan, hydroqunone, sorbic and propionic acids compare their native materials were prepared and tested against mycelial linear growth, morphology, of highly pathogenic isolates of fungi causing root rot disease of cucumber Fusarium oxysporum and Fusarium solani. Different rates of nanoemulsion formulations and application methods as soil drench before and after cultivation in plastic houses were tested. Data obtained indicated that application of nanoemulsion formulations of chitosan/hydroquinone by the rate 0.5% as soil drench of transplantings 2 days before cultivation in greenhouse it highly suppress wilt and root rot syndromes on cucumber plant and highly significantly increased morphological characters such as plant hight, fresh weight and yield fruits. So, nanoparticles as eco-friendly agents of environmental resources will be consideration in programme as alternative fungicides for controlling plant diseases and enhance plant growth

and productivity.
Keywords: nanoparticles, chitosan, fungi, root rot, cucumber

P4.1-002

TRANSGENIC CITRUS EXPRESSING A TRUNCATED ONCOCIN ANTIMICROBIAL PEPTIDE SUPPRESSES ASIAN CITRUS PSYLLID (DIAPHORINA CITRI) AND REDUCES CITRUS CANKER

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Text
The Asian citrus psyllid (ACP) transmits the bacterium Candidatus Liberibacter asiaticus (CLas), the putative cause of citrus Huanglongbing disease (HLB), the most serious threat to citrus production worldwide. A 10 amino acid peptide derived from the Oncocin antimicrobial peptide was evaluated for its ability to control HLB in Carrizo trifoliate citrus through transgenic expression. Detached leaf studies demonstrated up to 80% mortality of CLas+ psyllids when fed for 7 days. Whole plant studies demonstrated a significant delay in nymph development and reduction in nymphs (number/shoot) over 14 days for transgenic cloned lines (TCL) as compared to non-transgenic controls. TCL # 48 caused the greatest delay in nymphal instar development, while also reduced nymphal emergence by 60.4%. TCL #14 reduced insect emergence from nymphs by 54%. The mortality rate for ACP in transgenic plants individually caged with adult psyllids for two weeks was 10X higher than the mortality rate for controls. The antimicrobial effect of oncocin was confirmed by challenging transgenic leaves with citrus canker (Xanthomonas citri). Canker symptoms were reduced >10X in some TCLs compared to the controls. Based on these results, it can be concluded that transgenic expression of the truncated oncocin might provide commercially viable tolerance to HLB by inducing mortality in both the insect vector and the disease associated bacteria, and that it may also provide tolerance to citrus canker disease.

P4.1-003

CAN RAPID DETECTION METHODS HELP TARGET PATHOGEN CONTROL MEASURES?

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Text
Early detection of pathogens is crucial for enabling the deployment of interventions and can

contribute to reducing synthetic pesticide use, and facilitating more effective use of bio-pesticides.
Vertical farms (VF) have potential to address challenges around global food security and sustainable crop production, in particular growing nutritious crops more closely to where they are consumed. They provide an ideal environment for the proliferation of some pathogens due to highly intensive growing conditions. Disease risk is mitigated by growing short cycle crops, to achieve production in the presence of pathogens. Production of longer cycle and more nutrient dense crops may be limited if sustainable disease control cannot be achieved. In this paper we will present data on remote imaging technologies and machine learning applied to VF grown crops for the detection of biotic and abiotic stress ahead of visual symptoms, facilitating effective interventions. We will also discuss rapid DNA diagnostic methods based on automated disease detection and high throughput sequencing (HTS), exploring how these tools can better target fungicide applications in arable field crops.
It seems unlikely that any one technology can be used to direct crop management interventions, we will likely need to incorporate several technologies, operating at different spatial/temporal scales. Data processing and presentation to growers will increasingly be important to realise impact from these technologies.

P4.1-004

ADAVELT ACTIVE (FLORYLPICOXAMID), A NATURALLY INSPIRED SOLUTION FOR BROAD SPECTRUM DISEASE CONTROL

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Text
Adavelt™ active (florylpicoxamid) is a second generation picolinamide fungicide (FRAC group 21) from Corteva Agriscience that offers broad spectrum activity against key diseases in a wide range of crops. Adavelt controls major ascomycete pathogens such as powdery mildews, Alternaria spp., Botrytis cinerea, Cercospora spp., Colletotrichum spp., Monilinia spp., Mycosphaerella musicola, Zymoseptoria tritici and more. As a Quinone Inside Inhibitor (QiI), Adavelt inhibits mitochondrial respiration in fungi by blocking electron transfer in the respiratory chain. This is a novel target site in many crops with no cross-resistance to other modes of action. Due to its unique or under exploited mode of action, Adavelt will form a critical new tool to manage fungicide resistance. Quick plant uptake and effective translocation provide Adavelt with excellent acropetal redistribution and translaminar properties on cereals and dicots. Adavelt acts on various life cycle stages of the targeted pathogens with market-leading curative control vs Z. tritici, thus offering growers a flexible solution for managing diseases throughout the season. Adavelt will be offered in a range of formulations either straight or in pre-mixes for use in cereals, row crops, oilseed, top fruit, tree nuts, grapes, tropical fruit, vegetables, turf and ornamentals and others.

P4.1-005

SELECTING PHYTOPHTHORA-TOLERANT CITRUS ROOTSTOCKS

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Text

Phytophthora species affecting citrus in Morocco are *Phytophthora parasitica* and *Phytophthora citrophthora*. They cause trunk and foot rots, gummosis, branch canker, feeder roots rot, damping-off of seedling and brown rot on citrus fruits. The use of resistant rootstocks is the most economical and sustainable control measure of Phytophthora. Thus, grafting citrus varieties on sour orange may impair the damage caused by Phytophthora. In addition, this rootstock has a high aptitude for grafting and a remarkable compatibility with numerous commercial varieties. For these reasons, Sour Orange is predominantly used as a rootstock. However, this rootstock is highly sensitive to Tristeza disease caused by Citrus Tristeza Virus (CTV). Therefore, Moroccan citrus industry is seeking alternative rootstocks. To address this issue, seeds of several citrus rootstocks were introduced from USA and from France, and they were tested under Moroccan conditions. Seedlings of the rootstocks raised from introduced seeds were inoculated with the fungus directly on the stem of the seedling plant. Inoculated plants were maintained under adequate growth conditions in the greenhouse. The length of the lesions was measured sixty days post inoculation (DPI). This presentation will cover results of the evaluation of two series of rootstock introductions, made by a private producer and by INRA-Morocco.

P4.1-006

LORANTHUS LIGUSTRINUS IS AN EMERGING PARASITE CAUSING DECLINING THE YIELD OF CITRUS RETICULATA ORANGE IN INDIA AND ITS SUSTAINABLE MANAGEMENT METHODS

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Text

The production and productivity of Citrus reticulata are mainly affected by the Loranthus stem parasite. It lowers the yield by 47 percent and kills the trees within 4 to 5 years. We have identified two types one is the single-haustoria type, with 6–8 branches at the tip, and the other is the climber type, with multiple haustoria and coils around the stem. The first type of Loranthus has a stem length of 30–42 cm, and it caused a decline within 3–5 years. The second type of Loranthus runs faster and causes a decline within 2 to 3 years. To manage the loranthus foliar spraying of diesel 60% was found to be more effective followed by stem injection with 2, 4-Dicholorophenoxy acetic acid 5%, and foliar spray of diesel 30%. The treatments of diesel 60%, 2, 4-D 5%, and diesel 30% have reduced the loranthus infection by 97.5%, 90%, and 87.5%, respectively. The average yield per tree was increased to 37.5 kg in treated trees compare to the control. We have removed the loranthus by pruner 10 cm away from the base and pasted a 10% Bordeaux mixture. Birds could transmit the parasite through seeds. Birds scaring objects are installed in the field viz. bird deterrents sound ranges from 65-105 decibels, erection of false man-like toys made from stuffing the shirts with straw, and

third is the coiling of colored ribbon in the periphery of the field. Bird deterrents were found to be the best way to keep the birds away from October to March during fruiting time.

P4.1-007

GREEN SYNTHESIS OF FERROCENYL CHALCONES AND THEIR EVALUATION AGAINST PLANT PATHOGENIC FUNGI AND ROOT KNOT NEMATODE

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Text

Green synthesis of ferrocenyl chalcones and their evaluation against plant pathogenic fungi and root knot nematode

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A new microwave method (MM) has been developed for the synthesis of a series of thirty-four substituted ferrocenyl chalcones and comparing it with conventional method (CM). Characterization of synthesized compounds was done by different spectroscopic techniques. A series of ferrocenyl chalcones were synthesized and evaluated for fungicidal activity against Sclerotium rolfsii & Alternaria solani and for nematicidal activity against root knot nematode, Meloidogyne incognita. In vitro study revealed that 4-bromophenyl, 2,6-dichlorophenyl, and 5-chloro-2-hydroxyphenyl ferrocenyl chalcones derivatives were found to be most active against S. rolfsii. Based on in vitro study, these three most effective derivatives were chosen for pot experiment. The percent disease incidence was significantly decreased as compared to control & it was found to be minimum in plants treated with 4-bromophenyl derivatives @ 1000 ppm. In case of nematicidal activity, also based on in vitro study, ten most effective derivatives were taken for pot experiment. The activity was highest in 4-methoxyphenyl, 4-methylphenyl, and 3,4,5-trimethoxyphenyl ferrocenyl chalcones derivatives @ 80 ppm.

P4.1-008

FIELD EFFICACY OF VARIOUS SYSTEMIC AND BIOLOGICAL FUNGICIDES AGAINST COFFEE LEAF RUST IN HAWAII

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Text

Coffee leaf rust (Hemileia vastatrix, CLR) is considered the world's most devastating pathogen of coffee and is found in nearly all coffee growing regions. Hawaii was one of the

last locations to grow coffee without the disease until CLR was confirmed on Maui in October 2020. Within a year, it spread to all the islands. CLR damage reduces the tree's photosynthetic capabilities and causes premature defoliation of infected leaves. In turn, future growth and yield can be lost, and in severe cases, CLR can cause tree death. Coffee is Hawaii's no. 3 agricultural commodity. Currently Hawaii's coffee growers can only contact fungicides and one translaminar fungicide to battle CLR. Considering the urgent research need, this project aimed to identify effective systemic and biological fungicides against CLR in Hawaii. This was a 2 year project in which we tested 7 systemic fungicides in 2021 and 8 fungicides (6 systemic and 2 biological) in 2022 in replicated field plots (Randomized Complete Block Design) at two major coffee farms in Kona, Hawaii. CLR disease incidence (% infected leaves) and infection severity (0-5 scale) were measured at least 5 months post initial treatment in each year. Our results showed that inpyrfluxam and azoxystrobin were both very effective against CLR. Although both biological fungicides tested showed some efficacy, they were not as effective as inpyrfluxam and azoxystrobin. This research provided critical foundation for CLR management in Hawaii and beyond.

P4.1-009

USE OF BIOLOGICAL PRODUCTS-BASE MANAGEMENT PROGRAMS FOR THE CONTROL OF SWEET CHERRY DISEASES

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Text

Bacterial canker and pre- and postharvest fruit rots are diseases affecting sweet cherry worldwide. Use of biological products is a suitable alternative to reduce agrochemical applications. However, there is a lack of information on the interaction between these products and their effect on the population dynamics of these pathogens when incorporated into integrated disease management programs. This study evaluated the phytosanitary impact of biological based management programs on disease control in sweet cherry. Half hectare units were established under two biological-based programs, one based only on chemical control and the other under the local grower program, in a ´Sweetheart´ sweet cherry orchard in Chillán, Chile. Population counts and incidence and severity measurements were made on cherry at specific phenological stages. Results showed that biological-based programs, which included *Pseudomonas protegens, Bacillus* spp. and *Trichoderma* spp., reduced populations of *Pseudomonas syringae* pv. *syringae*; *Botrytis cinerea* and *Alternaria* spp. at specific phenological stages, with no differences, regarding chemical and grower programs. Between programs, no differences in incidence and severity of bacterial canker were detected, but the incidence of *Alternaria* spp. rot was 87% lower in the chemical program compared to biological-based programs, in the ripe fruit stage. These results allow to promote bioproducts inclusion in commercial disease management programs.

P4.1-010

BACTERIAL SPOT INCITED BY XANTHOMONAS CUCURBITAE: A SERIOUS EMERGING DISEASE OF CUCURBITS

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Text

Bacterial spot of cucurbits, caused by *Xanthomonas cucurbitae*, is a serious emerging disease in the United States (U.S.) and worldwide. The pathogen infect leaves and fruits. *X. cucurbitae* is identifies based on the colony characteristics on yeast dextrose agar, PCR test using RST 2 and RST 3 primers, and pathogenicity test. Evaluation of isolates of *X. cucurbitae* from the North Central Region of U.S. showed that *X. cucurbitae* isolated from Illinois, Michigan, Kansas, Ohio, and Wisconsin were more virulent than the reference ATCC 23378 strain. In a four-year rotation with nonhost crops, development of bacterial spot was delayed only by two weeks. The pathogen survived for more than 24 months in infected pumpkin debris buried in the field. Although applications of some chemicals reduced incidence and severity of leaf and fruit infection, sprays did not provide effective protection of plants against the pathogen. We screened 81 commercial cultivars of gourds, pumpkins, and squashes, and 300 accessions of *Cucurbita* spp. for their resistance to *X. cucurbitae* under greenhouse and field conditions. All commercial cultivars were susceptible to *X. cucurbitae*. Only 9 and 21 accessions were identified as resistant and less resistant, respectively. Resistant and less resistant accessions belong to the species *Cucurbita maxima*, *C. maxima* subsp. *maxima*, *C. maxima* subsp. *andreana*, and *C. okeechobeensis* subsp. *martinezii*. This is the first report of resistance to the bacterial spot.

P4.1-011

USING RNA INTERFERENCE TO PROTECT CROPS AGAINST FUNGAL PATHOGENS

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Text

Sclerotina sclerotiorum, the causal agent of white mold, infects over 600 species of plants worldwide. Sclerotinia is a persistent problem for global food production that has traditionally been managed using broad-spectrum fungicides. However, current fungicide strategies have proven less effective and crop rotations fail due to the promiscuous host range of Sclerotinia and the formation of durable resting structures known as sclerotia. Thus, there is an immediate need to manage Sclerotinia using novel species-specific control methods. Our strategy exploits the inherent cellular defense process known as RNA interference (RNAi). Upon encountering a double stranded RNA (dsRNA) molecule, the cell processes the dsRNA specifically targeting transcripts with sequence homology. Using a re-designed bioinformatics approach, we identified Sclerotinia-specific target genes. RNAi knockdown was confirmed using quantitative real-time PCR on RNA isolated from fungal liquid cultures. dsRNA molecules were screened for growth inhibition on the plant using a system representative of field conditions that showed up to 85% reduction in lesion spread. We then generated transgenic Brassica napus (canola) over-expressing good quality dsRNA and showed a more profound and prolonged tolerance to the fungus. Finally, I will provide insight into the uptake

mechanisms and utility of next generation molecular fungicides and their applicability to control plant pathogens.

P4.1-012

SUGAR BEET CULTIVARS (CR+) WITH C. BETICOLA RESISTANT GENE CAN IMPROVE THE ECONOMIC VIABILITY OF THE INDUSTRY WHERE CERCOSPORA LEAF SPOT IS A MAJOR PROBLEM

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Text

Cercospora beticola causes Cercospora leaf spot (CLS), a devastating disease of sugar beet (Beta vulgaris, L). From 1999 through 2015, growers in Minnesota and North Dakota, USA, used QoI, DMI, and triphenyltin hydroxide to manage sensitive populations of C. beticola. In 2016, US growers lost over $200 million because of fungicide resistance and a CLS epidemic. CR+ cultivars with a C. beticola resistant gene from B. maritima recently became available. Field trials using approved moderately tolerant cultivars (non-CR+) and improved CR+ cultivars were recently conducted in the USA. Fungicide applications were done on a calendar basis, and on an only when needed based on the presence of symptoms and favorable environmental conditions. In a dry and warm 2021 season, fungicides were necessary to provide protection for the non-CR cultivars, but were not necessary for the CR+ cultivars. In 2022, conditions were not favorable for development of C. beticola until late August. One or two timely fungicide applications based on the presence of symptoms and thresholds resulted in low disease severity and recoverable sucrose similar to fungicide applications on a calendar basis. The availability and use of the newer CR+ cultivars will improve the economic viability of the sugar beet industry, and will help to reduce the inoculum load that may facilitate the use of non-CR+ cultivars. However, fungicides will have to be used judiciously to prolong the usefulness of the CR+ cultivars.

P4.1-013

A PLANT DISEASE COMPLEX BETWEEN A PLANT PARASITIC NEMATODE AND A FUNGUS - REEVALUATING PRATYLENCHUS CAPSICI DISEASE ETIOLOGY

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Text
During the last years we have identified a new nematode species *Pratylenchus capsici* in Israel leading to devastating damage on pepper crop resulting in stunted growth and significant yield reduction. Molecular phylogeography suggests that contemporary gene flow is prevented among different agricultural farms, while population dispersal from weeds (Chenopodium album and Sonchus oleraceus) to pepper occurs on a relatively small scale. Metabarcoding analysis of soil microbial community from *P. capsici* infested roots indicated that Olpidium species are widely presented in *Pratylenchus* introduced root-lesion, and might be a faithful companion associated with roots infected by *P. capsici*. The migratory potential of these nematodes is still under study. Altogether, results obtained through our research will facilitate with developing innovative management strategies through tailoring them within the agricultural practices and according to *P. capsici* etiology and characteristics.

P4.1-014

EVALUATION OF ORGANIC MATERIALS REVIEW INSTITUTE (OMRI) PRODUCTS AND VARIETIES FOR FUSARIUM HEAD BLIGHT (FHB) MANAGEMENT IN ORGANIC WINTER WHEAT IN INDIANA, UNITED STATES OF AMERICA

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Text
Fusarium head blight (FHB), caused by Fusarium graminearium, is one of the most important wheat diseases in the world. In Indiana, limited information is available regarding the efficacy of OMRI approved products. Field trials were conducted in 2021 and 2022 to investigate the efficacy of four OMRI products in the control of FHB. Treatments consisted of two wheat cultivars (Kaskaskia and Harpoon) as main plot and Reynoutria sachalinensis 12%, Bacillus pumilus QST 2808, Streptomyces lydicus WYEC 108, copper hydroxide, prothioconazole + tebuconazole, and a nontreated control as sub-plots. F. graminearum was inoculated 24 hours after treatment, at Feekes 10.5.1. Disease ratings were assessed at Feekes 11.2. In 2021, FHB incidence, severity, and index were similar for all treatments. The concentration of deoxynivalenol (DON) and percent fusarium damaged kernels (FDK) in Harpoon were reduced by 80 and 43%, respectively, compared to Kaskaskia. Prothioconazole + tebuconazole reduced FDK by 28% over the nontreated control. Yield was similar across all treatments in 2021. In 2022, FHB severity from Harpoon was reduced by 48% compared to Kaskaskia, but no differences were detected for FHB incidence and index. When treated with prothioconazole + tebuconazole, the DON concentration was 43% lower in the variety Harpoon compared to Kaskaskia. There was no difference between treatments and varieties for FDK. The variety Harpoon yielded 14% more than Kaskaskia in 2022.

P4.1-015

MANAGEMENT OF TAR SPOT IN CONVENTIONAL AND ORGANIC CORN SYSTEMS IN INDIANA, UNITED STATES OF AMERICA

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Text

Tar spot is caused by Phyllachora maydis, an obligate fungal pathogen that reduces corn grain yield and quality. P. maydis was first reported in the U.S. in 2015 and has since been confirmed in 18 states. Data regarding optimal application timing of foliar fungicides in conventional systems is limited. In addition, efficacy data for tar spot management in organic systems is yet to be published. Conventional corn trials were established from 2020 to 2022 to test the fungicides Mefentrifluconazole + pyraclostrobin and flutriafol + bixafen applied at various timings. Organic corn trials were established in 2021 and 2022 to test Bacillus amyloliquefaciens MBI 600, Streptomyces lydicus WYEC 108, copper oxychloride + copper hydroxide, hydrogen peroxide + peracetic acid, and pyraclostrobin + metconazole, all applied at R1 corn growth stage. Percentage of stromata per ear-leaf was assessed on five plants per plot and used to calculate the area under the disease progress curve (AUDPC). Reductions in tar spot severity reached up to 99.6% after the application of mefentrifluconazole + pyraclostrobin at VT and at R3 fb 3 weeks after first application (WAT). In organic trials, tar spot severity was reduced by up to 52% after the application of pyraclostrobin + metconazole. Yield increases were observed after application of mefentrifluconazole + pyraclostrobin at R3, V8 fb 3 WAT, VT fb 3 WAT, R3 fb 3 WAT and after the application of flutriafol + bixafen at V8 fb 3 WAT.

P4.1-016

RESISTANCE TO FUSARIUM OXYSPORUM F. SP. LUFFAE IN LUFFA GERMPLASM DESPITE THE PATHOGEN COLONIZATION

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Text

Fusarium wilt of *Luffa*, caused by *Fusarium oxysporum* f. sp. *luffae* (Folu), causes great losses in *Luffa* plants worldwide. In this study, 45 accessions of *Luffa* germplasm were used to determine their resistance to Folu isolates (FOLUST, FOLUSC, Fomh16, and Fol114) in two independent trials. In the first trial, only FOLUST was used to preliminarily identify resistant accessions. Nine accessions of *L. acutangular* and five of *L. aegyptiaca* were resistant to the FOLUST isolate. In the second trial, the other three isolates were then used to reevaluate the 14 resistant accessions. The results indicated that the 14 accessions were resistant to FOLUSC but exhibited variable resistance to the Fomh16 and Fol114. Eight accessions of *L. acutangula* and one accession of *L. aegyptiaca* were resistant to Fol114. Seven accessions of *L. acutangula* and one accession of *L. aegyptiaca* were resistant to

Fomh16. Although resistant accessions showed symptomless, the Folu isolates could colonize hypocotyls at 28 days post inoculation, except for isolates FOLUSC and FOLUST on accession LA140. A green fluorescent protein (GFP)-tagged isolates (FOLUSC-A and Fomh16-A) were used to study the Folu distribution and colonization in *Luffa* accessions. In addition, fifteen *Luffa* hybrids were obtained from reciprocal crosses between five resistant lines, four hybrids were successfully grafted with bitter gourd plants and significantly reduced disease incidence caused by *F. oxysporium* f. sp. *momordicae* and Folu.

P4.1-017

INVESTIGATING THE MODE OF ACTION OF A NEW BIOFUNGICIDE

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Text

The ascomycete *Botrytis cinere*a and the oomycete *Phytophthora infestan*s are two important plant pathogens affecting several crops. These phytopathogens are commonly controlled by the frequent use of chemical fungicides with possible detrimental effects to human health and the environment. The present study aims at characterizing the efficacy and mechanism of action of a new biofungicide based on choline pelargonate (TMAP) against these phytopathogens. The chemical properties of TMAP were evaluated at different concentrations in water solution, such as critical micelle concentration, pH, and conductivity. TMAP efficacy was then evaluated against *B. cinerea* and *P. infestan*s at different concentrations *in vitro* and the inhibitory effects were assessed on mycelium growth and spore germination to identify minimum inhibitory concentration and minimum fungicidal concentration. Overall, TMAP reduced pathogen growth at low concentrations. In order to clarify the mechanism of action, TMAP effects on the fungal cell membranes are under evaluation, by analyzing the electrolyte leakage, pH variations, and nucleic acid release from *B. cinerea* and *P. infestans* after TMAP treatment. Results from these experiments will be discussed to further characterize the efficacy of TMAP against the two phytopathogens *in planta*.

P4.1-018

STUDY OF THE EFFECT OF DISEASES ON OIL PLANTS: A CASE STUDY OF THE SUNFLOWER PLANT

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Text

The sunflower plant (Helianthus annuus L.) has become among the vital popular sectors in the Mediterranean regions and at the level of most regions of the world, as this type of sunflower plant is characterized by large, easy-to-grow and multi-colored flowers. This descriptive study aims to find out the main diseases that threaten the sunflower plant. The sunflower plant (Helianthus annuus L.) is native to America. Fortunately, many pathogenic microorganisms and insect pests raise the challenge for growing production. If needed, genetic progression can be easily transferred to oil plants. The major diseases that threaten oil plants such as the 'Helianthus annuus L.' type sunflower can affect the leaves, stems, seeds, flowers and cells of the entire plant. Some microorganisms and insects play a major role in protecting oil plants from disease and thus contribute significantly to the increase in vegetable oils across the world.
Keywords: sunflower plant, major diseases, Microorganisms, insects, genetic progress.

P4.1-019

A SNAPSHOT OF SENSITIVITY OF SOUTHEASTERN UNITED STATES MONILINIA FRUCTICOLA ISOLATES FROM PEACH TO PROPICONAZOLE AND THIOPHANATE METHYL

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Text

The strategic use and rotation of single-site fungicides during preharvest season is an essential component of IPM practices for control of peach brown rot in the Southeastern United States. However, every management program needs to be assessed over time for efficacy and sustainability. We collected more than 240 Monilinia fructicola isolates from South Carolina, Georgia, and Alabama commercial orchards and investigated sensitivity to propiconazole and thiophanate methyl. Results show that most isolates from South Carolina and Georgia were resistant to propiconazole (78.4%), whereas most isolates from Alabama were sensitive (83.8%) based on relative growth (RG) values at a discriminatory dose of 0.3 mg/L propiconazole. RG values for resistant isolates ranged from 21.1% to 90.0%. Resistant isolates contained the genetic element Mona located upstream CYP51 as determined by PCR and gel electrophoresis. In contrast, the most sensitive isolates (RG ranged from 0% to 18.7%) did not possess Mona. The RG values for our resistant isolates demonstrated no additional shift in sensitivity compared to a similar study conducted 15 years ago likely due to strict compliance of producers to resistance management recommendations. Surprisingly, all isolates from commercial orchards, except for one, were sensitive or reduced sensitive to thiophanate methyl using the discriminatory dose of 1 mg/L, opening the possibility of using this chemistry again carefully and strategically in IPM programs.

P4.1-020

IDENTIFICATION OF 7-HYDROXYTROPOLONE AS AN ACTIVE MOLECULE PRODUCED BY PSEUDOMONAS PA14H7 AGAINST DICKEYA, CAUSAL AGENT OF BLACKLEG ON POTATO.

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Text

Seed potatoes are subject to a strict control of quality for certification. In France, blackleg, a bacterial disease caused by *Pectobacterium* and *Dickeya*, is regularly the main cause of rejection of seed potatoes during field inspection. So far, neither conventional nor biocontrol product are available on the market to control this disease. *Pseudomonas* PA14H7, a bacteria isolated from potato rhizosphere, was identified as an antagonist agent against *Dickeya*.[1] The objective of this study is to identify the main active(s) molecule(s) produced by PA14H7 in order to understand the mechanism involved in this antagonism. In that way, supernatant of PA14H7 culture was extracted and analyzed by LC/MS, GC/MS and NMR. We have putatively characterized the presence of 7-hydroxytropolone (7-HT). This molecule has already been described in other *Pseudomonas*[2] and is known for antibacterial and antifungal activities. We have synthesized 7-HT[3] in order to determine the amount produced by PA14H7 and to compare its in vitro efficiency vs PA14H7 supernatant one. The final objective is to understand the key role of 7-HT in the antagonist activity of PA14H7 as a potential biocontrol agent.

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P4.1-021

OPTIMUM TIMING OF FUNGICIDE APPLICATIONS FOR MANAGING KERNEL SMUT OF RICE

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Text

Kernel smut, caused by *Tilletia horrida*, is an emerging disease of rice that represents a major threat to the U.S. rice industry. Because of the unavailability of resistant cultivars, farmers heavily depend on midseason preventive applications of fungicides. Timely application of fungicides is the key to managing kernel smut and maximizing production returns. The objective of this study was to determine the best timing of fungicide applications for management of kernel smut in rice. A field trial was conducted in 2020, 2021 and 2022 to evaluate the efficacy of applications of Amistar Top (azoxystrobin plus difenoconazole) and Tilt (propiconazole) made at 1) panicle differentiation (PD) + 7 days, 2) mid-boot, 3) heading, 4) PD + 7 days plus mid-boot, 5) mid-boot plus heading, and 6) unsprayed control. Plots were spray inoculated with secondary sporidia of the fungus (10^5 spores/ml) at both boot and

heading stages. Over the years, both fungicides were effective in reducing kernel smut, with Amistar Top being slightly more effective than Tilt. Applications made at the mid-boot stage were most effective with up to 80% control efficacy, followed by those at PD + 7 days. Applications made at the heading stage were ineffective. The results demonstrate that the mid-boot stage is the optimum time to apply a fungicide for managing kernel smut of rice.

P4.1-022

EXPLORING ALTERNATIVES AND SYNTHETIC FUNGICIDES FOR APPLE BITTER ROT MANAGEMENT IN THE MID-ATLANTIC U.S.A.

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Text

In search of biorational options to manage apple bitter rot caused by different *Colletotrichum* species, we evaluated 18 fungicide treatments on cultivars ‘Idared’ (ID) and ‘Golden Delicious’ (GD). Out of 18 treatments 17 were a single-material spray programs applied throughout the summer. In this inoculated experiment, testing one fungicide with multiple applications until harvest allowed us to determine the whole season protection efficacy. Out of 8 treatments with biorational alternatives, such as giant knotweed extract, *Swinglea glutinosa* extract, acibenzolar-*S*-methyl, laminarin, citric acid, and phosphites none provided satisfactory management of apple bitter rot allowing 37-67% disease incidence on ID and 11-33% disease incidence on GD fruit. In July 2022, all synthetic fungicides including ferbam, captan, ziram, fluazinam, benzovindiflupyr, pyraclostrobin and trifloxystrobin, but not kresoxim-methyl, were effecive with zero to 13% disease incidence on ID fruit and only 0.6 to 3% disease incidence on GD fruit. Untreated inoculated and untreated non-inoculated controls exhibited 63 and 75% disease incidence on ID, respectively. Both controls exhibited 28% disease incidence on GD fruit. Second rating in August confirmed these results, but showed more disease incidence in biorational materials (78-89% on ID; 21-48% on GD) and in synthetic fungicides (12-39% on ID; 6-11% on GD). Future same and new experiments will serve to find new options for bitter rot control.

P4.1-023

EVALUATION OF BURKHOLDERIA GLUMAE CONTROL IN RICE (ORYZA SATIVA) VARIETY 67 WITH SILVER NANOPARTICLES (AGNPS)

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Text

Bacterial panicle blight caused by Burkholderia glumae is one of the world's most serious seed-borne bacterial rice diseases, with an impact on food sustainability in the future. It is a

typical example of the change from a minor plant disease to a major disease due to changes in environmental conditions. Despite their economic importance, effective control measures and rice varieties with complete resistance to this disease are not yet available. In this study, the effect of silver nanoparticles (AgNPs) electrochemically synthesized and applied by spraying in rice plants was evaluated to determine if they constitute an effective control method against B. glumae under greenhouse conditions compared to commercial control. The study was done in a completely randomized design with 5 treatments: T1. Preventive, T2 curative, T3. Positive control, T4. Negative control, T5. Complete control. It was found that the preventive treatment with 5 ppm of AgNPs presents a promising phytoprotective effect for the control of B. glumae in rice variety 67. Further studies are required to assess their effect on field and possible environmental effects.

P4.1-024

DEVELOPMENT OF RESOURCES FOR CONTROL OF STRIPE RUST ON WHEAT AND BARLEY IN THE UNITED STATES

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Text

Stripe rust, caused by *Puccinia striiformis*, is one of the most destructive diseases of cereals worldwide. To control the disease on a yearly basis, potential yield losses are predicted before stripe rust development based on weather data, field surveys are conducted during the crop season, and recommendations are made for implementing appropriate control measures for individual cultivars based on the yield loss prediction, field surveys, and cultivar resistance. For improving control of stripe rust, various studies have been conducted for new knowledges of virulence, genomics, and population genetics of the pathogen, epidemiology of the disease, host resistance, and measures for integrated control. Races of the pathogen have been identified using a set of differentials, and selected races are used in greenhouse tests, together with field tests, to screen wheat germplasm and breeding lines for developing new resistant cultivars. SSR, SNP, and KASP markers have been developed for monitoring the pathogen populations. To discover more genes for resistance to stripe rust, bi-parental populations and assembled panels of wheat germplasm have been studied using QTL mapping and GWAS approaches, respectively. KASP markers have been developed for new resistance genes, and new wheat germplasm lines have been developed for resistance genes and combinations of genes for more efficiently use in breeding programs.

P4.1-025

DETECTION OF ERWINIA AMYLOVORA IN APPLE ROOTSTOCKS: A CASE STUDY IN HIGH-DENSITY APPLE ORCHARDS

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Text

Fire blight infection of apple rootstock can lead to canker development or asymptomatic infections. Cankers can girdle the rootstock leading to tree death and production losses, especially in high-density orchards. Confirming fire blight infection in apple rootstock is a challenge for apple growers because fire blight cankers can be visually misdiagnosed with cankers caused by several Oomycete and fungal pathogens. Accurate fire blight diagnosis is necessary to inform the efforts to remove infected trees from the orchard, can assist in replanting the orchard, and help prevent further pathogen dissemination. We used PCR to detect *Erwinia amylovora* in symptomatic and asymptomatic rootstocks for two years. Rootstock canker incidence and tree death were rated in 3 to 6 infection foci per orchard on 7 farm sites in New York state. Each focus consisted of a central rootstock-cankered tree and the nearest surrounding edge trees with no canker on the rootstock. In the first year, most orchards showed *E. amylovora* detection rates of 10.7 – 45.3% in the asymptomatic rootstocks on the edge trees in infection foci. One year later, 20.8 – 56.3% cankered rootstocks were detected on the edge trees and from zero to 35.4% dead edge trees were recorded. However, PCR showed no pathogen detections in sampled edge rootstocks one year later. Our results elucidate latent fire blight infections in rootstock and indicate that whole trees need to be removed as rootstocks express cankers with delay.

P4.1-026

IN VITRO AND IN SITU ANTIFUNGAL ACTIVITY OF THREE PLANT EXTRACTS AND TWO SYNTHETIC FUNGICIDES FOR THE CONTROL OF VASCULAR WILT OF OIL PALM CAUSED BY FUSARIUM OXYSPORUM F. SP. ELAIEDIS.

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Text

The most important oil crop in the world is oil palm whose production is reduced yearly by diseases with a greater percentage being Fusarium oxysporum f. sp. Elaiedis (Foe) which is the causal pathogen for vascular wilt. Plant based fungicides appear to be better alternatives because they are eco-friendly and are not dangerous to consumers in contrast to synthetic pesticides. In an approach towards the development of eco-friendly management schemes, an in vitro and in situ antifungal assay was conducted against four morphotypes of Fusarium oxysporum f. sp. elaiedis with extracts of Tithonia diversifolia, Azadirachta indica and Voacanga africana. The hexane, methylene chloride and methanol extracts of these plants were tested at five concentrations (10%, 15%, 20%, 25% and 30%). Also, Benomyl® and Mancozeb® (synthetic fungicides) were equally tested at five concentrations to do a comparative study of their efficacy. The results revealed that 30% concentration of methanolic extract of Azadirachta indica inhibits all four morphotypes in vitro and in a greenhouse experiment. Since plant extracts are a source of cost effective and non-

hazardous fungicide against Foe, Azadirachta indica is therefore recommended by this study as a good antifungal efficacy, used at 30% concentration for formulating new, safer and ecofriendly fungicides.

P4.1-027

INNOVATIVE OPTICAL DEVICE FOR REAL-TIME SPORE DETECTION APPLIED ON GRAPEVINE DOWNY AND POWDERY MILDEW

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Text

Downy and powdery mildews, caused respectively by the oomycete Plasmopara viticola and the ascomycete Erysiphe necator are the major threat for Swiss grapevine. As both polycyclic pathogens, they produce abundant number of spores and cause multiple infections throughout the season.

Decision support systems as infection forecast are only based on weather data and lack biological data such as spores load. For this purpose, an innovative spore detector device was developed. Through holographic imaging, it allows us to detect aerial particles in real time. A trained artificial intelligence identifies and counts Plasmopara viticola and Erysiphe necator spores 24/7. Holographic imaging allows us to be far more specific than classic optical imaging by getting 3D shape, density and weight. An artificial intelligence is trained by a neural network on images of pure spores cultivated in laboratory. Experiments on field during 3 seasons gave satisfactory results, linked to the diseases observed on vineyards. Quantitative spore detection data will be integrated in the decision support system and tested in field trials with the goal to avoid unnecessary sprays.

Aims of the use of this device are to improve performances of the Vitimeteo forecasting model by integrating spore quantitative data, to monitor the first appearance of a target spore and to monitor disease progression on site. Finally, this new technology could be applied to other relevant fungal diseases caused by spore dispersion.

P4.1-028

THE PLANT PATHOLOGY IN THE TROPICAL PLANT HEALTH NETWORK SCOPE

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Text

The Brazilian agribusiness supply chains now have an even stronger support for scientific research on diseases, pests and weeds in tropical agricultural systems of grain and fiber crops such as cotton, corn, soybeans, and wheat. A nationwide initiative called Tropical Plant Health Network (RFT) arises to organize, strengthen, and optimize existing collaborative networks. This national cooperative research network has developed a website (https://www.fitossanidadetropical.org.br/) to centralize important information that was previously spread among different research groups. Our work proposal aims to optimize existing collaborate networks for multiple biological targets between research centers to develop applied studies in plant health. The idea was to consolidate almost 20 years of experiences in collaborative research, and at the same time form a basis for the development of future research networks. RFT already brings together more than 34 public and private research centers and several supporters from funding organizations and industry sectors. This document aims to present to the scientists from 12th International Congress of Plant Pathology, in Lyon/France, the advantages and benefits, the potential reach and structuring process of the RFT. **Support:** FAPEAGRO. **Acknowledgement:** National Cooperative Network in Soybean, Corn, Wheat and Cotton.

P4.1-029

SCREENING OF BIOCONTROL SOLUTIONS AGAINST BLACK-ROT (GUIGNARDIA BIDWELLII), AMONG REGISTERED ANTI-MILDEWS BIOFUNGICIDES TO DESIGN BLACK ROT CONTROL STRATEGIES FOR CONVENTIONAL AND ORGANIC VITICULTURE AND THE DEPLOYMENT OF RESISTANT VARIETIES.

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Text

Black rot (*Guignardia bidwellii*), considered as a secondary disease may be an emerging problem in the near future in grapevine. It can cause losses of up to 80% at harvest, and may re-emerge owing to the reduction or even withdrawal of chemical compounds, the deployment of varieties resistant to mildews, but also because of climate change.
To date, there is no biocontrol product approved against this disease in France and we have very few candidates for the development of alternative control methods.
As part of the Zero Black-rot project (France Agrimer funding), the aim was to identify active biosolutions and integrating them into the technical itineraries of winegrowers.
To this end, a set of products or substances already approved and marketed in French vineyards against downy and powdery mildews and/or Botrytis) have been selected, preferably from the lists of approved biocontrol products, of basic substances or biostimulants or fertilizers.
Out of a total of 42 products tested under controlled in vitro conditions, only 16 products have

shown a significant efficacy against black rot. At the end of the greenhouse in-vivo further testing, only 8 caught our attention.
Two candidates were further tested for integration into alternative strategies in vineyards: potassium bicarbonate and potassium phosphonate. They displayed significant, yet partial, effectiveness. These products can already be integrated into the vineyard, in combination with sulfur or (modulated) copper.

P4.1-030

BROAD-SPECTRUM CONTROL OF FOLIAR DISEASES WITH AN ENZYME-BASED BIOCHEMICAL PESTICIDE

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Text
Field trials were conducted in 2022 to evaluate the efficacy of an enzyme-based biochemical pesticide (AX), with potential for organic registration, for control of foliar diseases on cabbage, cucumber, and pumpkins in Phelps, NY, USA. Plots for all crops consisted of 10 plants using a complete randomized block design with four replicates, and all data were collected from the center 5 plants. Non-treated and commercial controls were included in all trials. Experimental AX formulations and commercial controls were applied with a CO_2 hand-held pressurized sprayer. Cabbage plants were inoculated with *Xanthomonas campestris* pv *campestris* grown from cultures. Cucumber (downy mildew) and pumpkin (powdery mildew) plots were not inoculated; infection was induced from natural field inoculum. On all rating dates, AX formulations and the commercial control Kocide 3000 (Certis, USA LLC) reduced the severity of black rot on cabbage compared to the non-treated control. At 20 days after the first application, the severity of downy mildew on cucumbers treated with each AX formulation was reduced significantly compared to both the non-treated and commercial control Stargus (Pro Farm Group, USA) treated plants. In addition, on all rating dates, AX formulations and commercial control Regalia (Pro Farm Group, USA) significantly reduced the severity of powdery mildew on pumpkins. These trials demonstrate that AX proprietary enzyme formulations have broad-spectrum biocidal activity.

P4.1-031

NOVEL SOURCES OF RESISTANCE TO SOYBEAN SEEDLING DISEASE PATHOGEN PYTHIUM IRREGULARE

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Text
Soilborne seed rot and seedling diseases of soybean are the number two cause of soybean yield loss in the United States behind soybean cyst nematode. Over 200 soybean varieties

were selected from the USDA soybean germplasm collection for resistance screening against predominant Midwest species *Pythium irregulare*, *P. ultimum* var. *sporangiiferum* and *P. ultimum* var. *ultimum*. Varieties scoring higher on both stand counts and adjusted root weight measures significantly greater than the population mean were discovered in the *P. irregulare* screenings, while no significant resistance was observed in in the *P. ultimum* screenings. A continuous distribution of phenotypes was observed among the selected varieties indicating a likely polygenic quantitative resistance. GWAS uncovered two marker trait associations (MTAs) explaining ~10% each of the variation observed in both emergence and root weight after inoculation. Loci in linkage with these MTAs show both susceptibility and resistance functions. Comparison to the published Williams82 reference genome indicated that the resistance MTA on chromosome 10 is located in a polygalacturanase-like gene in a region also predicted to produce cupins, germins, and polyketide synthesis enzymes. The susceptibility MTA on chromosome 15 was not located within any annotated regions but within 100 kbp of a leucine-rich repeat protein kinase.

P4.1-032

EXOGENOUS APPLICATION OF METHYL SALICYLATE INDUCES DEFENSE IN BRASSICA AGAINST PEACH POTATO APHID MYZUS PERSICAE

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Text

Phytochemicals play a critical role in insect-plant interactions, insect herbivores use their sense of smell to detect the volatile compounds released by host plants. The change in phytochemistry of host plants alters the insect-plant tritrophic interactions. Several natural plant derived compounds have been found capable that affect plant's chemistry by activating cascade of defense pathways on their exposure to host plants. Methyl salicylate (MeSA) is a natural plant derived compounds that has been used as a plant defense elicitor on several crop plants. The aim of this study was to investigate the effects of different concentrations (75 mg/l and 100 mg/l) of MeSA treatment of Brassica rapa subsp. chinensis cv. 'Hanakan' on interactions with peach-potato aphid *Myzus persicae* and its natural enemies *Diaeretiella rapae*. To test the responses of *M. persicae* and its natural enemies *D. rapae*, performance and behavioural bioassays were performed. Our results showed that brassica plants treated with MeSA (100 mg/l) significantly reduced aphid performance due to high mortality and low larviposition in cage bioassay. In aphid settlement bioassay, significant lower number of aphids were settled on MeSA treated plants. While parasitoid *D. rapae* spent significant longer time on plants treated with MeSA (100 mg/l) in foraging bioassay. The current findings can be utilised in developing new sustainable approaches for the management of peach-potato aphid.

P4.1-033

A SUPER ABSORBENT POLYMER CONTAINING COPPER FOR CONTROLLING MAL SECCO DISEASE OF LEMON

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Text

Mal secco of lemon is a highly destructive tracheomycosis caused by the fungus *Plenodomus tracheiphilus*. The management includes pruning of symptomatic branches and their sanitation by spraying aqueous suspensions of Cu^{2+}-based fungicides. However, these treatments cause the dispersion of Cu^{2+} in the environment, with toxic effects on living organisms and negative impact on soil microbiome and contamination of food.
This study investigated the effectiveness of a super absorbent polymer (SAP) containing copper (SAP-Cu) in controlling mal secco. This new polymer, thanks to its physical properties, acts as reservoir for the controlled release of Cu^{2+} ions ensuring a longer lasting effectiveness of the treatment and preventing, at the same time, the excessive soil and groundwater contamination resulting from the leaching of the metal.
SAP-Cu was characterized by AAS, UV-VIS spectroscopy and ToF-SIMS. *In vitro* tests were performed to determine the inhibitory effects of SAP-Cu against *P. tracheiphilus* on both PDA medium and naturally infected lemon cuttings. Results showed that SAP adsorbed up to 30 times its weight of Cu^{2+} solution at 236 mM; when SAP-Cu was in contact with lemon twigs it transferred Cu^{2+} ions along the xylem vessels. *In vitro*, SAP-Cu significantly inhibited the viability of *P. tracheiphilus* both in PDA medium and in naturally infected lemon twigs. Overall, this study highlighted that the SAP could be a suitable carrier for Cu^{2+} or other antifungal compounds.

P4.1-034

APPLICATION OF YEAST, RESISTANCE INDUCTORS AND MULTI-SITE FUNGICIDES IN THE CONTROL OF DISEASES IN COTTON

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Text

Cotton is one of the main products exported by Brazil. But the main production costs is the application of fungicides to control ramularia spot (*Ramulariopsis gossypii*) and target spot (*Corynespora cassiicola*). The present work evaluated the effect of multisite fungicides, resistance inducers and yeast in the control of target spot and ramularia in cotton and their impact on yield. The experiment was conducted in the municipality of Sorriso/MT, in the

2019/20 harvest, experimental design was randomized blocks, consisting of eight treatments and five replications. Multisite fungicides (mancozeb, copper oxychloride and chlorothalonil), resistance inducers (salicylic acid and micronutrient “copper”) and yeast Pichia sp. associated with application of site-specific fungicides. Disease severity was evaluated, and the area under disease progress curve (AUDPC) calculated. To evaluate productivity and measure fiber quality, bolls were collected from all plants in the two central lines of each plot. In the evaluation of the AUDPC of the target spot there was no difference between the evaluated treatments, however for ramularia all treatments differed from the control treatment. The fiber quality was not affected by the evaluated diseases. Increase in yield was observed in the treatment with site-specific fungicide plus the addition of the multi-site fungicide chlorothalonil, providing an average increase of 88.3% in yield.

P4.1-035

IDENTIFICATION AND FUNGICIDE SENSITIVITY OF XYLARIA SP. GROWN ON SHIITAKE WOOD LOG IN TAIWAN

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Text

Shiitake is high value mushroom in Taiwan, and usually be planted by sawdust bag or wood log. Because of the climate factors in Taiwan, the diseases, pests or competitors occurred easily during shiitake cultivation. However, the effect of pathogens or competitors on the yield and quality of shiitake remain to be resolved. This study focused on a fungus which was isolated from the shiitake wood log. After comparing the fruiting bodies in the field and data of ITS sequence analysis, the fungus was preliminarily identified as *Xylaria* sp. According to the results of the dual culture assay involving *Xylaria* sp. (CYX-1) and *Lentinula edodes* (Br-1), the mycelium growth of Br-1 was significantly suppressed. After 14 days culturing, mycelium of Br-1 was covered by CYX-1, and the backsides on PDA turned brown. It showed that CYX-1 was detrimental to the mycelial growth of Br-1 and could be the competitor of Br-1 in wood log. In the fungicide sensitivity test, the mycelium of CYX-1 could not grow on PDA with 30 ppm of thiabendazole and be inhibited in filter paper-disk with 1,000 dilution fold of thiabendazole (60% SC). Therefore, thiabendazole can be used as a potential chemical control agent for *Xylaria* sp. Future work will focus on the identification and phylogenetic analysis of CYX-1 by artificial culture of fruiting bodies and multiple-gene sequencing, respectively. The mechanism of pathogenesis in *Xylaria* sp will also further to understand.

P4.1-036

EFFICACY OF INORGANIC CHEMICALS AS PLANT RESISTANCE INDUCER ON THE CONTROL OF POTATO COMMON SCAB DISEASE

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Text
Potato common scab caused by several members of the genus *Streptomyces* is an economically important disease worldwide, and can cause significant reduction in the market value of potato. As there are no effective pesticide and limited results of crop rotation and resistant potato cultivars for managing the disease, this study explored the possibility of inducing defense by inorganic chemicals such as salicylic acid (SA) and neutralized phosphorous acid (NPA) to control *S. europaeiscabiei* in the greenhouse. Application of 0.05% SA or 0.1% NPA was conducted on potato by soil drench and tuber treatment, respectively. The results showed that the disease severities of inoculated potato plants by soil-drench with SA and NPA were 20.85% and 39.75%, whereas the disease severity of tuber treatment with SA and NPA were 35.18% and 38.77%. Reduced disease severities of potato common scab were observed in case of both soil drench and tuber treatment, but there was a significant difference only between soil drench with SA and the untreated control (47.30%), indicating that soil drench with SA provided better disease control than tuber treatment.

P4.1-037

AN INTEGRATED PRODUCTION CHAIN FOR CERTIFIED OLIVE, CITRUS AND FIG PROPAGATION MATERIAL IN GREECE

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Text
In the frame of the national “Rural Development Programme 2014 – 2020”, an integrated chain for the production of certified propagating material for olive, citrus and fig trees has been initiated for the first time in Greece. Starting from mother tree plantations of the official plant variety maintainer, the Hellenic Agricultural Organization “Demeter”, pre-basic plantations were established in Chania, Crete and Kalamata, Peloponnese. Basic plantations are being established by two cooperating nurseries and finally certified plantations will be created by the Agricultural Cooperative of Kavousi, Crete. At all stages varietal authenticity and plant health are ensured by appropriate testing. Within this framework, the National Reference Laboratory of Virology of Benaki Phytopathological Institute has applied and verified current molecular detection methodologies for four olive viruses (OLYaV, ArMV, CLRV, SLRSV), seven citrus viruses/viroids (CTV, CVV, CPsV, CLBV, CiVA, CEVd, HSVd), as well as four fig viruses (FMV, FLMaV-1, FMMaV, FFkaV) to meet Commission Implementing Directive (EU) 2020/177 requirements. Virus infected olive and citrus pre-basic or basic trees were only scarcely found. The completion of the proposed integrated production chain promotes the use of certified propagating material of olive, citrus and fig trees, including important local varieties, currently quite limited in the country and thus reduces the necessity for imports of mother plants and seedlings.

P4.1-038

DECIPHERING THE INFLUENCE OF CO-INOCULATION TIMING ON ANTAGONISTIC EFFECTS OF LEPTOSPHAERIA BIGLOBOSA ON L. MACULANS

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Text

Phoma stem canker is an economically damaging disease of oilseed rape, caused by two co-existing pathogens *Leptosphaeria maculans* and *L. biglobosa*. *L. maculans* produces a phytotoxin called sirodesmin PL. Our previous work showed that *L. biglobosa* has an antagonistic effect on the production of sirodesmin PL if it was simultaneously co-inoculated with *L. maculans*. Here, we investigated the effects of sequential co-inoculation on interactions between the two pathogens in terms of sirodesmin PL production. Clarified v8 broths were inoculated with *L. maculans* first, then *L. biglobosa* sequentially with 1, 3, 5, 7 days in-between, and vice versa. Controls were *L. maculans* only, *L. biglobosa* only, and *L. maculans* & *L. biglobosa* co-inoculated simultaneously. Secondary metabolites were extracted from culture filtrates at 14 days post inoculation and analysed with HPLC. Mycelia were freeze-dried, weighed, and homogenised for DNA extraction and qPCR. Results showed no significant differences in mycelial weight between treatments. Both sirodesmin PL and its precursors were not produced if *L. biglobosa* was inoculated before *L. maculans*, this was due to *L. biglobosa* inhibiting the growth of *L. maculans*, confirmed by qPCR. However, the antagonistic effects of *L. biglobosa* were lost if it was co-inoculated 5 days after *L. maculans*. There is a need to investigate the mechanisms of the antagonistic effects of *L. biglobosa* to develop new strategies for sustainable control of phoma stem canker.

P4.1-040

EARLY BLIGHT COMES LATE IN SWEDISH WARE POTATO

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Text

Early blight is one of the most common foliar diseases in potato and is caused by the fungus *Alternaria solani*. Long-term field inventories revealed that the outbreak of early blight starts in mid-August and can be severe in mid-September in starch potato crops, especially in the south-eastern parts of Sweden. Inventories of the incidence of early blight in ware potato grown in central Sweden showed another pattern as just a few lesions could be found in August and September. However, most of the ware potato haulm are destroyed in late August to early September to ensure desired quality. Since dehaulming often is done at the same time as the epidemic of early blight starts, the effect, and necessity, of fungicide use on ware potato yield were evaluated in four field trials. The fungicide strategy for the two field trials in Skåne (2015) was an alteration of a triazole and a mixture of a strobilurin and boscalid. The treatments in the two field trials in central Sweden (2015 and 2016) were either a strobilurin or a mixture of a strobilurin and boscalid. The fungicide efficacy was compared to an untreated control in all field trials.

There was no effect on yield in neither of the four trials suggesting that fungicide use against *A. solani* is not needed in Swedish ware potato production. One concern for the unnecessary use of fungicides in ware potato is the prevalence of resistance genes toward fungicides based on strobilurins and SDHI in the Swedish population of *A. solani*.

P4.1-041

EVALUATING THE EFFICACY OF PHOSPHITE AND METALAXYL IN PHYTOPHTHORA ROOT ROT CONTROL IN AVOCADO

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Text

Phytophthora root rot, caused by the oomycete *Phytophthora cinnamomi*, is the most economically important disease in avocado world-wide. Field and glasshouse trials have evaluated effects of timing and method of application of anti-oomycete chemistries phosphite and metalaxyl, for management of Phytophthora root rot in avocado. Tree health and trunk diameter of grafted nursery trees planted in a field site infested with *P. cinnamomi*, were significantly greater as early as 3 months after planting, for trees which had received phosphite as pre-planting drench, phosphite applied as sprays pre- and post-planting, or pre- and post-planting metalaxyl granules applied to the surface of soil, compared with untreated control, and phosphite as standalone pre- and post-planting sprays. In glasshouse trials with seedlings, phosphite foliar sprays applied at 2 time points significantly reduced % root necrosis following inoculation with *P. cinnamomi* in one of the two experiments. However, metalaxyl granules applied on the surface of the potting mix significantly reduced both % root necrosis and % frequency of *P. cinnamomi* recovered from roots, and significantly increased plant height, diameter, leaf number, both above-ground and root biomass, compared to that of *P. cinnamomi*-inoculated control plants in both trials. Further experiments are in progress and results will be presented.

P4.1-042

CHEMICAL PRIMING OF DEFENCE IN FOREST HEALTH: OAK DISEASES CASE OF STUDY

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Text

Plants are continually exposed to (a)biotic stresses potentiated by climate change. One plant defense strategy, to cope with these threats, is priming, a sensitisation of plant defence mechanisms for a faster and/or stronger activation after subsequent attack. Studies on priming in oak seedlings are lacking. We aim to determine whether oak seedlings can

express chemical-induced priming and the potential trade-off in growth. Oak seedlings were treated with salicylic acid (SA), jasmonic acid (JA) and beta-aminobutyric acid (BABA) 7 days before infection with Erysiphe alphitoides, the powdery mildew (PM) causal agent. To investigate the mechanisms behind the specific SA and BABA priming of defence, untargeted metabolome and transcriptome analyses were performed. Metabolites were subjected to LC-MS/MS. Spectra were filtered using the XCMS R script and MarVis was employed to putatively identify metabolites and pathways. Fold changes versus water treatment were applied to isolate primed metabolites. In addition, RNA-seq was performed using the same leaf samples from the PM experiment. Transcriptome data was analysed using the Quercus robur genome as reference (www.oakgenome.fr) with Python (HTSeq) and R (DESeq2) scripts. We have identified molecular markers of priming in oak seedlings. Our results, in terms of enhanced resistance to PM and early/late responses depending on the chemical applied, provide valuable information to fight pathogens in oak seedlings.

P4.1-043

TOMATO SEED PRIMING WITH WATER-SOLUBLE POLYSACCHARIDES FROM JANIA ADHAERENS PROMOTES PLANT GROWTH AND INCREASES PLANT RESISTANCE TO SOILBORNE PATHOGENS

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Text

Marine macroalgae are a source of natural bioactive compounds, such as polysaccharides, that deserve exploitation in the field of plant disease management. Moreover, current European regulations require that priority should be given to alternative products than synthetic pesticides.

In agriculture, algae have been mostly used for their beneficial properties on plant development.

In this study, we showed that tomato seed biopriming with water-soluble polysaccharides (WSPs) from the alga *Jania adhaerens* protects seedlings and adult plants from the soilborne pathogens *Fusarium oxysporum* f.sp. *lycopersici*, *Pythium ultimum* and *Rhizoctonia solani* artificially inoculated in a growing substrate.

First, WSPs were characterized by FT-IR spectroscopy and seedling emergence, disease severity, and expression of genes related to phenylpropanoid, chlorogenic acid, SAR and ISR pathways, and chitinase and *β*-1,3 glucanase activities were investigated after seed priming.

WSPs FT-IR spectra showed typical bands assigned to alduronic acids and glycosidic linkage formation in polysaccharides. Depending on the WSPs dose, seed treatment enhanced seedling emergence, reduced disease severity and increased plant growth. Moreover, HQT, HCT, PAL, PR1 and PR2 genes were significantly upregulated together with β-1,3 glucanase activity.

These results show that algal WSPs have the potential for being considered as natural compounds for soilborne pathogens control in sustainable agriculture.

P4.1-044

STUDY OF ANTHRACNOSE FRUIT ROT & BLACK LEAF SPOT OF STRAWBERRY IN PAKISTAN AND THEIR BIO-MANAGEMENT USING INDIGENOUS PLANTS EXTRACTS

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Text

During 2017-18 and 2019-20 surveys were conducted in 12 strawberry producing districts of Pakistan, of which 8 in Punjab province, 3 in Khyber Pukhtunkhwa province and Islamabad. Disease incidence ranged from 17-55 % for anthracnose fruit rot and 11-32% for black leaf Spot respectively. 90 isolates of *Colletotrichum* spp. (69-*C. acutatum* & 21-*C. gloeosporioides*) and 82 of *A. alternata* were identified morphologically . For molecular identification, PCR of 19 highly pathogenic isolates *C. acutatum* (12); *C. gloeosporioides* (7) & 12 *A. alternata* were amplified by ITS, BT, ACT, TEF1-α and EndoPG primers and submitted in GenBank and subjected to phylogenetic analysis. This is 1st detailed study of these strawberry diseases from Pakistan and these diseases also been reported from almost all major strawberry producing countries. The inhibitory effects of 3 plant extracts viz. *Eucalyptus camaldulensis, Cannabis sativa* and *Polygonum afghanicum* were evaluated against the mycelia growth of these fungal pathogens. Results showed that all plant extracts brought about some inhibition in the mycelial growth. However, the highest concentration caused maximum inhibition followed by lower concentrations of plant extracts. The extract of *P. afghanicum* leaves proved highly effective in inhibiting the mycelial growth of these fungi followed by *E. camaldulensis* & C. *sativa extracts*. These indigenous plants thus may have potential as the new natural fungicide for management of these fungal pathogens

P4.1-045

MONITORING AND FUNGICIDE RESISTANCE ALLELE DETECTION OF THE WHEAT BLAST PATHOGEN IN PARANÁ STATE, BRAZIL

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Text

Wheat blast is considered one of the most devastating fungal diseases affecting wheat crops worldwide. The disease is caused by the ascomycetous fungus *Pyricularia oryzae Triticum* lineage (*PoT*l). In Brazil, populations of the pathogen have shown the prevalence of high levels of resistance to fungicides used intensively for managing wheat diseases. To rationalize fungicide inputs, we need high-performance monitoring tools, enabling quantitative measurement of pathogen´s inoculum levels and detection of fungicide resistant alleles. Therefore, using an automated spore sampling device positioned in a major wheat cropping region (Londrina, Paraná), coupled with a real time qPCR assay, our objectives were to i) monitor specific fungal DNA from *PoT*l airborne ascospores continuously released from 2019 to 2021, and ii) reveal the prevalence of QoI resistant (QoI-R) and QoI sensitive (QoI-S) *cyt*B alleles on samples. *PoT*l inoculum was consistently detected during the 2019 and 2020 within wheat cropping seasons with higher amounts detected in 2019. However, high peaks of *PoT*l DNA off-seasons also were continuously detected in both 2020 and 2021. Relative prevalence of QoI resistant (QoI-R) and QoI sensitive (QoI-S) *cyt*B alleles in airborne ascospores samples was detected by a combination of PCR-amplification and pyrosequencing in all the subset of 14 *PoT*l DNA randomly chosen for this assay. **Support:** FAPESP, Araucaria Foundation and IDR-Paraná (Brazil), and BBSRC (UK).

P4.1-046

EVALUATING HARD SQUASH CULTIVARS FOR SUSCEPTIBILITY TO POWDERY MILDEW AND FRUIT ROT

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Text

Michigan is an important producer of squash, pumpkins, and cucumbers in the United States. Powdery mildew (PM) (*Podosphaera xanthii* and *Golovinomyces cichoracearum*) affects cucurbits causing foliar blight, defoliation, and reduced fruit yield and quality. Fruit rot (*Phytophthora capsici*) occurs during production and postharvest and is a top grower concern. Pathogen resistance to site-specific fungicides limits mitigation strategies. We evaluated 12 *Cucurbita maxima* and *Cucurbita moschata* hard squash cultivars available for processing and the fresh market in separate, replicated, and controlled field plots under high disease pressure for susceptibility to these destructive pathogens. Age-related resistance (ARR) to *P. capsici* fruit rot occurred for all *C. moschata* cultivars 21-40 days post-pollination (DPP). For the *C. maxima* cultivars evaluated, only the Kabocha type 'Thunder' exhibited ARR at 40 DPP. When foliage was assessed for PM (% diseased foliage), all cultivars developed PM with a final disease assessment of 66 to 97%. The Kabocha type *C. maxima* cultivars Thunder (69%) and Sunshine (66%) had significantly less PM compared to the *C. maxima* cultivar NK-580 (97%) and *C. moschata* cultivar Dickinson (95%) at the end of the season. According to data from the area under the PM disease progress curve, 'Thunder' and 'Sunshine' had significantly less disease than 'Ultra' (*C. moschata*), 'New England Cheddar', and 'NK-580'. Results may inform growers and breeding programs.

P4.1-047

IDENTIFICATION OF DEFENCE MECHANISMS IN DORMANT SEEDS

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Text

Dormancy is an adaptive strategy that allows seeds to persist in the soil in the face of (a)biotic stresses to ensure germination and dispersal of the species. Our team's work has shown that imbibition of dormant *Medicago truncatula* seeds leads to the activation of a defence response (Bolingue *et al*., 2010). However, the defence pathways and their regulation during dormancy remain poorly understood. Here, we set out to identify the molecular pathways underlying defense activation in dormant tomato (*Solanum lycopersicum*) seeds. As a measure of seed defence, a method was developed that determines the antimicrobial activity in exudate from imbibing seeds against *Alternaria brassicicola* using nephelometry. Exudates from imbibing seeds that are dormant show antimicrobial activity. In contrast, during imbibition of seeds that are germinating, this activity is not detectable and only becomes evident at the seedling stage. Using the accessions of the tomato MAGIC population, we identified a large variation in the level of antimicrobial activity in the dormant seed exudates and this activity appears to be tissue specific. Current research focusses on dissecting the molecular pathways in dormant tomato seeds using transcriptomic and metabolomic analyses. These results will contribute to a better understanding how seed defends themselves and will serve to develop new strategies of seed-borne pathogens management and plant breeding.

P4.1-048

A WEATHER-BASED MODEL TO PREDICT THE POPULATION OF AUREOBASIDIUM PULLULANS AND TO IMPROVE GREY MOLD BIOLOGICAL CONTROL

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Text

Botrytis cinerea causes countless damages on an exceptionally wide range of crops. This disease is difficult to control because of the ability of the pathogen to survive under adverse environments, to profusely produce wind dispersed spores, to infect different plant tissues under a wide range of environmental conditions, to stay in a quiescent stage for up to several weeks, and to develop resistance to fungicides. Alternatives to synthetic fungicides are available but their optimal use is generally not well documented. One of the barriers to their adoption is that fungicide application recommendations are mainly based on synthetic fungicides. The objective of this study was to develop a weather-based model to predict the population temporal dynamic of the biocontrol agents Aureobasidium pullulans. Controlled

conditions experiments were conducted to determine the influence of temperature (10 to 30oC) on A. pullulans populations, estimated using a qPCR, on both strawberry leaves and flowers. Populations of A. pullulans on flowers parts were monitored under field conditions. These data were used to develop a hydrothermal model to predict the population of A. pullulans. The next step will be to integrate this predictive algorithm into a decision support tool to improve grey mould control by better timing of A. pullulans applications.

P4.1-049

MECHANISM OF ?-1,3-GLUCANASE COOPERATING WITH HSAF IN LYSOBACTER ENZYMOGENES TO ANTAGONIZE PYTHIUM

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Text

Plant diseases cause serious crop loss. Biocontrol is one of the effective measures to control plant diseases caused by pathogens. *Lysobacter enzymogenes* is an environmentally ubiquitous bacteria against a variety of pathogenic fungi and oomycetes. Its antagonistic ability mainly comes from the production of secondary metabolites HSAF and extracellular lytic enzymes such as β-1,3-glucanase. In this study, we found that when incubation *L. enzymogenes* OH11 with the pathogen *Pythium aphanidermatum*, the production of HSAF significantly increased. The same results were obtained when incubated with β-1,3-glucan, which is one of the components of oomycete cell wall. Further study showed that *L. enzymogenes* OH11 uses four β-1,3-glucanases (GluA, GluB, GluC, and endoglucanase) to degrade β-1,3-glucan into glucose, then provides energy for itself, thus increasing the production of HSAF. In this process, GluB is the last step degrading β-1,3-glucan into glucose. We also found that when targeting *P. aphanidermatum*, the transcription induction of β-1,3-glucanases genes is earlier than that of HSAF biosynthesis genes, and even the transcription of β-1,3-glucanases genes is inhibited at last. The above results indicated that when *L. enzymogenes* encounters oomycetes, it will preferentially use β-1,3-glucanase as a weapon to degrade oomycetes cell wall to gain more energy, thus launching its ultimate weapon HSAF to kill oomycetes.

P4.1-050

INTEGRATED MANAGEMENT FOR CONTROLLING VASCULAR-STREAK DIEBACK ON COCOA IN INDONESIA

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Text

Vascular streak dieback (VSD) is the main disease of cocoa in Indonesia that affected the decrease of production until the death of plant. This disease could decrease more than 40 % of photosynthetic activity of cocoa plant. Recently, the technique for controlling VSD was

vary in Indonesia. Good Agricultural Practices implementation in the controlling VSD are the usage of superior cocoa that resistant to VSD, the canopy replacement, shade trees management, specific nutrient application, applying the biological control, and fungicide application. This review shows that the integrated GAP implementation control for VSD needed to reach this goal.

P4.1-051

INTEGRATED MANAGEMENT OF INSECT VECTORS OF PIERCE'S DISEASE IN TAIWAN

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Text

Suspected symptoms of Pierce's disease (PD) of grapevines were first observed in the mountainous vineyards of Nantou, Taiwan in 2002. To monitor potential vectors, sticky yellow cards were used and detected more than 10 species of cicadelline leafhoppers and spittlebugs. Among these species, *Kolla paulula* (Walker) and *Bothrogonia ferruginea* (Fabricius) were confirmed as vectors via transmission assays between 2012 to 2016. Both species are native to Taiwan while their host plant ranges and migration patterns were not identical, and *Bidens pilosa* L. var. *radiate* Sch. is one of their preferred host plant rather than grapevines. The investigations for the relationship between field vector population dynamics and infected grape plants showed the population density of these two vectors was highly correlated to the existence of weed hosts surrounded by the vineyard. The population density can be controlled effectively by using a riding mower regularly. PD infection rate declined effectively through farmers' inspection and removal of infected plants. So far, PD was rarely reported for decades. As to the case in the plain vineyards of Changhua, PD-infected grapevines have not been detected in the area where the grapevines have to renew every 7-10 years because the groundwater level in the area was high which is harmful to the root system development of grapevines.

P4.1-052

SCREENING OF NOVEL PLANT ACTIVATORS THAT PROMOTE RESPONSE TO PATHOGENS EVEN UNDER THE EUTROPHIC CONDITIONS

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Text

We have developed a high-throughput screening (HTS) system for the defense gene

expression monitoring system using bioluminescent reporters and for discovery of plant activators. A promoter fragment of a tobacco salicylic acid (SA) inducible pathogenesis-related gene, *PR-1a* isolated from the genomic DNA of tobacco BY-2 cells, was fused to the luciferase reporter gene and introduced into Arabidopsis. To detect the *PR-1a* promoter expression as a luciferase activity, transgenic Arabidopsis seedlings were treated with chemicals with luciferin solution and the bioluminescence levels were monitored *in vivo* using a bioluminescence imaging system. Using the assay system, we have been successfully engaged in the discovery and evaluation of a wide variety of plant defense gene inducing agents (Ono et al. 2011; Watakabe et al. 2011). In this process, we found that the induction pattern of *PR-1a* gene expression changes depending on the nutrition conditions. Specifically, we found that the induction of *PR-1a* gene expression in response to the treatment with SA or acibenzolar S-methyl is strongly suppressed in Arabidopsis seedlings grown under the eutrophic conditions. Furthermore, by using the HTS system, we have succeeded in discovering compounds that have a *PR-1a* gene induction activity even under the eutrophic conditions.

P4.1-053

AN OVERVIEW OF ALTERNARIA BLIGHT IN BROCCOLI IN GEORGIA, USA

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Text

Alternaria leaf blight and head blight (ABHR) caused by Alternaria species complex is an emerging threat to brassica crops in the eastern United States particularly in Georgia. Based on a two-year survey of multiple commercial broccoli fields in Georgia, USA it was found that nearly 85% of the isolates were A. brassicicola and only 15% were identified as A. japonica. Conidial germination assay for a sub-set of isolates on azoxystrobin-amended agar medium indicated that majority of the isolates of both Alternaria species were sensitive at both concentrations (10 and 100 µg/ml). Further field studies indicated that high nitrogen application rates and avoiding overhead irrigation or irrigating in the morning (6 AM) resulted in reduced levels of percent head rot. Interestingly, it was found that older leaves are more susceptible to A. brassicicola infection and is related to reduced wax deposition compared to the younger leaves. Further it was also demonstrated that the pathogen can be seed-borne and seed transmitted under controlled conditions. The pathogenic isolates of Alternaria spp. were recovered from naturally infested commercial broccoli seedlots, which further provide indications of potential movement of pathogen through infested seedlots. Together, these studies provide information for refining management practices to reduce ABHR outbreaks in broccoli.

P4.1-054

CONTROL EFFECT AFTER TRUNK INJECTION WITH OXYTETRACYCLINE ANTIBIOTIC FOR ELAEOCARPUS SYLVESTRIS DECLINE DISEASED IN JEJU ISLAND

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Text

Since 2013, *Elaeocarpus sylvestris* planted in street trees, native habitats, and natural monuments has turned yellowing leaves, weakening branches, and eventually dying in Jeju Island. It was known as the phytoplasma that causes the decline of *E. sylvestris*, in order to reduce decline symptoms and control phytoplasma, we used oxytetracycline antibiotics including oxytetracycline wettable powder for trees, ultramycin for livestock, and aqua terra for fish, diluted to three concentrations including the standard amount, and applied gravity trunk injection method. From 2018 to 2020, we were conducted trunk injection for 40 trees per test regions in 4 regions of Jeju Island. After trunk injection, we compared vitality of trees, before and after trunk injection using Jun's Meter (PURUMBIO Co.). As a result of measuring vitality, all treatment groups showed 82.2mΩ in April (before injection), and was showed 85.2~95.5mΩ in May (after injection), and these results were indicating that the vitality of trees were improved. In addition, through visual inspection, the symptoms of decline were clearly reduced and the vitality was recovered, but several trees died in the untreated control group. The decline symptoms were recovered in the year of trunk injection, but the symptoms began to recur the following year. It is necessary to discover technology that can control phytoplasma by selecting new antibiotics which are sensitive to phytoplasma.

P4.1-055

PREVENTION OF STOMATAL ENTRY AS A STRATEGY FOR PLANT DISEASE CONTROL AGAINST FOLIAR PATHOGENIC PSEUDOMONAS SPECIES

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Text

The genus Pseudomonas includes some of the most problematic and studied foliar bacterial pathogens. In Japan, bacterial blight on Brassicaceae crops caused by *Pseudomonas cannabina* pv. *alisalensis* (*Pcal*) are causing severe problems. Generally, in a successful disease cycle, there is an initial epiphytic lifestyle on the leaf surface and a subsequent aggressive endophytic stage inside the leaf apoplast. Leaf-associated bacterial pathogens enter intercellular spaces and internal leaf tissues by natural surface opening sites, such as stomata. Currently, treatments with copper-containing compounds and antibiotics are commonly used against bacterial plant pathogens including *Pcal*. However, *Pcal* strains

resistant to these chemicals already occur in the fields. Therefore, the demand for alternative control strategies has been increasing. We have demonstrated that these three strategies prevent the entry of Pcal into plants, leading to disease reduction: 1) Cellulose nanofibers, 2) Plant activators, and 3) Amino acids. We here would like to discuss these efficient strategies for bacterial disease control to prevent bacterial entry.

P4.1-056

GENOME-WIDE ASSOCIATION MAPPING OF RESISTANCE AGAINST RICE BLAST STRAINS IN SOUTH CHINA AND IDENTIFICATION OF A NEW PIK ALLELE

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Text

Background: Effective management of rice blast, caused by the fungus *Magnaporthe oryzae*, requires an understanding of the genetic architecture of the resistance to the disease in rice. Rice resistance varies with *M.oryzae* strains, and many quantitative trait loci (QTLs) affecting rice blast resistance have been mapped using different strains of *M. oryzae* from different areas. However, little is known about the genetic architecture of rice resistance against the *M. oryzae* population in Hunan Province, which is a main rice production area in South China.
Results: In this study, we used three isolates from Hunan Province and the rice diversity panel 1 to perform a genome-wide association study (GWAS) of blast resistance in rice. A total of 56 QTLs were identified. One of the QTLs is localized with the resistance gene *Pik* locus which confers resistance to all three isolates. Genomic sequence analysis of the resistant cultivars led to the identification of a new *Pik* allele, which we named *Pikx*. Yeast two-hybrid and co-immunoprecipitation assays between AvrPiks and Pikx confirmed that *Pikx* is a new allele at the *Pik* locus.
Conclusions: Our GWAS has identified many new blast resistance QTLs. The identified new *Pik* allele *Pikx* will be useful for breeding cultivars with high resistance to blast in Hunan and other South China provinces. Further research on the relationship between *AvrPiks* and *Pikx* will provide new insights into the molecular mechanism of rice resistance to *M. oryzae*.

P4.1-057

NEW SOURCES OF RESISTANCE AND HERITABILITY TO WILT/ROOT ROT COMPLEX DISEASES IN KABULI CHICKPEA

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Text

Chickpea wilt/root rot complexes are the most important yield-limiting factors in spring planted chickpea in the Mediterranean region, south Asia, and East Africa highlands. In WANA, Fusarium wilt caused by *Fusarium oxysporum* f.sp. ciceri (*Foc*) is a dominant pathogen in the disease complex. ICARDA Kabuli chickpea breeding program evaluates breeding lines in sick plots dominated with *Foc* pathogen for global elite lines supplies. In the 2021–2022 growing season, 240 genotypes were evaluated in sick plots at ICARDA research station in Lebanon and naturally infested experimental field at ICARDA research station in Morocco. The experiment was laid out in an alpha lattice design with two replications. Percent plant mortality was scored on plot bases once the average mortality of the susceptible line (ILC482) reached over 95%. The analysis of variance (ANOVA) analysis has indicated that the genetic variation among genotypes was caused by genetic variation as broad sense heritability was high (h2 = 0.76). However, only 51.3% of the changes were attributable to the genotypes, and only 10.0% to the environment (locations). Only four genotypes (S180005, S180022, S180071, and S180079) showed good levels of resistance (<20%) at both locations. These genotypes will be utilized for pyramiding additional resistance genes into other FW races and producing high-yield breeding varieties to be shared with the national chickpea breeding programs in CWANA countries.

P4.1-058

STRAWBERRY BOTRYTIS CINEREA CONTROL BY PLANT EXTRACTS

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Text

Plant extracts can replace chemical fungicides in managing plant diseases due to their antibacterial and antifungal action, low toxicity, and biodegradability. They can inhibit diseases in their early development stages and increase plant defense response to the pathogen. The aim was to evaluate different plant extracts in various concentrations against Botrytis cinerea. Research was carried out at the LAMMC Institute of Horticulture in Lithuania. The essential oils (EO) used in this study Thymus vulgaris, Coriandrum sativum and CO2 plant extracts (PE) Mentha spicata, Nigella sativa. The B. cinerea single spore isolates obtained from infected strawberries. Evaluated concentrations: 200 µl/l, 400 µl/l, 600 µl/l, 800 µl/l, 1000 µl/l. The results showed that T. vulgaris EO achieved highest B. cinerea mycelium inhibition at concentrations from 200 µl/l. The lowest concentrations of C. sativum EO did not affect B. cinerea growth, but 800 µl/l concentration had a slight effect. All evaluated concentrations of N. sativa PE had quite low inhibition of B. cinerea mycelium. A similar tendency observed in M. spicata PE. Our data indicate that PE should be used in higher concentrations. However, essential oils are promising as bio-fungicides against strawberry B. cinerea. Acknowledgement. This project has received funding from European Regional Development Fund (project No 01.2.2-LMT-K-718-03-0035) under a grant agreement with the Research Council of Lithuania (LMTLT).

P4.1-059

SUSTAINABLE HORTICULTURAL CROPS PROTECTION IN LITHUANIA

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Text

Long-term and inadequate pesticide use can increase the environmental load and resistance risk. Integrated plant management (IPM) encourages all solutions for environmentally safe plant protection solutions that are effective and efficient. LAMMC Institute of Horticulture evaluates sustainable plant protection management systems for horticulture, reducing the usage of the same active ingredient during horticultural crop's vegetation season and prolonging the preharvest interval up to 1.5 times. Diseases control program was based on internet supported forecasting system iMETOS® (Pessl Instruments, Austria). Forecasting models for diseases are validated and adapted to local conditions. Therefore model-based applications of fungicides are more precise. Every year, crop production area managed by IPM rules increases. However, we observed several variations in national standards for crop protection and control methods, plant nutrition, and soil management. In Lithuania, a lack of resistant/tolerant cultivars and a limited pesticide supply are the major issues in integrated fruit production. Applications based on forecasting models allow reducing plant protection costs, especially when the meteorological conditions are not favourable for disease development. Acknowledgement. This research received funding from the Lithuanian Ministry of Agriculture Research and development project "Development of integrated pest management guidelines for horticultural crops" Contract MT-22-8

P4.1-060

INTEGRATED MANAGEMENT TO CONTROL BLAST DISEASE OF WHEAT

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Text

Field trials were conducted on four components: (i) seed treatment, (ii) splitting of nitrogen fertilizer, (ii) foliar spray of additional fertilizer, and (iv) fungicidal spray, under environmental conditions highly conducive to wheat blast in Bangladesh. Seed treatments with Xelora (Thiophanate-methyl + Pyraclostrobin), Vitaflow (Carboxin + Thiram), and Talc based Trichorderma harzianum were equally effective for reduction of disease severity on susceptible variety BARI Wheat 26 and partially resistant variety BARI Wheat 32. Three split of nitrogen fertilizer (basal 1/2 + crown root initiation 1/4 + maximum tillering 1/4) reduced blast severity for both varieties. Spray of additional fertilizer SiO2 at booting stage reduced disease severity and increased yield in optimum and late planting conditions for both varieties. Fungicidal spray with Opera (Epoxiconazole + Pyraclostrobin) and Cabrio (Pyraclostrobin) were more effective for reduction of disease severity and increase of yield among fifteen fungicides. Finally, two combinations of four components: (i) seed treatment with Xelora, splitting of nitrogen fertilizer, spray of additional fertilizer SiO2 and one spray of Opera (pre-heading) and Cabrio (after heading), and (ii) seed treatment with Vitaflow, splitting of nitrogen fertilizer, spray of additional

fertilizer SiO2 and one spray of Opera (pre-heading) and Cabrio (after heading) were significantly effective for integrated management of wheat blast disease.

P4.1-061

STRAWBERRY BOTRYTIS CINEREA CONTROL BY LED-LIGHT

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Text

Nowadays, challenges in plant protection demand new solutions. It's known that specific light-emitting diodes (LED) are suitable for targeted plant protection and could resolve pathogen resistance problems. The aim is to evaluate the effects of pulsed LED-light wavelengths on Botrytis cinerea inhibition. Research carried out under controlled environmental conditions. Strawberry B. cinerea single spore isolates maintained in the Petri centre with Potato dextrose agar. Conditions: 22±2°C temp., 4 and 8 h photoperiod, 20±2 µmol m−2s−1 PPFD, 32 Hz, monochromatic LED-light (red 627 nm, yellow 590 nm, cyan 505 nm, blue 470 nm and royal blue 455 nm). Mycelial growth rates (mm) evaluated daily for four days after inoculation (1-4 DAI). Results revealed that B. cinerea acted differently at 4 and 8 h photoperiods. The lowest mycelium growth was at 4 h photoperiod under red after 1 DAI, at 2-3 DAI – under cyan and at 4 DAI – under blue. However, royal blue increased mycelium growth at 2-4 DAI. The development of B. cinerea at 8 h photoperiod suppressed by blue and cyan at 1 DAI, at 2 DAI – royal blue, and at 3-4 DAI under blue. Pulsed light affects B. cinerea growth depending on the photoperiod. Our findings raise new questions about developing B. cinerea control strategy by LED-light. Acknowledgement. This project has received funding from European Regional Development Fund (project No 01.2.2-LMT-K-718-03-0035) under a grant agreement with the Research Council of Lithuania (LMTLT).

P4.1-062

STRAWBERRY INTEGRATED PLANT PROTECTION IN LITHUANIA

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Text

Botrytis cinerea is one of the major diseases of strawberry, reducing yields and requiring large amounts of pesticides during the growing season. Therefore, the EU focused on integrated plant protection to decrease pesticide use and recommended preparing IPM guidelines for crops in each country depending on environmental conditions. The aim was to evaluate a conventional plant protection system and based on a forecasting model. Field experiments carried out at LAMMC Institute of Horticulture. First year in conventional and

forecasting where 3 applications in each treatment. Second year, in conventional, was 4 and in forecasting, only one. Fungicides applications based forecasting model were more precise regardless of favourable conditions for disease development and ensured yield and quality of fruits. Results showed that conventional yield increased by an average of 2.3 and 2.7 t ha-1 in different years compared to untreated. In addition, forecasting model showed a yield increase of 4.3 and 3.2 t ha-1 in different years compared to untreated. As a result, the strawberry forecasting model-based plant protection system lowers plant protection expenses, especially when the environment is unfavourable for disease development.
Acknowledgement. This research received funding from the Lithuanian Ministry of Agriculture Research and development project “Development of integrated pest management guidelines for harmful organisms control in main greenhouse crops” Contract MT-21-9.

P4.1-063

LOCAL CONTROL CASE STUDY FOR FIRE BLIGHT ON APPLES

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Text
Fire blight (FB), caused by *Erwinia amylovora*, is a fatal disease in pome plant. Based on the case of local control of fire blight on apples, effective managing strategy for first outbreaks area is to remove infected plant after precision monitoring and to follow control manual. In June 2021, FB first occurred in the Gyeongbuk region of the Republic of Korea. Suspicious samples were confirmed using real time(RT)-PCR. All trees in the orchard were buried if the incidence rate was 5% or more. Orchards within a radius of 5 km from the outbreak site were precisely monitored, and infected trees in 6 orchards with FB were removed. Infected trees were found at a distance of up to 1.5 km from the original source. Surveillance was conducted twice more at one month intervals, but no more infected strains occurred. In February and March of the following year, farmers cut suspicious branches and trunk cankers away and then infected trees were removed through RT-PCR test. Including antibiotics, protecting materials were carried out four times from pre-blossom to fruiting period. As a result of epidemiological investigation, it was analyzed that first occurrence was most likely introduced through contaminated seedlings. Rapid spread of inner orchards be caused by frequent pruning at growth initial period.

P4.1-064

PERSPECTIVES ON THE APPLICATION OF COLD ATMOSPHERIC PRESSURE PLASMAS IN PLANT PROTECTION AGAINST SOFT ROT PECTOBACTERIACEAE

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Text

Plant pathogenic bacteria belonging to the soft rot *Pectobacteriaceae* (SRP) group are responsible for high economic losses in the production of crops, vegetables and ornamentals. No control methods are available to combat SRP infections. Thus, indirect and direct applications of cold atmospheric pressure plasmas (CAP) were investigated to eradicate these pests. In terms of the indirect approach, we synthetized the post-plasma solutions of antibacterial and plant growth promoting properties by treating mineral salts solutions with direct current atmospheric pressure glow discharge. The application of post-plasma solutions diminished soft rot disease symptoms. The antibacterial properties of these liquids were associated with deeply penetrating, reactive oxygen and nitrogen species. Moving to the direct implementation of CAP, a 2 min dielectric barrier discharge (DBD) plasma exposure eradicated >3.07 logs of SRP cells from the surface of mung bean seeds. The antimicrobial properties of DBD were linked with denaturation and aggregation of bacterial DNA and proteins in addition to rupturing of the cellular membrane leading to outflow of the cytoplasm contents. Also a 3-4% stimulation of seed germination and by 13.4% early seedlings growth were noted in the DBD-exposed seeds. Implementation of the CAP-based innovative and eco-friendly technology into agricultural practice might limit the spread and economic impact of SRP.

Funding: National Science Centre 2019/33/B/NZ9/00940

P4.1-065

EFFECTS OF LIME SULFUR MIXTURE TREATMENT CONCENTRATION AND TIME ON ORGANIC WHEAT SEED DISINFECTION AND SEEDLING GROWTH

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Text

Seedborne pathogens such as fusarium head blight (FHB) and smut are the most problematic and important biotic limiting factors for organic wheat seed production. This study aimed to evaluate the effect of treatment concentration and time of lime sulfur mixture (LSM), an organic agricultural material, on the disinfection effect and seedling growth of Keumgang wheat seeds. The fungal and bacterial infection rates of untreated Keumgangmil seeds were 45% and 0%, respectively, and the germination rate was 80%. When 0.2% LSM was treated for 3, 5, 7, and 10 minutes, the germination rate of the wheat seeds treated for 5 minutes was 100%, and the sterilization effect of fungi and bacteria was 100%, respectively. After 28 days of disinfection with 0.2% LSM, the plant height of the seedlings treated for 5 minutes was significantly longer by 10.4% compared to the untreated ones. When 0.4% LSM was treated for 3, 5, 7, and 10 minutes, the germination rate of the wheat seeds treated for 3 minutes was 100%, and the the sterilization effect of fungi and bacteria was 100%, respectively. After 28 days of seed disinfection treatment with 0.4% LSM, the plant height of Keumgangmil seedlings was examined, and the length of seedlings treated for 3 minutes was significantly longer by 12.0% than that without treatment. Based on the above results, it is considered that immersion treatment with 0.2% and 0.4% lime sulfur mixture is the most effective method for disinfecting Keumgang wheat seeds.

P4.1-066

USE OF OLIGONUCLEOTIDES FOR THE CONTROL OF BOTRYTIS CINEREA IN HORTICULTURAL CROPS

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Text

Botrytis cinerea, the causal agent of the gray mold disease, is one of the main limiting factors of horticultural crops production worldwide, consuming up to 40% of fungicides in its control. However, this fungus has been categorized by FRAC (*Fungicide Resistance Action Committee*) as a phytopathogen with a high risk for fungicide resistance development, a fact that has been demonstrated in our country. In addition, and according to the "farm to fork" strategy of the recent European Green Deal, the diversity of fungicides available to growers will be reduced by 50% in 2030. For this reason, alternative control tools and molecules with fungicide activity are needed to *B. cinerea* control. In this study, the efficacy of emerging strategies using oligonucleotides with antifungal effect has been explored. Preliminary results, obtained in *in vivo* assays, have shown a significantly reduction of the fungal development, demonstrating the potential of these oligonucleotides to be novel candidates to include in the different strategies of integrated control programs of the gray mold disease.

This work has been funded by: AYUDAS A LA I+D+i, EN EL ÁMBITO DEL PLAN ANDALUZ DE INVESTIGACIÓN, DESARROLLO E INNOVACIÓN (PAIDI 2020). Project code: PY20_00048

P4.1-067

GRAPEVINE ENDOPHYTIC BACTERIA AS POTENTIAL BIOCONTROL AGENTS AGAINST GRAPEVINE TRUNK DISEASES PATHOGENS

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Text

Grapevine trunk diseases (GTDs) are an unsolved problem in the disease management of grapevine. In the present study we aimed to screen, identify and characterize grapevine endophtic bacteria with biocontrol potential against GTD pathogens.
A total of 54 bacterial isolates were obtained from 15 healthy trunks and screened for antifungal activity against the Esca pathogen P*haeomoniella chlamydospora*. Ten positive isolates were further tested in dual culture assays against *P. chalymdospora*, *Eutypa lata*, *Botryopshaeria dothidea* and *Diaporthe eres* pathogens. One isolate (ID: 3/1) with efficient antibiosis against the above mentioned pathogens was identified as a member of *Bacillus*

amyloliquifaciens species group according to 16S rDNA sequence. Experiments with the steril culture filtrate of this isolate pointed out its fungistatic and fungicide activity, while no phytotoxicity was detected in leaf disk assays. The antifungal effect could be recovered in both the organic solvent extracts and ammonium sulfate precipitate of the culture filtrate, suggesting that the active agent is a lipoprotein.
According to the above results, isolate 3/1 can be an efficient biocontrol agent against GTDs with a special emphasis on its ability to grow in the host tissues and the secretion of effective antimycotic molecules.

P4.1-068

EVALUATION OF WILDFIRE DISEASE CONTROL EFFECT BY VARIETY ACCORDING TO BORDEAUX MIXTURE TREATMENT IN ORGANIC SOYBEAN SEED PRODUCTION

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Text
It is necessary to develop control technologies for problematic diseases such as wildfire for organic soybean cultivation. This study evaluated the efficacy of 4-4 formula Bordeaux mixture (BM) treatment on wildfire disease control efficacy by soybean variety before and after the rainy season. The soybean varieties announced in the test were Daewon, Daechan, and Nogpoong, and 4-4 BM was prepared and used in the laboratory. 4-4 BM was sprayed three times at 7-day intervals from the end of July, the rainy season, and was additionally treated once after seed formation. The occurrence of wildfire disease was significantly lower in the three soybean cultivar fields treated with 4-4 BM than in the non-treated field until harvest season. Among the three soybean cultivars, Nogpoong soybean was found to be susceptible to wildfire disease. The incidence of wildfire disease in the Nogpoong soybean block treated with 4-4 BM was significantly lower than that in the untreated block. Through the above results, it was revealed that 4-4 Bordeaux mixture treatment will be an organic agricultural material that can effectively control wildfire disease in the organic Daechan and Daewon soybean farming.

P4.1-069

INGADOSIDES A-C, ACACIC ACID-TYPE SAPONINS FROM INGA SAPINDOIDES WITH POTENT INHIBITORY ACTIVITY AGAINST GRAPEVINE DOWNY MILDEW AS POTENTIAL ALTERNATIVES TO COPPER FUNGICIDES

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Text

The reduction of copper-based fungicides has a high priority in European policy as well as in organic agriculture. To successfully reduce copper use, preventive strategies have to be fully implemented, and several substitution products need to be brought to the market. Plant-derived plant protection products could provide sustainable and environmentally friendly alternatives. As part of a project aiming at the discovery of such new products, we screened a library of more than 3000 plant extracts against important plant pathogens. One of the extracts with promising activity against grapevine downy mildew (*Plasmopara viticola*) was an 96% ethanolic extract from the leaves of *Inga sapindoides* (*in vitro* MIC_{100} 25 µg/mL). On grapevine plantlets under controlled conditions, compared to non-treated plants, the *I. sapindoides* ethanolic extract reduced grapevine downy mildew by 96%-97% at 0.5 mg/mL, and its efficacy was comparable to a standard copper treatment (two independent experiments). Targeted isolation of the active constituents resulted in the characterization of three acacic acid-type bidesmosidic saponins with high antifungal activity (in vitro MIC_{100} values of 3 - 6 µg/mL). *I. sapindoides*, a tree which is often cultivated for shading coffee plantations in Central America, may represent a sustainable source of fungicidal products to be used in the replacement of copper.

P4.1-070

OZONATED WATER APPLICATION AS AN INNOVATIVE TOOL FOR ELICITATION OF PLANT DEFENSE RESPONSE: THE CASE OF BEGONIA HYBRIDA-BOTRYTIS CINEREA PATHOSYSTEM

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Text

Ozonated water (OW) represents an innovative and eco-friendly solution for inhibiting pathogens in pre- and post-harvest. When bubbled into water, ozone dissolves partially forming reactive oxygen species, that can exert positive effects against microorganisms and contaminants. It can be directly applied as soil drench or sprayed on leaves by controlling plant diseases and avoiding chemical residues. Ozone-treated products are safe and sustainable, and this makes OW a promising approach when low impact management practices are requested. This is the case of edible flowers, known to be highly perishable and susceptible to several fungal pathogens. Here, the application of OW at different concentrations (200-800 ppb) was firstly tested in vitro on *Botrytis cinerea*, *Fusarium oxysporum*, and *Verticillium dahliae* showing a high inhibitory effect on spore germination in liquid solution (-60% at 400 and 600 ppb). According to these preliminary results, in *Begonia hybrida* plants artificially inoculated with *B. cinerea*, pot irrigation supplemented with OW (400 ppb, for 2 weeks) resulted increased number of flowers (+50 compared to uninoculated and untreated ones) with higher water content (+32%). Considering the scarce development of visible injuries associated with *B. cinerea* on petals, the obtained data indicate the priming effects of OW treatment and the potential of this sustainable technique in limiting the damage of necrotrophic fungi in edible flowers.

P4.1-071

SUSTAINABLE STRATEGIES TO MANAGE YELLOW RUST IN NORWEGIAN SPRING WHEAT

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Text
Yellow rust, caused by the fungus *Puccinia striiformis*, f.sp. *tritici* (*Pst*), is one of the most yield-reducing diseases in wheat. Employment of resistant varieties has been an effective and sustainable strategy to manage this disease. However, new *Pst* races emerged and infected previously resistant varieties in Norway in 2014. Since then, *Pst* races were monitored every year until 2022. A three-year field trial was established to test the effect of variety resistance, fungicide timing and dosage, and a combination of these factors to develop sustainable and practical management strategies in Norwegian spring wheat. We registered *Pst* infections on the digital information platform VIPS (www.vips-landbruk.no) and the RUSTWATCH crowdsource app (https://arcg.is/1zTHTS). Infected leaf samples were sent to the Global Rust Reference Center (https://wheatrust.org) for race typing. Results showed that the Norwegian *Pst* population has been dominated by PstS10 since 2014. Field trials were conducted at three different locations in the southeast of Norway over three years. We chose three varieties with different levels of *Pst* resistance, three different spraying times (early, late, and at 1% infection) and two different dosages (1/2 and ¾ dose) of fungicides. We assessed *Pst* and leaf blotch diseases, yield quantity and quality. Varieties and location had a strong effect on *Pst* development over all years. Timing and dosage of fungicides appeared less important.

P4.1-072

TEMPERATURE IMPACTS THE PROTECTIVE EFFICACY OF MICROBIAL BIOCONTROL AGENTS

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Text
The development of biocontrol agents could be a promising option to decrease reliance on fungicides for the control of white mold caused by Sclerotinia sclerotiorum on oilseed rape . Two bacterial strains have shown promising activities against this pathogen. Their effective use in the field requires that various factors be considered, which may interfere with their protective efficacy. For example, knowledge of the impact of temperature on treatment efficacy could guide recommendations for more reliable application in the field.
The aim of this study was to determine the level of efficacy of these two bacteria against S. sclerotiorum under different temperature conditions. To this end, detached leaves of oilseed rape were treated with the bacteria and incubated during 6 or 24 hours at temperatures

representative of those encountered in the field at the time of flowering, the favorable period for pathogen infection (5, 13, 21 or 28°C). Two strains of S. sclerotiorum with different levels of aggressiveness were inoculated on the leaves 24 hours after treatment and incubated at 21°C, a temperature favorable to the development of the pathogen. Temperature at the time of treatment had a substancial effect on the protective efficacy of biocontrol, depending on the strain of S. sclerotiorum. These results emphasize the need to know the factors modulating the efficacy of biocontrol agents before their use in the field.

P4.1-073

IMPACT OF THE SCN COALITION PUBLIC-PRIVATE PARTNERSHIP ON USA SOYBEAN GROWERS

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Text

The Soybean Cyst Nematode (SCN), *Heterodera glycines*, is the greatest yield-limiting biological factor in North American soybean production. This is due in part to low active management of SCN among soybean growers. In 2015, “The SCN Coalition,” a public-private partnership (PPP) of agro-chemical and seed companies, soybean grower organizations and universities formed. The SCN Coalition utilizes the multi-media strength of its partners to inform soybean growers about the increasing threat of SCN, with the objectives to increase active SCN management and reduce yield losses. Since 2018, The SCN Coalition generated over 70 million potential impressions in the U.S. agricultural media, 2 million video views, and millions of social media and personal contacts. To measure the impact of The SCN Coalition, U.S. national market research of soybean grower awareness and behavior was conducted in 2015 and 2020. In that time, over 50% of growers recalled all primary SCN Coalition messages and reported 6% to 18% increases in use of SCN management tools. Using conservative economic estimates, grower-reported increases in management and yield impacts suggest The SCN Coalition resulted in financial gains to U.S. soybean growers far exceeding $100M USD. The SCN Coalition serves as a model of a how a University-led PPP can facilitate national changes in grower behavior and economics when public and private sector partners are committed to a common vision and goal.

P4.1-074

EVALUATION OF FUNGICIDE EFFICACY AND TIMING ON PHOMA BLACK STEM OF SUNFLOWER IN THE UNITED STATES

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Text
Phoma black stem (PBS), caused by *Phoma macdonaldii* Boerema (teleomorph *Leptosphaeria lindquistii* Frezzi), is an important sunflower disease in North America, South America, Europe and Asia. Active management of PBS in the United States is rare, due partially to grower perception that PBS does not cause yield loss and limited available information on the efficacy of modern fungicides. The objectives of this study were to determine fungicide efficacy and optimal application timing for management of PBS on sunflower. Efficacy of ten fungicides from FRAC groups 3, 7, and 11 were evaluated in four field experiments in 2018 and 2019. Fungicide timing was evaluated by applying single and/or sequential applications of pyraclostrobin fungicide at three sunflower growth stages in six field experiments between 2017 and 2019. All experiments were arranged in a randomized complete block design with four replications. Efficacy was determined by calculation of PBS disease severity index (DSI) and harvested yield. Efficacy of nine of the ten fungicides evaluated, and all fungicide timings that included an early bud application, resulted in a DSI reduction when compared to the non-treated controls. The DSI was negatively correlated to sunflower yield only in high-yield environments. Results of this study provide information about the yield impact and fungicide management of PBS to sunflower growers and the sunflower industry.

P4.1-075

REAL TIME ADAPTED CANOPY VIGOR APPLICATION OF COPPER IN VINEYARD

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Text
Adaptation of canopy sprays based on the real vine vigor, determined by proximity sensors, is a promising solution to reduce fungicide use. This solution must provide a crop protection at least equal to conventional sprayers to be widely adopted. The aims of this study were to: i) test the on-the-go system for variable rate application, and ii) optimize copper distribution in vineyard. The trial was carried out within the PSR-project RIPRESO in a commercial vineyard in Colli Piacentini (Italy) area, using a low-volume sprayer. Conventional spraying (C) was compared to a variable rate (VR) application, reducing the volume by 50%, based on Canopy Index (CI) value, measured by MECS-Vine sensor. Vines growth, downy mildew incidence and severity were assessed at stages BBCH 59,73 and 79. Water sensitive papers were used to evaluate ground spray drift and spray efficacy, in terms of canopy coverage (%) and spray deposit (µg cm-2). VR allowed 15.4% reduction of Cu solution compared to C, keeping a similar canopy coverage (25-30%) and ground drift. Moreover, VR provided a similar disease control, both in terms of incidence and severity, on both leaves and bunches, of C. At harvest, yield and grape composition were not affected by treatment. This study adapted a traditional sprayer for real-time spraying adjustment based on actual CI; and

demonstrated the effectiveness of VR spray in reducing PPP use, maintaining similar crop protection, yield and fruit composition than C spray.

P4.1-076

OPTIPLASM : OPTIMISATION OF THE OFFICIAL EVALUATION OF OILSEED RAPE VARIETIES AGAINST CLUBROOT (PLASMODIOPHORA BRASSICAE).

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Text
OPTIPLASM project, started in 2019, is a project funded by CASDAR. The main objective of this project led by GEVES, in partnership with Terre Inovia and IGEPP, is to optimise the official CTPS tool for evaluating oilseed rape varieties under controlled conditions against Plasmodiophora brassicae (cruciferous clubroots). The current tool is based on a set of population isolates corresponding to the main pathotypes (P1*/P1/P2*/P3) identified in France in 2010-2013. These pathotypes tend to evolve during successive multiplications, which requires repeated characterisation to provide a robust result. The production of genetically homogeneous single-spore isolates that are stable over time is the chosen approach to overcome this difficulty. These have been produced and characterised for the different pathotypes during the 3 years of the project.
In parallel, a study of the correlation between the official CTPS test conducted under controlled conditions and the varietal response observed in the field was carried out. The idea was to evaluate the capacity of the test carried out in the laboratory to predict the behaviour of varieties in the field by comparing the behaviour of a panel of varieties under controlled conditions and in the field. As an exploratory measure, the possibility of evaluating the level of partial resistance of varieties should also be explored in order to enhance the value of genetics currently classified as sensitive.

P4.1-077

ACTIFOL : EVOLUTION OF KNOWLEDGE ON LETTUCE FUSARIUM OXYSPORUM F. SP. LACTUCAE

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Text
Fusarium oxysporum f. sp. lactucae on lettuce creates a serious threat for growers and a challenge for breeders with three main topics: emergence of a new race, the possibility or not of seed transmission and treatment.
The Actifol project, funded as CASDAR by the French Ministry involving 9 partners, studies

among other things, the possibility of seed transmission of Fusarium oxysporum f. sp. lactucae and the experimentation of alternatives methods of treatment against Fusarium on lettuce. The evaluation of seed transmission is conducted on commercial seed lots and on seeds harvested from artificially inoculated plants (from susceptible, intermediate resistant and resistant varieties). To date, of the 49 seed lots tested for detection of Fusarium, all have been negative (even those from artificially inoculated mother plants). Other seed lots are still being harvested.
Eight marketed products from different companies have been tested in controlled conditions with inoculation by Fusarium on a susceptible variety of lettuce to define the level of protection at the stage of transplantation of plantlets in 2021. After various results obtained race 1, new assays on same products are planned in 2023 and adapted to race 4 tested on two different genotypes. Results of the task will be present to the posters.

P4.1-078

INSIGHTS TOWARDS THE CONTROL OF RAMULARIA LEAF SPOT — AN INVESTIGATION OF THE RELATIVE ROLES OF BARLEY VARIETY, SEED SOURCE, FUNGICIDE TREATMENT, AND GEOGRAPHIC LOCATION

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Text
Ramularia leaf spot (RLS) caused by Ramularia collo-cygni (Rcc) is an emerging disease of barley in temperate regions including Europe. Although the disease results in a yield loss of ~30%, and reduces grain quality, there are currently no effective controls. To address knowledge gaps about the disease and identify approaches for its control we investigated the interactions between RLS and barley variety, seed source, fungicide treatment, and geographical location. Our approach was to select two barley cultivars, sourced from three different sites, and cultivate them at two locations with differing disease pressure. To evaluate the role of seed in RLS pathogenesis, seed samples were analysed for Rcc biomass through qPCR, further grown under controlled conditions, and the seedlings quantified for pathogen biomass. For the field trials, all treatments except the untreated controls, received two applications of fungicides in addition to a fungicide seed treatment. Specifically, the treatment comprised of QoI (GS 29) followed by either an RLS-targeted and non-RLS-targeted fungicide (GS 49). Treatment plots were sampled before and after the second fungicide application. Plots were scored for visual RLS symptoms, quantified for pathogen biomass, and yield data was recorded. Preliminary data suggest correlations between environment and fungicide in RLS pathogenesis. Future work will focus on interactions between plant microbiome, RLS and fungicide treatment.

P4.1-079

REDUCTION OF EUTYPA LATA IN CABERNET SAUVIGNON WOOD BY BIOCONTROL AGENTS NATIVE TO CENTRAL CHILE

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Text

The Maule Region in central Chile is one of the main producing areas of wine grapes, which is a significant commercial activity supporting economic and social development. Grape Trunk Diseases have been recognized as limiting vineyard longevity and productivity in the last 12 years. Since the first detection of *Eutypa lata* in 2020, efforts have been made to comprehensively know its epidemiology under Chilean edaphoclimatic conditions and develop sensible management practices. This study investigated the antagonistic activity of formulated biological control agents native to Chile against *E. lata*. Mamull® (*Bionectria ochroleuca* strain Mitique; *Trichoderma gamsii* strain Volqui; *T. virens* strain Ñire) and Coraza® (*T. virens* strain Ñire; *Bacillus licheniformis* strain Copihue; *B. ochroleuca* strain Mitique) were tested under field conditions, spraying or painting on wounds made by pruning 1-year-old Cabernet Sauvignon wood. Each wound was inoculated on the day of pruning with a mycelial plug of *E. lata* isolate 2B including those pruning cuts of the control plants without any treatment. Inoculations were made in August 2021 and the extent of stained wood was assessed in June 2022. Our results showed a 60 and 69% reduction in staining spread for Mamull® (sprayed) and Coraza® (painted) respectively. No foliar symptoms were observed at that time. Biological control agents could be integrated with other management strategies to control *E. lata* and Grape Trunk Diseases efficiently.

P4.1-080

INFLUENCE OF SOIL-BORNE INOCULUM FO PLASMODIOPHORA BRASSICAE MEASURED BY QPCR ON DISEASE SEVERITY OF CLUBROOT-RESISTANT CULTIVARS OF WINTER OILSEED RAPE

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Text

Brassica oilseeds have been valuable crops in Sweden for the past 80 years, and injuries of clubroot caused by the soil borne pathogen Plasmodiophora brassicae constitute a permanent threat due to the persistence of the resting spores in the soil. Access to resistant cultivars is considered the most effective tool in managing clubroot, and guidelines for managing clubroot as a part of IPM were developed based on soil analysis of P. brassicae DNA. Three clubroot resistant (Cr) commercial cultivars of winter OSR and a susceptible ´Cultivar mix´ were evaluated for disease severity (DSI) and yield performance in field soils, selected for varying abundance of natural inoculum of P. brassicae in seven field experiments 2017-2019. For cultivar mix a negative correlation (y=-252.4ln(x) + 58897.6) was found between inoculum density and seed yield, whereas no correlation was found for resistant cultivars. In comparative bioassays performed in a growth chamber ´Cultivar mix´ exhibited a high correlation between DSIb and number of gene copies g-1 soil (R2=0.72). For resistant cvs. Mentor and Alister results indicate that resistance was under pressure. The best long term control strategy for clubroot is an extensive soil testing based on DNA

technology and to use Cr cultivars in situations where the abundance of P. brassicae exceeds 1300 gene copies per g -1 soil but < 100 000 gene copies per g -1 soil, as there is a risk of losing efficacy of resistance at high inoculum densities.

P4.1-081

THE INFLUENCE OF LIQUID FERTILIZERS ENRICHED WITH BIOACTIVE COMPOUNDS ON THE DEVELOPMENT AND HEALTH OF SELECTED AGRICULTURAL CROPS – PROJECT FERTI UP

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Text

An important goal of the European Green Deal strategy is to reduce use of chemical plant protection products, including fungicides. The purpose of the project in a consortium with Grupa Azoty Zaklady Azotowe Pulawy S.A. (POLAND) and the Institute of Plant Protection – NRI in Poznan (POLAND) is to develop and implement fertilizing products based on bioactive compounds of microbial and plant origin and liquid nitrogen fertilizers that will stimulate the growth and development of plants in the initial period of plant growth and increase their resistance to biotic and abiotic stresses. The tests conducted under laboratory conditions were aimed at the efficacy of selected bioproducts in limiting the growth of mycelium of economically important pathogenic fungi. Selected bioactive compounds were then verified in terms of their compatibility with urea-based liquid fertilizers (including RSM® - urea-nitrate solution and RSM®S - urea-nitrate solution with sulfur). In the next stage, the effect of selected bioproducts applied together with appropriate liquid fertilizers on the development of oilseed rape, wheat and corn plants and the efficacy of diseases control in greenhouse and field experiments was analyzed. Works were carried out as part of the project "Supporting the development and resistance of plants using liquid fertilizers enriched with bioactive compounds", POIR.01.01.01-00-1265/20, co-financed by the NCBiR, Operational Program - Intelligent Development 2014-2020.

P4.1-082

EFFECT OF CEREAL-PULSE ROTATIONS ON FUSARIUM AVENACEUM PATHOGENICITY

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Text
Pulse root rot is caused by oomycete and fungal species, of which Fusarium avenaceum (Fa) is an important fungal pathogen. Since Fa is a common pathogen of pulse root rot and cereal head blight, we hypothesized that cereal-pulse rotations can influence Fa pathogenicity. As a first step to test this, we are assessing the natural variation in aggressiveness of Fa isolates collected from wheat, pea and lentil. Isolates collected from wheat (16) and pea (19) were screened for aggressiveness in wheat (cv Langdon) by point inoculation and pea and lentil (cvs CDC Meadow and CDC Proclaim) by soil and seed inoculations. Point inoculation of Langdon, soil inoculation of CDC Meadow and seed inoculation in CDC Proclaim had higher disease severity for pea isolates compared to wheat isolates. A similar difference in disease was not observed for a repeated trial of CDC Proclaim seed inoculation. Likewise, CDC Proclaim soil inoculation did not have a statistically significant difference between pea and wheat isolates. Thus, the general trend is that the pea isolates evaluated are more aggressive in pulses and wheat. Currently, we are screening 7 lentil isolates for aggressiveness in pulses and wheat as described above. The next step will be to select a subset of isolates which will be evaluated for altered aggressiveness after serial passage through wheat spikes or pulse roots, to determine whether passage through one crop type can influence pathogenicity on another crop type.

P4.1-083

REPEATED APPLICATIONS OF POTASSIUM BICARBONATE SUPPRESSES DOLLAR SPOT ON AMENITY TURFGRASS

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Text
Dollar spot (Clarireedia jacksonii) is the most economically important disease of amenity turfgrass in temperate climates. Increasing fungicide resistance and the declining number of novel chemistries entering the market has led to widespread interest in more integrated dollar spot control strategies. Past research has indicated that C. jacksonii requires an acidic leaf environment for infection and that there may be a metabolic cost to the fungus to modify the leaf surface pH. Our study attempted to suppress C. jacksonii infection by increasing leaf surface pH through repeated applications of potassium bicarbonate. The study was conducted at the OJ Noer Turfgrass Research Facility in Madison, WI during the summer of 2022. The results demonstrated that high rates of potassium bicarbonate applied every 7 days effectively suppressed dollar spot to a commercially acceptable level. Lower rates of potassium bicarbonate, including those found in the commercial formulation Kaligreen, demonstrated little to no suppression of the disease. Future work will focus on optimizing the application rate, reapplication interval, and water carrier volume for potassium bicarbonate and other compounds to support the development of more sustainable disease management strategies.

P4.1-084

NOVEL PLANT DEFENSE INDUCERS AND ANTIMICROBIALS FOR MANAGING HUANGLONGBING (CITRUS GREENING) AND CITRUS CANKER DISEASES

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Text

Huanglongbing (HLB), putatively caused by *Candidatus* Liberibacter asiaticus (CLas), and citrus canker, caused by *Xanthomonas citri* subsp. *citri* are the two most devastating bacterial diseases in Florida, USA. Yet, there is no HLB control and only limited control measures against canker. Thus, there is a pressing need to develop novel therapeutics for long-term disease control in citrus orchards. This study assessed new strategies for combating HLB and canker with novel class plant defense inducers (PDIs) and antimicrobials. Foliar spray applications of several PDIs on the two-year-old healthy citrus trees a week before exposure to CLas-infected Asian citrus psyllid (ACP) effectively delayed CLas infection and HLB symptoms for six months. All non-treated trees and those treated with bacteriostatic oxytetracycline were infected with CLas, two to four months after ACP exposure, with high bacterial titers. In separate experiments, PDIs and Actigard (acibenzolar-S-methyl) significantly reduced leaf canker lesions relative to non-treated control and were comparable to copper sulfate pentahydrate. Additional experiments with trunk injection of an antimicrobial compound into the CLas-infected trees reduced CLas titers and HLB symptoms in the greenhouse and field 3-6 months after injections. The disease prevention mechanisms and potential use of PDIs and antimicrobials for simultaneous management of economically important citrus diseases are currently being evaluated.

P4.1-085

THE EFFECT OF SLIGHTLY ACIDIC ELECTROLYZED WATER FOR CONTROLLING CUCURBITS POWDERY MILDEW (PODOSPHAERA XANTHII) USING VOLATILIZED METHOD UNDER GREENHOUSE CULTIVATION

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Text

Electrolyzed water has recently been used due to its potential use for controlling various plant diseases including cucurbits powdery mildew. However, developing proper application technique and efficiency for farmers are becoming a concern. This study aimed to investigate the future potential of slightly acidic electrolyzed water (SAEW) using the volatilized method focusing on the capability to suppress the disease severity of cucurbits powdery mildew and HOCl distribution inside the greenhouse. The greenhouse was divided into four (4) blocks consisting of volatilized SAEW, mist nozzle + SAEW, volatilized tap water, and mist nozzle + tap water. Cucurbit plants such as melon, cucumber, and squash were used as samples. The samples were sprayed automatically for 60 seconds during nighttime, intervals of 5 minutes for 3 hours long (only for volatilized, the continuous spray was conducted). The results indicated that SAEW using the volatilized method still effectively suppressed the disease severity only on cucumber and melon for 3 weeks consecutively, compared to tap water treatment. The concentration of HOCl inside the greenhouse showed a range of 1.4 – 5.8 μg m-2min-1. Moreover, this study revealed that SAEW has no impact on chlorophyll content, and also no water droplets were found on the leaves. This study demonstrates the possibility of controlling powdery mildew on cucurbit in a wide area using a volatilized SAEW as an alternative to pesticide use.

P4.1-086

PREPARATION OF NANO SUSTAINED RELEASE FUNGICIDE BASED ON ZIF-8 MATERIALS TO CONTROL STRAWBERRY ANTHRACNOSE

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Text

Preparation of nano sustained release fungicide based on ZIF-8 materials to control strawberry anthracnose

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Zeolitic imidazolate framework-8 (ZIF-8) is a rhombic dodecahedral crystal type cage coordination compound formed by the reaction of zinc ions as transition metals with 2-methylimidazole. In this study, prochloraz was loaded in ZIF-8 by a one pot method (Pro@ZIF-8), due to the acidic degradation of ZIF-8, making it pH responsive for drug release to control strawberry anthracnose. Dynamic light scattering results showed the particle size of Pro@ZIF-8 was 129.6 ± 14.8 nm, and scanning electron microscopy indicated the crystal structure was not changed after drug loading observed. TGA tests showed the loading capacity of prochloraz was 10.8%, the quantity of prochloraz released under pH 5.0 was 89.8 ± 4.6%, significantly higher than that pH 7.0 conditions (20.4 ±1.1%). In vitro results showed that the EC50 of Pro@ZIF-8 was 0.0150 ± 0.006 μg/mL, which was significantly different from that of prochloraz (0.0429 ± 0.01 μg/mL) in 4 days. Pot experiments showed that Pro@ZIF-8 could effectively postpone the occurrence of strawberry anthracnose in 14 days, which was better than prochloraz. Fruit preservation experiments indicated Pro@ZIF-8 could inhibited the spread of pathogen and relatively safe for fruits.

P4.1-087

INJECTING OXYTETRACYCLINE: AN EFFECTIVE APPROACH FOR MANAGING HLB DISEASE IN CITRUS

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Text

Huanglongbing (HLB) is a destructive bacterial disease associated with Candidatus Liberibacter asiaticus and vectored by the Asian citrus psyllid, for which there are no effective control measures. It is challenging for growers to manage HLB with foliar sprays since the pathogen lives within the trees' vasculature. Trunk injection ensures treatments are delivered directly into the tree's vascular tissue, allowing for improved uptake and transport. This study assessed the efficacy of injecting Oxytetracycline (OTC) in managing HLB and its effects on tree health parameters, including yield and fruit quality. We also assessed its efficacy against citrus canker (Xanthomonas citri subsp. citri), which causes disease on leaves, fruits, and twigs. In a field study where both diseases are prevalent, mature 'Duncan' grapefruit trees were injected with OTC (0.79 g active ingredient/tree) in the spring and/or fall of 2021 and 2022, and tree health and bacterial titer were assessed. OTC injection increased fruit yield and improved tree health relative to the water-injected control; however, it did not reduce the incidence of citrus canker. These findings suggest that OTC injection could be a promising tool for citrus growers to combat HLB and maintain tree health and productivity when combined with other best management practices, such as vector control and proper nutrition. The long-term effect of injection on tree wounding and recovery is currently being investigated.

P4.1-088

CONTROL OF CHICKPEA ASCOCHYTA BLIGHT USING CURATIVE FUNGICIDE STRATEGIES AND CULTIVAR RESISTANCE

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Text

Worldwide chickpea production is constrained by the fungal disease Ascochyta blight (*Ascochyta rabiei*). Disease management is often reliant on foliar fungicides to reduce grain yield losses, due to limited cultivar resistance. To develop management strategies to reduce the impact of Ascochyta blight this study, across nine experiments, investigated: 1) cultivar resistance, 2) applying fungicides preventatively (before rainfall events), or curatively (after the first signs of disease), and 3) interrow sowing in standing or slashed cereal residue. Four experiments showed a significant benefit of cultivar resistance where the moderately susceptible cultivar had grain yield losses of 38-64% as compared to 74-96% in the susceptible cultivar. In most seasons, all fungicide strategies reduced disease severity.

Comparisons between preventative or curative applications of dual active fungicides produced mixed results for grain yield. In drier environments, there was no significant difference in grain yield, but in wetter environments, yield losses were significantly greater with the curative compared to the preventative treatments. In both environments there was greater disease severity observed with curative fungicide strategies. Sowing chickpeas in standing cereal residue significantly reduced grain yield losses to disease.
This study highlighted the benefits of cultivar resistance, curative fungicide applications in drier environments, and interrow sowing in standing cereal residue.

P4.1-089

SMALL PEPTIDES AS POTENTIAL ENHANCERS OF A PLANT'S RESPONSE TO PATHOGEN ATTACK – INVESTIGATING THEIR UPTAKE INTO PLANT CELLS FOLLOWING EXOGENOUS APPLICATION.

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Text
Small peptides (<100 amino acids) are involved in a wide range of biological responses in plants to both biotic and abiotic stimuli, including Programmed Cell Death (PCD). In the presence of the exogenously applied, plant-derived small peptide Kiss of Death (KOD_{pep}), *Arabidopsis thaliana* root hairs undergo a morphological PCD-like response at low KOD_{pep} concentrations and/or limited incubation periods. The efficacy of two cell penetrating peptide (CPP) fluorescein isothiocyanate (FITC) labelled tags was also investigated: a classic CPP tag (RQIKIWFQNRRAKWKK) and the more recently discovered amoeba-derived CPP tag (RRVQIWFQNKRAKVKR). The presence of either CPP tag resulted in increased intracellular uptake of KOD_{pep} as indicated by an increase in fluorescence activity. However, this was associated with a loss of a PCD-like morphological response which was superseded with a necrotic-like response at high peptide concentrations and/or longer incubation periods. Biological activity of the KOD_{pep} to induce a PCD-like morphological response in the absence of a CPP tag was detected, with novel intracellular uptake of KOD_{pep} observed. The potential role of KOD_{pep} in a plant's response to a pathogen and the utility of small peptides to participate in the arsenal of plant defence strategies will be presented.

P4.1-090

FUNGICIDE APPLICATION METHOD IMPACTS ON MAIZE GRAIN FILL DURATION, KERNEL DYNAMICS, AND GRAIN YIELD

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Text
Climatic variability and emerging maize (Zea mays L.) disease has driven farmers toward using multiple fungicide applications to enhance grain yield. Despite observed fungicide yield increases, minimal research has addressed the physiological mechanisms behind observed yield increases. Specifically, how does disease control impact grain fill duration and kernel formation. Trial objective(s) were to assess how fungicide application methods can impact maize grain fill, kernel formation, and yield. In 2022, 6 trials were initiated in Indiana, Kentucky, and Michigan, USA. Treatments include: 1) control (C), no fungicide applied, 2) C + starter fungicide (Flutriafol) applied at planting, 3) C + foliar fungicide (prothioconazole, trifloxystrobin, and fluopyram) applied at the R1 growth stage, and 4) C + starter fungicide and foliar fungicide. Preliminary results observed yield increases at 2 of 6 site-years and 4 of 6 site-years from starter fungicide and R1 fungicide, respectively. In addition, starter fungicide and R1 fungicide increased kernel number at 3 of 6 and 4 of 6 site-years, respectively. Furthermore, starter fungicide, R1 fungicide, and their combination increased grain fill duration by 4, 5, and 4 days, respectively and increased maximum kernel weight by 5, 5, and 10%, respectively. Overall, preliminary data shows the ability of different fungicide application methods to reduce leaf disease severity, increase kernel number, grain fill duration, and kernel weight.

P4.1-091

EVALUATION OF BACTERIAL ISOLATES FOR BIOLOGICAL CONTROL OF BROWN SPOT IN RICE PLANTS

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Text
Biological agents can control brown spot (Bipolaris oryzae) in rice (Oryza sativa), increasing sustainability and food quality. Our aim was characterize biochemically and identify the most effective microorganism antagonist to B. oryzae. Two assays (E1 and E2) were conducted, composed of twenty-two treatments and three replications in completely randomized design. E1 characterized all 21 isolates for production of extracellular enzymes, siderophores, biofilm, indoleacetic acid (AIA) and phosphate solubilization. E2 tested in vitro antagonism between 21 isolates and B. oryzae. All isolates produced Lipase, N-fixation, phosphorus, sideophere, AIA and biofilm, Protease produced twenty isolates (95.24%), Pectinase and cellulose produced sixteen isolates (76.20%), Amilase produced fourteen isolates (66.67%), Potassium produced eight isolates (38.10%) lacase produced four isolates (19.05%), zinc produced two isolates (9.52%) while ligninase produced only isolate (4.8%), the antagonist results showed that Bacillus cereus (BRM65921 and BRM65922) and Prestia megaterium (BRM65915) reduced radial colony growth of B. oryzae by 98.28, 98.28 and 90.35 % respectively

P4.1-094

EFFICACY OF A ZERO-RESIDUE STRATEGY AGAINST FIELD AND POSTHARVEST DISEASES ON STRAWBERRIES

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Text

In recent years, growing concerns associated to pesticide residues in fruit have led to develop alternative crop protection strategies, including zero-residue strategy (ZeR). We assessed the impact of a ZeR strategy on fruit quality and postharvest microbiota of strawberries (Fragaria x ananassa), compared to a conventional IPM strategy. Data on fungal pathogen incidence was gathered over two years in two Italian farms. Fruit picked from treated farms were sampled for yield, quality parameters and microbiota composition at harvest, after storage and at the end of shelf-life, while pesticide residues were quantified by accredited laboratories. Disease incidence showed no significant difference between the adopted strategies for either farm in both year. No systematic impact of the strategy was found on yield (commercial production, average fruit weight, number of fruit per plant), titratable acidity, total soluble solids. Minor differences were observed for fruit firmness and colour parameters. Quantification of residues on fruit returned lower levels for the ZeR strategy, although a long-term contamination by fosetyl-Al was noticed. Microbiota composition was unaffected by the crop protection strategy and resulted in very high levels of Botrytis at the end of shelf-life. The ZeR strategy showed comparable efficacy to the conventional strategy.

P4.1-095

CAN WE PREDICT WHETHER A BIOCONTROL PRODUCT WILL BE EFFICIENT?

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Text

In 2019, the scientific community of plant pathologists reflected on major scientific questions that remained to be solved in our field in the coming years (Harris, Balint-Kurti et al. 2020). Among these questions, the following had been identified: what is the impact of abiotic stresses on plant/microbe interactions, and more generally, how does the knowledge gained on simple interactions hold up in an ecological context? Indeed, for most interactions, we have very little idea of how interactions between plants and microbes are affected by the ecological environment in which they live. At the same time, the concept of eco-immunity has been described (Schulenburg et al., 2009). Broadly speaking, this concept aims to understand and explain variation in immune response, in other words, to determine why and how biotic and abiotic factors contribute to variation in the immunity of a living organism (Nobori and Tsuda 2019). In this presentation, we will elaborate on the effects of five factors in combination or not on the establishment of the septoria (Zymoseptoria tritici) infection cycle in wheat seedlings. Thus, we studied the impact of the partial resistance of the host variety, the water stress undergone by the seedling during the infection, two types of

treatments: a root biostimulant at sowing to promote host growth and a plant defense stimulator applied before inoculation to reinforce host defenses and finaly we tested the effect of a neighboring co-cultivated plant .

PROGRESS IN DISEASE CONTROL - Part2

C6.1-1

THE AUSTRALIAN WAY OF CROP DISEASE CONTROL IN AGRICULTURE AND HORTICULTURE

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Text

The history of extensive crop production in Australia only goes back to 1788 when the first British settlers set up their first colony in Sydney Cove. Since then, many broadacre and horticultural crops have been introduced to Australia from overseas and grown extensively there; in fact, all but one of the important crops grown in Australia are introduced species (macadamia, an Australian native, being the only exception). Inadvertently, many of the pathogens of those crops were also introduced to Australia before efficient plant biosecurity measures were set up and applied rigorously as part of border control and to prevent the spread of plant diseases within the country. As of today, the Australian crop production industry is valued at over AU$20 billion per year. Crop disease control is shaped by the climate, soil characteristics, technology, and other specific conditions, including the lack of important plant pathogens such as Xylella fastidiosa, Puccinia striiformis f. sp. hordei and Guignardia bidwellii that are kept out of the country as a result of the plant biosecurity system. This presentation will provide an overview of crop disease control practices in Australia; fungicide use; plant biosecurity; technology; and the research and development sector in this field.

C6.1-2

INTEGRATED CONTROL OF STRAWBERRY POWDERY MILDEW INCORPORATING THE USE OF A PREDICTION SYSTEM AND A BIO-AVAILABLE SILICON NUTRIENT

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Text
Strawberry powdery mildew (*Podospheara aphanis*) is the most serious epidemic disease of protected strawberries in many parts of the world. In order to control this disease many growers are spraying with fungicides every 7 or 8 days, resulting in up to 22 sprays in a 6 months season. This heavy use of fungicides may prevent epidemics developing but it has some deleterious consequences, for example expense, lack of appropriate active ingredients and the accumulation of a high number of pesticide residues in the fruit. The work presented here will demonstrate how the use of an on-farm, real-time decision support system can prevent epidemic development but also reduce the number of fungicides used by half. This system has now been in operation on commercial farms for 5 years and shows not only disease control but also a reduction in labour and fuel use which gives a financial saving. Furthermore the paper will demonstrate how the regular use of a bio-available silicon nutrient in the fertigation system enhances the passive defence pathway of strawberries and thus gives increased resilience to powdery mildew. This integrated use of different approaches enables strawberry growers to achieve their goal of increasing yield from each plant and also achieving sustainable production with reduced environmental impact.

C6.1-3

FUNGICIDE RESISTANCE MANAGEMENT, MONITORING AND ALTERNATIVES INVESTIGATED IN THE SOUTH AFRICAN CITRUS INDUSTRY

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Text
South Africa is one of the largest exporters of fresh citrus fruit. Production of high quality fruit is therefore critically important. Effective management of economically and phytosanitary fruit pathogens are therefore important. Preharvest and postharvest fruit pathogens such as *Alternaria alternata*, *Phyllosticta citricarpa*, *Penicillium digitatum* and *Geotrichum citri-aurantii* are managed using a combination of orchard practices, packhouse sanitation and fungicide applications. As these pathogens are prone to developing resistance to fungicides, resistance management practices include the application of fungicides with different modes of action either in tank mixtures or at different points in the packhouse. Resistance monitoring include regular testing of pathogen isolates for reduced sensitivity and characterisation of fungicide sensitivity within the populations of abovementioned pathogens. In cases where reduced sensitivity is detected, and a molecular resistance assay is not available, these assays are developed through research projects. However, with increasing pressure to reduce fungicide residues on fruit, research is also done into alternative, non-chemical management options. The resistance management and monitoring practices followed in the South African citrus industry will be described while some preliminary results of research into alternative management options will be outlined.

C6.1-4

A PERSPECTIVE FOR THE USE OF SYNTHETIC ANALOGS OF THE NATURAL PEPTAIBOL TRICHOGIN GA IV AS BIOPESTICIDES

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Text

Trichoderma spp. represent a rich source of bioactive molecules for controlling plant pathogens. As bioactive molecules, antimicrobial peptides (AMPs) are gaining interest as candidates for new eco-friendly plant protection products. Peptaibols are a peculiar class of AMPs with a well-defined helical structure but poor water solubility, which hampers their use in crop protection. Based on these premises, we used a biorational approach to synthesize peptide analogs of the natural peptaibol trichogin GA IV with Gly-to-Lys substitutions to enhance water solubility and antimicrobial activity. We obtained several water-soluble analogs with *in vitro* fungicidal and bactericidal activity. Some of these analogs completely inhibited the growth of the fungus *Botrytis cinerea* and the sporulation of the oomycete *Plasmopara viticola* at low micromolar concentrations. The peptide analogs also exerted bactericidal activity against the bacteria *Xanthomonas campestris* pv. *campestris* (Xcc), *Pseudomonas syringae* pv. *tomato* and *P. syringae* pv. *actinidiae*. In growth chamber experiments, the most effective peptides protected grapevine from *P. viticola* and *B. cinerea* and cauliflower from Xcc without phytotoxic effects. The microscopic analysis showed different alterations of the cell membrane and the cytoplasm depending on the examined plant pathogen. Finally, in a two-year field trial, one selected peptide resulted as effective as a cupric fungicide in protecting the vineyard from downy mildew.

C6.1-5

THE EVOLUTION OF FUNGICIDE RESISTANCE IN EUROPEAN CEREAL PATHOGEN POPULATIONS

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Text

Cereal disease control in Europe relies heavily on fungicides, combined with some varietal resistance. However, some pathogens that are key targets of control programmes, such as *Zymoseptoria tritici* on wheat, *Ramularia collo-cygni* and *Pyrenophora teres* on barley, and cereal rusts and powdery mildews, have proven to be highly adaptable in the face of crop protection measures. *Z. tritici* has evolved resistance against MBC and QoI fungicides and ongoing slides in sensitivity to azole and SDHI fungicides, as well as breakdown of host resistance such as that in Cougar-derived varieties.

The differing nature of resistance against the various fungicide classes has had different impacts on disease control. In some cases, a single mutation conferring high levels of resistance has spread rapidly through the pathogen population resulting in control failures

and the effective loss of that fungicide class for the control of that pathogen. In other cases, the gradual accumulation of mutations each contributing only partial resistance has been countered by the development of new, more active fungicides within that class. We will present data on the ongoing shifts and associated genotypes in *Z. tritici* sensitivity to azoles and SDHIs. We are also developing in vitro selection approaches to improve predictions of future resistance evolution, enabling more pre-emptive resistance monitoring and management.

C6.1-6

INTEGRATED PLANT DISEASE MANAGEMENT OF CEREALS IN FRANCE: FROM RESEARCH TO PRACTICE

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Text

Cereal diseases are numerous and need to be controlled for worldwide food safety from both quantitative and qualitative perspectives. This control must be effective, environmentally friendly, economically sustainable, and socially suitable. Integrated Plant Disease Management (IPDM) aims to answer those complex expectations by combining all solutions which are already available or to be developed. These strategies are based on three principles: prevention, risk assessment and control for which many researches are carried out in order to provide such innovative solutions for farmers. We can mention genetic resistances, decision support system, biocontrol products, diseases monitoring, remote sensing instruments, companion plants, new technical practices…
Here, we purpose to make a state of the art of the main diseases of cereals in France, the principal means of control available and how IDPM is currently implemented by farmers and stakeholders. We will focus on how characterizing the methods of integrated protection to help the producer to choose the best combination for him? How do producers adopt these new practices to deal with plant diseases as well as to face climate changes? What research should focus on the most to complement current solutions?
Such answers will highlight the significant advances arising in IPDM in France but also outline the gap we still need to fulfill by strengthening research and supporting farmers towards a successful agroecological transition.

P6.1-001

EFFICACY OF FOLIAR FUNGICIDES FOR THE MANAGEMENT OF FUSARIUM HEAD BLIGHT OF WHEAT AND MYCOTOXIN ACCUMULATION IN WHEAT GRAIN

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Text
The chemical control of F. graminearum s.s., causal pathogen of Fusarium head blight (FHB) of wheat and the mycotoxins it produces, is an integral disease management strategy. However, no fungicides are currently registered against FHB in South Africa. This study aimed to determine the efficacy of several foliar fungicides to control FHB and mycotoxin contamination in different production regions of South Africa. Commercial fungicides were evaluated during 2021 in a field trial each in the Western Cape (WC) and Free State provinces, respectively. Five wheat cultivars were evaluated in a randomised complete block design with three replicates at each location. Fungicides were applied to flowering wheat heads, two days prior to inoculation with F. graminearum s.s. Additional treatments included a fungicide only, fungus only and water only controls. Disease incidence, DON contamination and thousand kernel weight (TKW) was measured. All fungicides effectively reduced disease incidence and DON levels in field trials compared to the control while also increasing TKW. Significant differences between locations, cultivars and treatments for parameters were determined. All fungicide applications significantly reduced DON levels at Napier (WC) for inoculated bunches. The results obtained in this study confirms that foliar fungicides can effectively contribute to managing FHB and mycotoxins in wheat grain and thus represents a feasible disease management strategy.

P6.1-002

EFFECTS OF SEED PRETREATMENTS WITH MIXED BIOPRODUCTS FOR IMPROVING DISEASE TOLERANCE AND GROWTH OF PEPPER SEEDLINGS

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Text
Pre-sowing treatment of seeds is a very simple method for farmers' to increase growth, yield and also for pathogens management. Pepper seeds were subjected to combined application of Trichoderma isolate(T), chitin(CH) and salicylic acid(SA) for assessment of growth promotion and defense induction against Rhizoctonia solani, an important destructive soil-borne phytopathogenic fungus that causes severe loss of agricultural crops around the world. Effects of combined treatments with T+SA+CH, T+CH, T+SA, CH+SA on growth parameters, polyphenols, chlorophylls and carotenoids concentrations and on the activities of antioxidant enzymes, peroxidase (POX) and polyphenoloxidase (PPO), were examined in pepper seedlings leaves. The treatment of pepper seeds with all combinations used had a beneficial effect on the growth and development of pepper plants, both in the absence or in presence of the pathogen. Among treatments, the sequential applications of T+CH and T+SA+CH most effectively stimulated the plant's antioxidant system and thus resistance to the pathogen. The combination of SA+CH had no significant effect on plant growth nor on the antioxidant system. The use of natural compounds in combination with microbial suspensions may be recommended for application particularly to seeds in order to produce high quality crops due to the induction of disease resistance.

P6.1-003

VIRULENCE AND AZOXYSTROBIN SENSITIVITY ANALYSIS OF THE STRAWBERRY NEOPESTALOTIOPSIS CROWN ROT PATHOGENS FROM CHINA

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Text

In recent years strawberry crown rot caused by *Neopestalotiopsis spp* has occurred in many places in China, leading to an increasing economic loss. In order to understand their virulence and fungicide resistance profiles, 71 isolates of *Neopestalotiopsis spp* pathogens collected from six provinces (Fujian, Yunnan, Inner Mongolia, Sichuan, Jiangsu and Shandong) over China were determined for pathogenicity and sensitivity to azoxystrobin by a detached leaf assay and mycelial growth rate. The results showed that virulence differed significantly among the isolates from different geographical sources and was positively correlated to the annual mean temperature of the sampling sites. The EC50 values of azoxystrobin to the 71 *Neopestalotiopsis* isolates ranged from 2.89 µg/mL to 52.80 µg/mL, with an average of 44.57 µg/mL. Association between azoxystrobin sensitivity and conidia production was dose-dependent but there was no correlation between azoxystrobin sensitivity and virulence of the pathogens. The results of this study provide useless information for the formulation of azoxystrobin control plan for strawberry *Neopestalotiopsis* crown rot in China.

P6.1-004

DYNAMICS OF TOTAL, VIABLE AND CULTURABLE STATES OF TWO BACTERIAL BIOLOGICAL CONTROL STRAINS APPLIED TO GRAPEVINE AND FRUIT-TREE CROPS

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Text

The population dynamics of the biological control strains *Bacillus velezensis* A17 and *Lactobacillus plantarum* PM411 were evaluated after their release into grapevines in Penedès area (Catalunya, Spain) and apricot and peach trees in Torreilles (France). The population levels were quantified by viability qPCR, qPCR, and dilution-plate counting in leaves, flowers, and fruits over two growing seasons. *B. velezensis* A17 showed high survival rates in all crops. The viability of all A17 cells was confirmed since qPCR and viability qPCR estimations did not differ significantly. However, higher levels were estimated by dilution-plate counting due to the non-selective characteristics of the growth medium used. In contrast, the viability of *L. plantarum* PM411 was conditioned depending on the plant tissue, crop, and climate conditions.

In general, the viable population level of PM411 was higher in apricot than in grapevine. In general, the PM411 survival declined some days after application, indicating difficulties in its establishment. The population level of PM411 was made up of dead, culturable, and/or viable but non-culturable cells since significant differences between the three methods were observed. The size of each portion changed depending on the sampling time point and the field trial. In conclusion, A17 and PM411 differ strongly in their colonization and survival in grapevine, peach, and apricot.
Funding was provided by INTERREG POCTEFA EFA182/16/PALVIP.

P6.1-005

POTENTIAL OF AN EXPERIMENTAL BACILLUS VELEZENSIS STRAIN AS A BIOCONTROL AGENT TO CONTROL GRAPEVINE BLACK ROT DISEASE

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Text

Guignardia bidwellii (Ellis; anamorph *Phyllosticta ampelicida*), a hemibiotrophic fungus responsible for black rot (BR) disease in grapevine, could turn into a serious threat in the near future. Causing potential severe crop loss, it currently benefits from the withdrawal of several synthetic fungicides and the lack of commercial specific biocontrol alternatives. Within the framework of the French research project VITAE, combinations of management options, such as biocontrol and vine genetic resistance, are investigated.
To evaluate the efficiency and mode(s) of action of a biocontrol experimental candidate, *i.e*. a *Bacillus velezensis* strain, culture supernatants were tested via *in planta* efficacy bioassays in greenhouse (cv. Artaban & Marselan), and *in vitro* direct confrontation assays. The application of the bacterial supernatant significantly reduced both BR incidence and severity, with protection rates reaching 90% *in planta*. Two major modes of action accounting for the supernatant efficiency are put forward and deeply investigated: a direct effect against the pathogen and the elicitation of grapevine defense responses.
Thus, the *B. velezensis* supernatant could act as an efficient biocontrol tool in a future strategy of grapevine protection. The mechanisms of action and their relative importance will be further discussed by quantifying the antibiosis capacity under different conditions and at different infection stages, as well as deciphering the defense induction pattern.

P6.1-006

EFFECTS OF FLUBENETERAM ON INHIBITING THE DEVELOPMENT OF PUCCINIA STRIIFORMIS F. SP. TRITICI IN WHEAT LEAVES

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Text
Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is a serious threat to wheat production, and the application of fungicides is one of the most important means of controlling the disease. The purpose of this study is to determine effects of a new succinate dehydrogenase inhibitor (SDHI) fungicide, flubeneteram. Results showed that flubeneteram displayed significantly inhibitory effects on SDH enzymes of *Pst*. The baseline sensitivity of 173 *Pst* isolates from 13 provinces of China to flubeneteram was determined. Histological observations showed that after flubeneteram application, the formation and development of *Pst* hyphae and haustoria were significantly inhibited, and the structures were destroyed. The biomass of *Pst* in wheat leaves was significantly reduced at 18 h after flubeneteram application. Finally, it was found that flubeneteram could induce the upregulation of genes related to callose synthesis in wheat, thus increasing the number of callose deposition; moreover, flubeneteram also primed wheat for SA-induced defenses via upregulating pathogenesis-related genes (*PR1* and *PR2*), which play roles in preventing *Pst* infection. Altogether, our study is the first to provide evidence that flubeneteram induces wheat defense against *Pst* infection. The findings indicate that flubeneteram could be an effective fungicide for managing stripe rust.
Additional keywords: wheat stripe rust, flubeneteram; ultrastructure, histo chemistry, induced systemic resistance

P6.1-007

EVALUATION OF FUNGICIDES AND FUNGICIDE APPLICATION METHODS TO MANAGE PHYTOPHTHORA BLIGHT OF PIGEONPEA

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Text
Phytophthora blight of pigeonpea caused by Phytophthora cajani has been significantly increasing in major pigeonpea production regions of India. Limited information on pathogen infection, epidemiology and lack of adequate resistant cultivars is hampering the P. cajani management significantly. Therefore, five fungicides viz. metiram + dimethomorph, mancozeb + cymoxanil, cymoxanil + famoxadone, mancozeb and, mancozeb + metalaxyl-M were evaluated against P. cajani under control condition to control zoospore induction as well as infection of zoospores at seedling stage. Half maximal effective concentration (EC50) of fungicides for mycelial inhibition was calculated. Lowest EC50 was recorded in metiram + dimethomorph (0.17µg/ml) followed by mancozeb + metalaxyl-M (2.49µg/ml) and mancozeb + cymoxanil (8.23µg/ml) fungicides. The sporangium and zoospore formation significantly affected by mancozeb + metalaxyl-M followed by metiram + dimethomorph and mancozeb + cymoxanil on sporangia viability, zoospores germination and encystment. Further, under glasshouse conditions, different fungicide application methods (e.g. seed-treatment, soil-drench, foliar-spray either singly or in combinations) were evaluated with fungicides on susceptible (ICP 7119) moderately resistant pigeonpea (ICPL 99010, ICPL 20135 and ICPL 99048) cultivars. Among fungicide application methods seed-treatment + soil-drench, soil-drench + foliar-spray and soil-drench are effective in controlling of Phytophthora blight.

P6.1-008

CONTROLLING CUCUMBER POWDERY MILDEW AND ANTHRACNOSE BY BACILLUS VELEZENSIS STRAIN TCB43

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Text

Powdery mildew and anthracnose are important diseases during the harvest period of cucumbers, which could cause yield reduction, quality decline or shorter harvest period in severe cases. A bacterial strain, designated Tcb43, capable of inhibiting the germination of fungal spores was isolated from an organic red dragon fruit field. The phenotypes of Tcb43 cultured on nutrient agar was changeable, quite different from other *Bacillus* species. The Tcb43 strain was identified as *Bacillus velezensis* using whole genome sequencing. In the fungal inhibition tests, the Tcb43 could inhibit the mycelial growth of *Colletotrichum orbiculare* in confrontation assays, and the germination rate of the conidia of *Podosphaera xanthii* was significantly reduced by more than 85% after treating with the 200-fold dilution (200X) Tcb43 fermentation broth. Greenhouse assays revealed that treating cucumber plants with 200X Tcb43 reduced the incidence of anthracnose disease by 50% after treatment for three consecutive weeks. In field trial, the cucumber plant treated with 200X of Tcb43 fermentation broth by spraying continuous 5 weeks, reduced the incidence of powdery mildew disease up to 68% compared to the mock treatment. This work revealed that the effectiveness of the rhizobacterium *B. velezensis* Tcb43 to antagonize the anthracnose and powdery mildew fungus in greenhouse or field trial, indicating that Tcb43 is a potential biocontrol agent.

P6.1-009

TEACH ME HOW TO PROTECT YOU: THE GRAPEVINE LESSON ON DOWNY MILDEW CONTROL

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Text

In nature, plants have evolved several mechanisms to protect themselves from pathogens. Agriculture limited the defense possibilities by reducing the genetic variability of the plant and selecting the cultivated varieties, based more on quality traits than on pathogen resistance. This is particularly true for grapevine (*Vitis vinifera* L.), a woody plant that is susceptible to several diseases, such as downy mildew. In the past, the search for disease management tools has been focused on fungicides to target the pathogen primarily, and secondarily on introgressing resistant traits of other *Vitis* species into the cultivated grapevine. No attention has been paid to *V. vinifera* as a source of resistance. The recent discovery of a resistant

cultivar (Mgaloblishvili) in a grapevine collection of the *V. vinifera* domestication center (Georgia, South Caucasus), that possesses a high genetic variability, changed our perspectives on breeding. Moreover, it led to the development of biotechnological and natural fungicides from susceptibility/resistance mechanisms. dsRNA was designed to trigger RNA interference and transiently silence candidate susceptibility genes (*e.g.*, *VviLBDlf7*), and antifungal compounds (terpenes) were identified in Mgaloblishvili. An effective disease protection was achieved in both cases. Further implementations of these and other findings will shape a new era in grapevine downy mildew control, that will be possible thanks to the efforts in germplasm conservation.

P6.1-010

BISMUTH SUBSALICYLATE, A FUNGISTATIC COMPOUND AND PLANT DEFENCE STIMULATOR WITH POTENTIAL FOR MANAGEMENT OF GRAPEVINE TRUNK DISEASES.

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Text

Since use of sodium arsenite was banned in 2001, increasing numbers of grapevines affected by grapevine trunk diseases (GTDs) have been observed, and lack of highly effective control products has led research on bismuth subsalicylate (BSS). The antifungal capacity of BSS (which contains salicylic acid) was assessed against GTD pathogens, and for ability to stimulate plant defence genes. An objective was to design an appropriate formulation for BSS which had water solubility. A suitable formulation based on a liquid polymer was developed, with small particle size which increased the bioavaibility of the compound, an extremely important feature for eventual developments. Antifungal potency of the formulated BSS against GTD pathogens was confirmed, through growth inhibition of *Neofusicoccum parvum* (isolates Bt 67 and Bourgogne), *Diplodia seriata* (98.1) and *Fomitiporia mediterranea* (PHCO36). Stimulation of defence genes was analysed by RT-qPCR on grapevine callus (*VvPAL, VvEDS1, VvHSR1* overexpressed), and the non toxicity of BSS on grapevine cells was confirmed by fluorescence microscopy. BSS was then evaluated *in planta* using vertical plant endotherapy laboratory technique, where BSS was injected directly into grapevine rotten wood where mycelium complexes are concentrated. Preliminary observations will be presented for symptomatic grapevines treated (n=100) with BSS for 2 years in Alsace, taking into account the complexity of GTD symptom expression.

P6.1-011

COMBINED EFFECT OF ABIOTIC PARAMETERS ON TRICHODERMA SP. GROWTH WITH BIOCONTROL POTENTIAL ON STORED GRAIN PHYTOPATHOGENS

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Text

Temperature, pH, and water activity are key factors in the growth behavior, development and biocontrol activity of the fungal strains. These abiotic factors are relevant for different application in agriculture and biotechnology. *Trichoderma* spp. is well known as very effective biological mean for plant disease management. The present work aimed to establish the effects of environmental factors on the mycelial radial growth of *Trichoderma* sp. strain (isolate Tr.1) and its biocontrol capacity against stored grain phytopathogens. Also, it was evaluated the antagonistic effect of an isolate of *Trichoderma* sp. on five strains of *Penicilium* spp., *Fusarium* spp and *Botritys* sp. phytopathogens, by dual culture method. The pH used were 4, 5, 6 7, 8 and incubation temperatures were 10°C, 15°C, 25°C, 30°C, 35°C. The results of experiments showed that the most favorable pH was between 5 and 6, while pH 8 and pH 4 showed significant reduction in the growth parameters of *Trichoderma* sp. The results revealed that tested strain has grown better between 25°C and 30°C. Mycelial growth was reduced at 15°C and inhibited at 10°C. After six days of incubation at 28°C, the antagonistic capacity of *Trichoderma* sp. isolate was recorded. The results showed that *Trichoderma* sp. has high biocontrol potential as it inhibited the growth of all tested fungal strains associated with grain seeds, in percentage between 59.52% and 84.71%.

P6.1-012

BIOCIDAL ACTIVITY OF PLANT BY-PRODUCTS TO CONTROL PLANT DISEASES USING FOLIAR APPLICATIONS

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Text

Using substances of natural origin is a promising alternative to synthetic pesticides. The biocidal activity of organic extracts derived from waste streams of tomato shoots, chicory roots, wheat bran, celery, fennel, and sunflower stems was investigated against plant pathogens Phytophthora infestans, Botrytis cinerea, Blumeria graminis, Alternaria solani and A. alternata in the greenhouse. The dry waste sources were mechanically grinded for solid-liquid extraction using different types of solvents (water, ethanol, ethyl acetate and hexane). Foliar applications were implemented, at 10,000 ppm and 100,000 ppm dilutions of concentrated extracts on potato, tomato and wheat plants 24 h before inoculation. Disease assessment was measured based on disease severity index (DI). From a total of 58 extracts, water solvent-based extracts at 100,000 ppm exhibited the best inhibitory effect. Celery extract showed the best inhibitory effect (DI 11.11%) against P. infestans, followed by chicory root, wheat bran and fennel with DIs 20.74%, 22.96% and 23.70%, respectively. These extracts also displayed a moderate effect against B. graminis and A. alternata with DI ≈ 55%. A significant disease control (p-value < 0.05) was observed against B. cinerea on tomato plants using sunflower extract (DI 5.56%). The protective activity of these extracts against tested plant pathogens can be further processed as biopesticides to be applied alone or in combination with other disease management strategies.

P6.1-013

IMPROVING BLAST RESISTANCE OF THE RICE CULTIVAR ‘KAOHSIUNG 145’ USING MULTILINE VARIETY AND GENE PYRAMIDING STRATEGIES

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Text

Rice blast is an important limiting factor for rice growth. Deploying resistant varieties is an effective, economical, and eco-friendly way to manage rice blast. However, most resistant varieties possess only one major resistance gene, making the resistance easily to be broken down by field isolates after 3-5 years of monoculture on large scales. ‘Kaohsiung 145 (KH145)’ is an elite Taiwanese japonica rice cultivar with good rice quality and high yield, but moderate susceptibility to blast disease. To improve the resistance of KH145 and achieve durable resistance, this study aimed to develop KH145 multilines and pyramid lines by marker-assisted breeding and evaluate their blast resistance under field conditions. In farmers’ fields in Daliao and Meinong, Kaohsiung and Guanshan, Taitung in 2021 and 2022, a mixture of three KH145 monogenic lines carrying *Pi9*, *Pik-h*, and *Ptr* showed a much higher leaf and panicle resistance than the KH145 pure line. By testing the disease severity and genotypes of individual plants, we found susceptible-type lesions on some plants with Pik-h, indicating that this gene is being overcome. The three-gene pyramided lines, which were developed by hybridization of the KH145 monogenic lines, also exhibited high resistance to leaf and panicle blast in the upland and paddy blast nurseries. The lines are being selfed to increase the homozygosity. Also, lines with good agronomic traits and rice quality will be selected for commercial cultivation.

P6.1-014

EFFECT OF NANO ZINC LOADED PGPR BIOACTIVE FORMULATION ON PHYSIOCHEMICAL PROPERTIES AND ITS RELEASE EFFICIENCY AGAINST RHIZOCTONIA SOLANI KUHN

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Text

Rhizoctonia solani Kuhn is an ubiquitous in its distribution in worldwide and in India causing 8-50% yield losses to the farmers. Zinc oxide nanoparticles are generally regarded as GRAS materials due to its safe application and having antimicrobial activity. The present study focussed on the study of physiochemical properties by exploring the enzymatic activity on Superoxide dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) and on the release efficiency against R. solani against different concentrations of green engineered nano zinc loaded PGPR bioactive formulation (En-ZnO-NP-BF) from 1, 5, 10, 20, 50, 100, 150 and 200 ppm. The release efficiency was found to be increasing after treating the rice seeds with En-ZnO-NP-BF and placed in MS media. 200 ppm possessed the highest release efficiency with 47.46% followed by 150 ppm with 32.28% after 24 hrs. Sclerotia treated with En-ZnO-NP-BF showed highest mycelial inhibition of 98.81% at 200 ppm followed by 84.81% for 150 ppm compared to control 0.00%. No sclerotia was found to formed at 200 ppm of En-ZnO-NP-BF. This was followed by 150 ppm (11.67). In control maximum (53) sclerotia was found to form. The present study conclude that En-ZnO-NP-BF at 200 ppm was showing highest enzymatic activity against fungus and sclerotia formation and mycelial growth also reduced after treating sclerotia with En-ZnO-NP-BF.

P6.1-015

THE EFFECT OF SOIL MICROBIAL INOCULANTS ON THE SUSCEPTIBILITY OF GRAPEVINE TO PLASMOPARA VITICOLA INFECTION.

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Text

The control of Grapevine Downy mildew (GDM), caused by the oomycete Plasmopra viticola, strongly relies on applications of chemical fungicides, with negative impacts on the environment and human health. The use of beneficial microorganisms rises as an alternative in GDM management, in form of both microbial pesticides (biocontrol agents) and soil microbiota. The interest in the application of soil microbial inoculants (SMIs), including mycorrhizal fungi, has been increasing in recent years as biostimulants increase the growth and development of agricultural and horticultural crops, and mitigate environmental stresses. The effect of SMIs to augment the plant defense against pathogens has also been demonstrated. In 2021 and 2022, we implemented a RCB design experiment in a five-year vineyard in northern Italy, in which 7 commercial SMIs were applied at the rhizosphere level at the start of each season, in comparison with a nontreated test (NT). Between flowering and veraison, leaves were inoculated with a sporangial suspension of P. viticola in the field and under lab inoculations, and the resulting GDM severity was assessed; the inoculation experiment was repeated three times. Some SMIs were able to reduce disease severity compared to NT, with a maximum of 56 % efficacy. The results support the potential interest in SMIs to potentiate plant resistance against P.viticola in the frame of sustainable management of GDM in viticulture.

P6.1-016

EFICACY AND COMPATIBILITY OF BIOLOGICAL AGENTS AND CHEMICAL FUNGICIDES FOR THE MANEGEMENT OF SCLEROTINIA SCLEROTIORUM ON SOYBEAN.

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Text

The white mold caused by *Sclerotinia sclerotiorum* cause reduction of 20% to 70% of production of soybean in the South of Brazil and the control is based on fungicide sprays. Nowadays, biological control has been emerging in the manegement of the disease. The aim of this study was to evaluate the eficacy of *Trichoderma harzianum* (ESALQ1306), *Bacillus subtilis* (QST713) and *B. amyloliquefaciens* (CPQBA 040-11DRM 01) in the control of *S. sclerotiorum* isolates, alone and in combination with chemical fungicides. First, the sensitivity of biological agents to Bixafem+Protioconazol+Trifloxistrobin and Methyl tiofanate+Fluazinam was evaluated in vitro. This was followed by in vitro assays that evaluated the eficacy of biological agents and chemical fungicides (alone and in combination) in reduction the micelial growth of three isolates of *S. sclerotiorum*, by direct contact and pairing. *T. harzianum* inhibited the pathogen 70 to 90% (direct contact) and 100% (pairing) but was not compatible with fungicides; *B. subtilis* and *B. amyloliquefaciens* inhibited the pathogen just when paired (80 to 100%) and were compatible whit Methyl tiofanate+Fluazinam but not with Bixafem+Protioconazol+Trifloxistrobin. Fungicides inhibited 100% of pathogen micelial growth independently of isolate. These results are important for the adequacy of the disease management program, mostly about the simultaneous application of products, and should be validated in field tests.

P6.1-017

USE OF COPPER-BASED FUNGICIDES IN ORGANIC AGRICULTURE IN TWELVE EUROPEAN COUNTRIES

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Text
The reduction of copper-based plant-protection products with the final aim of phasing out has a high priority in European policy, as well as in organic agriculture. The aim of this survey was to provide an overview of the current use of these products in European organic agriculture and the need for alternatives to allow policymakers to develop strategies for a complete phasing out. Since there is a lack of centralized databases on pesticide use, we combined expert knowledge on permitted and real copper use per crop and country, with statistics on organic area in 12 European countries covering 83% of the organically managed horticultural area. We calculated that approximately 3258 t copper metal per year is used by organic agriculture in these countries, equalling to 53% of the permitted annual dosage. This amount is split between olives (1263 t y^{-1}, 39%), grapevine (990t y^{-1}, 30%), and almonds (317 t y^{-1}, 10%), followed by other crops with much smaller annual uses (< 80 t y^{-1}). In 56% of the allowed cases (countries × crops), farmers use less than half of the allowed amount, and in 27%, they use less than a quarter. At the time being, completely abandoning copper fungicides would lead to high yield losses in many crops. To successfully reduce or avoid copper use, all preventive strategies have to be fully implemented, breeding programs need to be intensified, and several affordable alternative products need to be brought to the market.

P6.1-018

EVALUATION OF MYCOPARASITIC FUNGI AS POTENTIAL BIOLOGICAL CONTROL AGENTS FOR WATTLE RUST (UROMYCLADIUM ACACIAE)

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Text
Wattle rust, caused by *Uromycladium acaciae* (*Cooke*) *P. Syd. & Syd*), is the most economically important disease currently affecting black wattle (*Acacia mearnsii*) plantations in South Africa. Available control measures for wattle rust consist of rust tolerant clones, azoxystrobin- based fungicides and difenoconazole fungicides. There is an urgent need to find alternative chemical and biological control measures that will comply with Forest Stewardship Council (FSC) regulations in the management of wattle rust. *Sphaerellopsis filum* and *Lecanicillium lecanii* are well known mycoparasites of rust. This study aims to evaluate the efficacy of S. filum and L. lecanii for the control of wattle rust through a series of potted nursery trials. Early results have shown that biweekly applications of S. filum significantly reduced rust disease progression. Although applications with *L. lecanii* did reduce rust disease progression, it did not produce a significant reduction relevant to the untreated rust control. This study demonstrates that *S. filum* has the potential to control wattle rust and could be used as part of an integrated pest management strategy for the disease.

P6.1-019

ANTIFUNGAL ACTIVITY OF BIOACTIVE MOLECULES ISOLATED FROM AGRICULTURAL WASTE AGAINST RICE BLAST FUNGUS

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Text

Developing chemicals with antifungal activity from agricultural wastes is a promising approach to reduce the environmental impact of disease management strategies in plant protection and potentially a valuable source of novel modes of action. Pyricularia oryzae is able to infect rice plants at all stages of development such as leaves, stems, nodes and panicles. The almost exclusive use of chemical fungicides, e.g. strubilurins, for rice blast managements increases the risk of emergence of fungicide resistant strains. The goal of our study was to extract bioactive compounds from grapevine wastes assessing their efficacy against P. oryzae strobilurin-resistant and -sensitive strains. We tested 28 compounds on two strobilurin-resistant and two sensitive P. oryzae strains, both on spore germination and mycelium growth inhibition. One compound inhibited the mycelial growth of resistant strains by 32% and that of sensitive strains by 58% at 0.06 mM concentration. Other two compounds completely inhibited spore germination of the resistant strains at 1mM without inhibiting mycelium growth. This preliminary work will pave the way for the synthesis of nature-derived compounds possibly acting on diverse cellular targets, exhibiting dual or multiple modes of action.

P6.1-020

EFFICACY OF IN-FURROW FUNGICIDE APPLICATION TO MANAGE SOUTHERN BLIGHT IN MISSISSIPPI PEANUT FIELDS.

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Text

Southern blight (SB), caused by Athelia rolfsii (AR) is a soil-borne fungus that can cause losses of up to 80%. Symptoms are water-soaked lesions, yellowing, wilting, and plant death. Currently, fungicides are used to manage the disease. This study was conducted to test the efficacy of in-furrow fungicide applications to control SB. In 2022, a field study was conducted in Stoneville, MS to evaluate the effect of in-furrow fungicides on the severity of AR, in peanut. Seven fungicides (Quadris, Solatenol, Omega, Velum, Proline, Elatus, Revytek) and a non-treated control were evaluated in a RCBD with four replications. Each plot was planted to four rows of ‘Georgia-06’ in Bosket fine sandy loam. During planting, two rows of each plot were inoculated with sterilized millet colonized by AR, and two rows were left non-inoculated. Stand counts, vigor, and phytotoxicity were collected at 15 and 30 DAP, and disease severity was recorded at 90 and 110 DAP. The data collected were analyzed using ANOVA in R studio software. No significant differences were observed in the severity of AR among the treatments; however, there was a numerical reduction of 11% in the incidence of AR in the plot treated with Elatus compared to the control. Despite no significant differences in yield, an average increase of 13% was observed in plots treated with Omega.

Further research is needed to assess the potential of in-furrow fungicide applications as a management strategy for SB in peanuts.

P6.1-021

ARABINOGALACTAN PROTEIN-LIKE PROTEINS FROM ULVA LACTUCA ACTIVATE IMMUNE RESPONSES AND PLANT RESISTANCE IN AN OILSEED CROP

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Text

Natural compounds isolated from macroalgae are promising, ecofriendly, and multifunctional bioinoculants, which have been tested and used in agriculture. We have characterized for the first time an arabinogalactan protein-like (AGP-like) from Ulva lactuca, which exhibits several features associated to land plant AGPs. In land plant, AGPs were shown to play a role in several plant biological functions, including cell morphogenesis, reproduction, and plant-microbe interactions. Here, we have evaluated its ability to (i) protect oilseed rape cotyledons against Leptosphaeria maculans, and (ii) its ability to activate immune responses. Preventive application of the Ulva AGP-like enriched fraction on oilseed rape, followed by cotyledon inoculation with L. maculans, resulted in a major reduction of infection propagation. The noticed reduction correlated with an accumulation of H2O2 in treated cotyledons and with the activation of SA and ET signaling pathways in oilseed rape cotyledons. In parallel, an ulvan was also isolated from Ulva lactuca. Preventive application of ulvan also enhanced plant resistance against L. maculans. Surprisingly, reduction of infection severity was only observed at high concentration of ulvan. Together, this study indicates that U. lactuca AGP-like glycoproteins exhibit promising elicitor activity and that plant eliciting properties of Ulva extract, might result not only from an ulvan-originated eliciting activities, but also AGP-like originated.

P6.1-022

BIOTECHNOLOGICALLY PRODUCED BIOACTIVE COMPOUNDS OF PLANT ORIGIN TO EXPLOIT IN PLANT PROTECTION

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Text

During their evolution, plants have developed mechanisms to adapt to their growth environment and deal with various types of stresses. The mechanisms that determine the composition of each environmental niche largely involve the creation of chemical heterogeneity by harnessing the metabolic flux from primary metabolism. Following this, plants produce species-specific chemical compounds through the exploitation of a network of interconnected biosynthetic steps. Eminent molecules of this are the compounds resveratrol and hydroxytyrosol both of which are directly connected to human health protection. Resveratrol, a stilbenoid, is mainly produced in grape skins, and hydroxytyrosol, a phenylethanoid, is produced in olive leave tissues and fruits. Various studies over the past decades have led to the identification of the genes involved in their biosynthesis. The latter, allowed the heterologous reconstitution of their biochemical pathways making possible their bulk production in microbial factories. After certain purification steps, such bioactive compounds of plant origin have been proven to be equally beneficial when they are utilized as antibiotics both in in-vitro as well as in-planta experimentation. Formulations of both types of compounds were able to inhibit either fungal or bacterial growth. Thus, their use as plant protection agents is investigated to replace existing commercial pesticides and contribute to environmental protection and consumer safety

P6.1-023

PSA3 VIRULENCE REDUCTION BY NATURAL MOLECULES: INSIGHTS ON SUBTLE REGULATION MECHANISMS

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Text

Pseudomonas syringae pv. *actinidiae* (Psa) is the causal agent of kiwifruit bacterial canker disease, currently, the disease management relies on copper-based compounds, which could force the insurgence of copper-resistance Psa strains, as already observed with other plant pathogens. Thus, new control strategies should target bacterial virulence mechanisms, such as the type III secretion system, to weaken pathogens rather than prevent their growth *in planta*. Exploiting a reporter system based on the GFP expression driven by the promoter region of the *hrpA1* gene, which encodes one of the major components of the T3SS, a chemical library was screened to seek molecules capable of reducing T3SS induction in the most aggressive Psa biovar 3. Among selected candidates, dicumarol was further characterized for its ability to dampen Psa virulence *in planta*. Moreover, a proteomic analysis suggested that dicoumarol could interfere with a regulatory mechanism common to

both ‘injectisome’ and flagellum-related T3SS.

P6.1-024

SPECTROSCOPIC DETECTION OF CROP AND FOREST DISEASES

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Text

Sustainable agriculture and forest management require advancements in high-throughput techniques to early and accurately detect plant stresses. Spectroscopy has emerged as a promising tool to monitor vegetation status (from leaf to landscape level), being a non-destructive, rapid, and relatively low-cost technique: it relies on the interaction of light with plant chemical/structural composition and water content. The present work highlights the potential of this technology for detecting and monitoring crop and forest diseases. First, it briefly reports basic concepts of vegetation spectroscopy. Then, it shows the capability of hyperspectral data to detect and monitor crop diseases, focusing on a case study on wheat blast disease [e.g., inoculated leaves were well discriminated by the analysis of spectral profiles (accuracy >0.75), even before the onset of visible symptoms]. Finally, it highlights that although the use of such spectral approaches has risen sharply, most studies have been carried out for agricultural applications, while the utilization for detecting and monitoring forest diseases has been less investigated. Indeed, although a number of forest diseases have been detected and quantified using optical sensors, some major issues need to be addressed, such as the lack in exploring several major diseases and geographic areas, and in the use of this approach for long-term monitoring.

P6.1-025

BIOCHEMICAL CHANGES IN VITIS VINIFERA LEAVES AND RESPONSES TO BOTRYTIS CINEREA INFECTION AFTER THE APPLICATION OF A YEAST EXTRACT FORMULATE

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Text

Grapevine (Vitis vinifera L.) is one of the most economically important crops worldwide, and the increase in wine production rate demand requires changes in the agricultural processing and manufacturing practices to make them sustainable. Issues associated with the large use of agrochemicals and consumer needs for residue free products have stimulated research

into new and eco-friendly tools for sustainable pest management and vine protection. The aim of the present study (supported by Kwizda Agro GmbH) was to characterize at functional level the “indirect” protective mechanisms induced by the application of a yeast extract formulate (YE) through the induction of defense responses in V. vinifera cv. Sangiovese plants artificially inoculated with Botrytis cinerea (Bc). In YE+/Bc+ leaves, germ tubes did not elongate well, and their hyphae were slightly spread over the leaves after 2 days. The hydrogen peroxide induction (observed from 1 h post inoculation; +40% compared with YE-/Bc- ones) triggered a production of ethylene and a concomitant accumulation of jasmonic acid (7 fold-higher and +34%, respectively). These results confirm a synergistic action in the regulation of defense reactions. The absence of oxidative stress (as confirmed by the unchanged values of malondialdehyde by-products) and the early activation of a signaling pathway suggest the capability of YE to induce resistance in grapevine against necrotrophic pathogens.

P6.1-027

DIVERSITY OF STRAINS OF RALSTONIA SOLANACEARUM SPECIES COMPLEX IN BENIN IN WEST AFRICA

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Text

Bacterial wilt, caused by Ralstonia solanacearum (Rs), is a major constraint to key cash crops in West Africa. In Benin, BW is particularly problematic in traditional leafy vegetables (Gboma and amaranth) and on key vegetable crops (tomato and pepper). In this study, the distribution of Rs across the main vegetable-growing regions in Benin was characterized for the first time, which is critical to implement targeted regional management. Overall, 27 localities were surveyed, and 50 strains were isolated and confirmed to be Rs-positive by species-specific primers. Rs diversity was broad, and strains from 3 phylotypes I, II & III were isolated. Most isolates (43) identified by PCR using a phylotype-specific primer were phylotype I and were isolated from tomato, pepper, bitter leaf, African basil, and weed. There were 1 strain in phylo I & II (tomato), 2 in phylo II (tomato), 1 in phylo III (pepper), and 3 in phylo II & III (tomato). The presence of Asian (phylo I) strain may be due to accidental introductions from humans. Sequevar analysis using egl primers showed that most strains belonged to sequences 17 and 18, while few others belonged to sequevars 14, 23, and 31. Given that peanuts are a significant source of protein for people in West Africa, greenhouse trials validated the pathogenicity of the seq. 14 strains on peanuts. The targeted approaches to control Rs, can help protect economically important crops and improve livelihood of subsistence farmers in Benin.

P6.1-028

INTEGRATED MANAGEMENT TO CONTROL BARLEY DISEASES

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Text

Barley (*Hordeum vulgare*) is the major cereal crop in Scotland. Many common barley varieties have only moderate resistance to the major foliar and ear diseases, especially in winter barley crops. Diseases can have significant impact on both the yield and quality of barley crops. Ramularia leaf spot has been increasingly difficult to control with one of the most effective plant protection products losing its registration in 2020.
Investigations into control of barley diseases in replicated field trials in both winter and spring barley crops using a combination of biologicals, elicitors and fungicides has been conducted over several years. Elicitors and biologicals were applied to seed and growing crops at the T0 timing (GS 24-30). Management programmes were designed to combine the use of the elicitors and biologicals with reduced rates of conventional fungicides.
Results indicate there is potential for new programmes and new products to maintain barley production whilst reducing our dependence on agrochemicals. Programmes with elicitors as a seed treatment and a T0 treatment followed by quarter dose rate fungicides gave significant yield increases over untreated crops and were only just behind a normal commercial fungicide programme in winter barley crops. Additionally, in Laminarin seed treated plots, the biological spray outperformed the fungicide and gave a significant reduction in disease and an increase in yield.

P6.1-029

NEW FUNGICIDE ALTERNATIVES TO FUMIGATION FOR MANAGING POTATO EARLY DIE

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Text

Potato Early Die Complex (PED) is an annual concern in potato growing areas worldwide causing up to 40% yield losses. The primary causal agent is Verticillium dahliae. However, several other pathogens may contribute, including root lesion nematodes (Pratylenchus spp.) and Colletotrichum coccodes. Pre-plant application of metam sodium has been the standard chemical practice for managing PED. However, high cost, worker safety and increasingly stringent regulations are motivating growers to look for alternative practices. New non-fumigant approaches are being developed to manage the various pathogens responsible for PED. Among these is fluopyram, developed by Bayer CropScience as Velum® Prime. To investigate the efficacy of fluopyram for managing PED, a study was carried out in grower fields in S Idaho from 2019 to 2022. Stems were collected from plants in grower fields treated with either Velum Prime or the grower standard three times (late July, mid-August and early September) and levels of V. dahliae and C. coccodes were quantified using qPCR. Plants treated with fluopyram showed a significant reduction in both V. dahliae and C. coccodes compared to the grower

standard. The plants treated with fluopyram also showed significant yield increases compared to the grower standard. This studysuggests that it is possible to manage PED using low dose at-planting and post-emergence applications of fluopyram.

P6.1-030

EVALUATION OF FUNGICIDE PROGRAMS FOR THE MANAGEMENT OF AERIAL STEM ROT IN POTATO.

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Text

Aerial stem rot (ASR) is an important foliar disease of potato worldwide and is predominantly caused by the pectolytic soft rot bacteria Pectobacterium carotovorum. It differs from black leg (caused by P. atrosepticum) as it is found later in the season after row closure. The pathogen primarily enters through wounds in the stem and expands outward leaving a distinct black lesion. These lesions spread down the stem as opposed to black leg lesions which spread up the stem from diseased tubers. Decaying stems appear watery and gooey and optimal conditions can spread lesions quickly and desiccate the entire stem. Ten years of field trials were conducted using susceptible varieties in a randomized complete block design. Treatments consisted of different formulations of copper containing fungicides starting at row closure and continuing on a 7-day schedule until vine kill. Plots were inoculated with P. carotovorium two weeks after the initial treatment. When disease symptoms started to show up in untreated plots, disease incidence was rated visually as the number of infected stems per plant every four days over a a two-to-three-week period. These results were used to calculate the Relative Area Under the Disease Progress Curve, showing the rate of disease progress. Results showed that treatments with the copper containing fungicides Previsto and Badge SC significantly reduced the incidence and severity of aerial stem rot compared to the non-treated control.

P6.1-031

ADAPTIVE MELANISATION AND TOLERANCE UNDER UV-C LIGHT

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Text

UV-C light (200-280nm) can combat a variety of microorganisms, including necrotrophic fungi like *Botrytis cinerea* (grey mould), offering a viable alternative to agrochemical control. Yet a balance must be struck between controlling infection and causing damage to plants. We exposed *B. cinerea* conidia and early vegetative mycelia to increasing UV-C doses (up to 8 minutes), logging survival data, and colony-forming units. We also applied a unique grey-

scale imaging method to measure melanin phenotypic changes post-treatment. Following that, we applied a non-toxic UV-C treatment (4 minutes) with incubation at 4°C (postharvest conditions) and 21°C (production conditions). We then analysed changes in gene expression in the melanin biosynthetic pathway. Melanogenic genes both up- and downstream in the pathway are targets for ongoing mutation survival studies. Initial survival trials confirm significant lethality in conidia with increasing UV-C dose, but that mycelial-stage *B. cinerea* not only demonstrated lower lethality rates but also a darker phenotype compared to untreated. Gene expression moreover shows significant and rapid upregulation in upstream gene (*YGH1*) within 30 minutes of UV-C treatment with an associated lag in upregulation of downstream genes (*BRN1*, *BRN2*). Adaptive melanisation to non-lethal UV-C treatment highlights the need for a more comprehensive understanding of grey mould pathogenic biology under current commercial UV-C treatment.

P6.1-032

A NEW PLANT PROTECTION CONCEPT BASED ON THE AUTOCIDAL CONTROL OF FUNGI

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Text

Apple scab caused by the fungus *Venturia inaequalis*, is the most important disease of apples in temperate countries. The main commercial apple varieties are susceptible to this disease and the phytosanitary treatments applied in the orchard to get rid of it represent a considerable economic and environmental cost (up to 30 fungicide applications/year). To combat apple scab, we are developing a totally new biocontrol strategy combining two inventions. The first invention which can be linked to autocidal control makes the fungus non-virulent. We target the sexual phase of *V. inaequalis* by forcing it to reproduce with non-pathogenic strains on apple belonging to the *forma specialis* pyracanthae of *V. inaequalis* (PYR strains), resulting in the generation of non-virulent progeny on apple the following spring. The second invention consists of applying the PYR strains in the spring to protect young apple leaves against a subsequent scab attack. It is expected that this breakthrough technical itinerary will cause a collapse in the size of the pathogen population in the orchard and thus allow reduction in the use of fungicides. This project involving phytopathologists, geneticists, orchards experimenters, economists and professionals of apple sector aims to prove the concept of this innovative strategy, to evaluate its sustainability as well as the conditions of acceptance and appropriation of the inventions by the apple sector

P6.1-034

CROSS-PROTECTION IN PLANT VIRUSES: HOW CLOSELY RELATED DO PROTECTING AND CHALLENGING VIRUSES NEED TO BE?

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Text
Cross-protection is a biocontrol method to protect plants from damage caused by pathogenic viruses. This strategy of cross-protecting a plant with a mild strain to prevent subsequent infection by related severe strains was first described in 1926. Although cross-protection was first evidenced nearly a century ago, the underlying mechanism(s) remain(s) poorly understood. In particular, although the genetic relatedness between cross-protective and challenge strains has been emphasized in a number of papers, the percentage of identity required for cross-protection remains unknown. This lack of data has led to a long and sometimes unsuccessful empirical search for cross-protective strains against some viral pathogenic ones. Recent papers have shown that cross-protection occurs only among variants of the same strain and not between variants of different strains for the citrus tristeza virus (Closterovirus) and the pepino mosaic virus (Potexvirus). In order to determine the percentage of identity required between the cross-protective and the challenge strains for cross-protection to be effective against the grapevine fanleaf virus (GFLV, Secoviridae), different cross-protective variants with decreasing sequence homology with respect to the challenge variant were selected and inoculated on Nicotiana benthamiana. Our results suggest that in addition to the genetic relatedness, the entry point of the cross-protective and challenge strains impacts the cross-protection success.

P6.1-035

MANAGEMENT OF WHITE RUST (ALBUGO CANDIDA) DISEASE OF RED AMARANTH FOR SEED PRODUCTION

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Text
Experiments were conducted for management of white rust disease of red amaranth (Amaranthus tricolor L.) for seed production during 2018 and 2019 at Dhaka, Bangladesh by following RCBD design with three replications. Eight treatments including chemicals, botanicals, bio-pesticides viz. Ridomil gold 68WG (Mencozeb + Metalaxyl @ 0.2%), Autostin 50 WP (Carbendazim @ 0.2%), Dithane M 45 (Mancozeb @ 0.2%), Goldton 50WP (Copper oxychloride @ 0.2%), Bordeaus mixture (CaO + CuSO4 @ 1%), G-Derma (Trichoderma sp.) @ 0.3%, Garlic bulb extract 1:1 (w/v) (Allium sativum @ 2%), and Allamanda leaf extract 1:1 (w/v) (Alamanda cathertica @ 2%) were considered for the management of white rust disease of Red amaranth (Amaranthus tricolor L.). Four foliar sprays were done at seven days interval, started from 7 days after disease appeared. Among the treatments, Ridomil gold 68WG gave best result. Moreover, Allamanda leaf extract showed better effect and Autostin 50WP had moderate effect against the disease. Growth and seed yield of red amaranth varied significantly among the treatments. In Rabi season of 2018, After 4th spray, the lowest plant incidence and severity were recorded in Ridomil Gold (24%; 1.83%) followed by Allamanda leaf extract (31%; 2.43%). Similar results also found in kharif season of 2019 where after 4th spray, the lowest plant incidence and severity were recorded in Ridomil Gold (18.66%; 1.93%) which was statistically identical with Allamanda leaf extract (22%; 2.23%).

P6.1-036

COMPARATIVE EVALUATION OF CHEMICAL FUNGICIDES AGAINST SHEATH BLIGHT DISEASE OF RICE IN AMAN SEASON

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Text

Two experiments were conducted to evaluate the efficacy of chemical fungicides for the management of sheath blight disease of rice in Bangladesh during 2019-20, RCBD design with 3 replications. Sheath blight disease susceptible variety BR 11 was used in these experiments. Fifteen treatments viz. T1 (Tilt 250 EC), T2 (Contaf 5 EC), T3 (Folicur 250 EC), T4 (Score 250 EC), T5 (Combi 230 EC), T6 (Filia 525 SE), T7 (Amister Top 325 SC), T8 (Acibin 28 SC), T9 (Awal 72 WP), T10 (Nativo 75 WP), T11 (Autostin 50 WP), T12 (Dithane M-45), T13 (Sunvit 50 WP), T14 (Control1 with inoculation) and T15 (Control2 without inoculation) were evaluated against Rhizoctonia solani causing sheath blight disease of rice (BR 11) in aman season. In field experiment, after the 4th spray the lowest disease incidence per hill (30%), disease incidence per tiller (10.80%), disease severity (10.80%) and percent relative lesion height (%RLH), (2.44%) was found in T2 treated plot (Contaf 5 EC). However the highest disease incidence per hill (93.33%), disease incidence per tiller (44.71%), disease severity (48.36%) and % RLH (9.11%) was found in T14 after the final spray. Similarly in case of yield contributing characters, the highest yield (6.94 ton/ha) and straw yield (7.12 ton/ha) was found in T2 treated plot while lowest yield (3.49 ton/ha), straw yield (4.32 ton/ha) observed in T14. Moreover, T3 (Folicur 250 EC) and T4 (Score 250 EC) were also significantly effective against this disease.

P6.1-037

CROSS-PROTECTION, A VIABLE METHOD TO FIGHT FANLEAF DEGENERATION? STUDY OF 14-YEAR-OLD PRIMARY-INFECTED VINES IN A VINEYARD PLOT

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Text

Fanleaf degeneration is a worldwide disease causing a severe decline of vineyards with serious economic losses. Its main causal agent is the grapevine fanleaf virus (GFLV), a virus transmitted from grapevine to grapevine by an ectoparasitic nematode. While GFLV is depicted as one of the most severe viruses of grapevines, infected-vines are not always symptomatic and show a wide range of symptoms with varying degrees of severity. The lack of effective measure to control this disease led us to explore mild strain cross-protection (MSCP) as a biocontrol method. In MSCP, primary infection with a mild isolate is used to prevent secondary

infection(s) by related severe variant(s). Efficient and long-term MSCP relies on the selection of isolates causing mild symptoms on the cultivar of interest and able to prevent subsequent infection by challenger variants present in its growing environment. In this study, the efficacity of MSCP was assessed by monitoring 2000 vines primary-infected with local mild GFLV isolates implanted in a severely diseased commercial plot. After 14 years, most vines remained mildly symptomatic. NGS analyses of 200 representatives of these vines (in terms of symptoms severity), suggest a great resilience of the primary infection and a moderate level of superinfection. We will discuss our results with the aim at developing MSCP as a workable approach to prevent fanleaf degeneration and its deleterious impact on viticulture.

P6.1-038

SIDE EFFECTS OF D-LIMONENE ON THE MEALYBUG DESTROYER , CRYPTOLAEMUS MONTROUZIERI MULSANT (COLEOPTERA: COCCINELLIDAE)

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Text

Before incorporating a pesticide into IPM programs, it is necessary to evaluate its impact on the main natural enemies. The lethal and sublethal effect of d-limonene (60 g/L) on adults and larvae of Cryptolaemus montrouzieri (Mulsant) were evaluated under laboratory conditions (26°C) through topical application and ingestion of treated individuals of Dactylopius opuntiae (Cockerell) female (a scale pest that caused damage to Opuntia spp. cactus crops in Morocco and many countries worldwide). The toxicity of this insecticide was compared to that of chlorpyrifos-methyl (480 g/L) and pyriproxyfen (100 g/L), which are both commonly known to be harmful to C. montrouzieri larvae and adults. D-limonene was slightly harmful when applied directly to C. montrouzieri adults (Percent reduction (E)= 66%) and moderately toxic to larvae (E= 88%). Chlorpyrifos-methyl was classi?ed as moderately toxic to the predator adults (C. montrouzieri) due to its effects on fecundity, egg hatching, and offspring survival (E= 90.7%). Pyriproxyfen was classi?ed as harmful to larvae because of its acute effect on pupal mortality (E= 100%). When C. montrouzieri adults were fed treated prey, d-limonene was classified as slightly harmful (E= 44.7%) and when the predator larvae were fed treated prey, d-limonene was classified as harmless (E= 9.8%). The results of this study indicate that D-limonene (60 g/L) may be compatible with augmentative releases of C. montrouzieri to control D. opuntiae in cactus crop.

P6.1-039

WORKING EFFICACY OF GREEN SYNTHESIZED SILVER AND COPPER NANOMATERIALS ON THE COMPONENTS OF ANTIOXIDANT DEFENSE SYSTEM OF CHILLI PLANT ATTACKED BY FUSARIUM OXYSPORUM F.SP. CAPSICI

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Text

Chilli production is significantly harmed by the interruption of fungal pathogens i.e., Fusarium oxysporum f.sp. capsici. In recent years application of green synthesized nanomaterials is documented as the best performer against various plant diseases. However, the knowledge about the application of green-synthesized Ag and CuNMs for the management of Fusarium oxysporum and its impact on the components of antioxidant defense system especially in chilli plant is still unknown. Therefore, in the current study, green synthesized silver and copper NMs were applied at three different concentrations to check their efficacy against fusarium wilt of chilli and the components of the antioxidant defense system of chilli plants. Results revealed that AgNMs were found most effective and exhibited disease incidence of F. oxysporum (22.79%) with a significant increase in chilli production (13.13%) along with number of fruits/plant (15%). Moreover, the application of AgNMs improved the concentration of ascorbic acid (1240, 997 µg/mL), total phenolic contents (950, 800µg/mL), flavonoids (111, 88mg/g), hydrogen peroxide (0.0013, 0.001U/mg), amylase (110, 89U/mL), chlorophyll a (0.31, 0.25mg/g), chlorophyll b (0.22, 0.16 mg/g) and total chlorophyll (0.61, 0.50 mg/g) in treated plants of resistant and susceptible varieties of chilli respectively than that of control. It is concluded that the application of green synthesized AgNMs may be a viable approach for disease management.

P6.1-040

NANOPARTICLES AS HAMMER OF THOR AGAINST PSEUDOMONAS SYRINGAE CAUSING APICAL NECROSIS IN MANGO

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Text

Apical necrosis disease of mango is becoming a potential threat for mango industry throughout the world. It causes severe yield loss depending on disease triangle. Various chemical, antibiotic, phytoextracts and biological management strategies have been adopted to overcome this malady. These control measures are either having adverse effects on environment or in slow action. Nanotechnology is one of the rapidly advancing and most fascinating science in the field of Agriculture. Use of nanoparticles (NPs) is an environment friendly and effective approach to control bacterial diseases of plants. Silver and copper nanoparticles have excellent antibacterial properties and used as an alternative to pesticides in management of bacterial plant diseases. NPs directly affect the bacterial pathogen and activate defense system of host plant through altering its nutritional status. Silver and copper can be directly toxic to bacterial pathogens while manganese, boron, silicon and zinc act as fertilizer in host defense. As demand for food production is increases globally under changing climate, nanoparticles will play a key role to mitigate new challenges in plant disease management by reducing chemical pesticides.

P6.1-041

DEVELOPMENT OF AN ASCOCHYTA BLIGHT SCREENING SYSTEM FOR THE SELECTION OF RESISTANT PEA (PISUM SATIVUM L.) ACCESSIONS

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Text

The cultivation of pea (*Pisum sativum* L.) among other leguminous crops has become more and more important in respect to biological nitrogen fixation for sustainable cropping systems and as important plant-based protein source for human nutrition. However, pea production is challenged by many biotic stresses, such as fungal and viral pathogens and insect pests. Among fungal pathogens, *Didymella pisi*, *D. pinodes* and *D. pinodella* contributing to the Ascochyta blight complex are causing severe yield losses in pea production. The disease is stubble-, air-, soil- and seed-borne, hence disease control includes certified seed production and fungicide applications. However, particularly in organic agriculture the latter is not available and disease resistant varieties are needed. In collaboration with an organic pea breeder, we have established a reproducible screening system for selection of resistant pea lines using artificial inoculation. Main achievements are the isolation and identification of *Didymella* strains which contribute most to Ascochyta blight under local conditions, and differential scoring scales of pea leaf or tendril symptoms caused by the different Didymella species used for inoculation. This screening system is fundamental for phenotypic selection of resistant breeding lines independent of the disease pressure in the field. Moreover, it can be employed for identification of resistance genes using genome-wide association studies or genomic prediction approaches.

P6.1-042

PHYTO-BIOPOLYMERS FOR INDUCING RESISTANCE AGAINST PEARL MILLET DOWNY MILDEW DISEASE AND TRANSCRIPT PROFILING OF DEFENSE GENE EXPRESSION

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Text

The year 2023 is recognized as the International Year of Millets underscoring the importance of millets. Pearl millet (*Pennisetum glaucum* (L) R. Br.) is projected to be one of the climate change compliant future crop. Pearl millet is a globally important cereal whose production is severely constrained by downy mildew caused by *Sclerospora graminicola* (Sacc.). In this

study, gum biopolymers of *Anogeissus latifolia, Gardenia resinifera, Lannea coramandelica, Buchanania lanzan, Boswwalia* spp., *Canarium strictum*, and T*erminalia tomentosa* were evaluated for their downy mildew disease resistance inducing efficiency and also their synergistic effects when combined with Metalaxyl (Apron 35 SD). Gum exudates of *Terminalia tomentosa* induced disease resistance against pearl millet downy mildew and reduced the disease up to 72% in comparison to the untreated control. *Terminalia tomentosa* gumbiopolymer elicited enzyme activities and transcript accumulation/gene expression of defense enzymes such as ß-1,3-glucanase, Phenylalanine ammonia lyase (PAL), Peroxidase (POX), Polyphenol oxidase (PPO), Lipoxygenase (LOX), and defense protein Hydroxyproline-rich glycoproteins (HRGPs). The very rapid and large changes in elicitor-treated seedlings, in contrast to the delayed, smaller changes in the untreated suscpeptible control seedlings suggests that rate and magnitude of defense gene expressions are important for the effective manifestation of defense.

P6.1-043

THE POTENTIAL OF AFRICAN NIGHTSHADE AS A 'DEAD END TRAP CROP' FOR MANAGEMENT OF POTATO CYST NEMATODES.

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Text

The potato cyst nematodes (PCN), Globodera rostochiensis is a major quarantine pest of potato (Solanum tuberosum L.)in Kenya, it was reported in Kenya in 2015, with contrywide surveys showing it was widespread in all potato growing regions.In this study, field experiments were conducted to evaluate the population dynamics of PCN under vegetable and potato crops rotation. Seven treatments, three African Indegenous vegetable crops, Solanum scabrum, Solanum villosum and Amaranths dubious, and three potato varieties 'Shangi', 'Mayan Gold' and 'Dutch Robin'and a Fallow (weed free) were established and replicated six times. Treatment were maintained on the same plots for first two seasons, the third season potato plots, S. scabrum A. dubius were maintained. Cyst density and viable eggs at planting and at harvest was evaluated. Results showed PCN populations declined significantly on vegetable crops,a significant natural decline noted under fallow. In S. villosum and S. scabrum, the viable eggs reduced by over 50% in season one and by 60 % and 70% in season two and three respectively.The number of cyst and viable eggs reduced by 30% in season 2 and 35% in season 3 in both dubius and weed free fallow. In potato plots increased by over 70% in potato plots in season 1 and 2,and dobled in season 3. The results indicate that African nightshade has potetial in capturing thus reducing PCN juveniles and eggs thus can be used as a trap crop in PCN management rotational programs.

P6.1-044

EFFICIENCY OF ELICITORS ON INDUCED RESISTANCE AGAINST CASSAVA ROOT ROT DISEASE

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Text

Cassava root rot disease (CRRD) is caused by one or several fungal genera. Fusarium species are an important component of the fungal complex that causes root rot disease in Thailand. Therefore, the aims of this study were to evaluate the efficacy of salicylic acid formulation and the mechanism of induced resistance in cassava plants against the CRRD. Zacha elicitor formulations at a concentration of 500 ppm and JN2-007 could reduce mycelial growth of F. solani. Also, the accumulation of hydrogen peroxide and activity of some enzymes related to plant defense mechanisms (peroxidase, polyphenol oxidase and catalase) revealed that the 500 ppm Zacha treatment showed an increased regulation in cassava plants at 24 hours after inoculation (HAI) compared to that of the noninfected plants. Cassavas sprayed with Zacha11 elicitor and inoculated with Fusarium suspension had a slight increase of β-1,3-glucanase activity at 24 HAI (15.62 µg min-1 mg-1 protein) compared to that of the negative control. For chitinase activity, the increase was similar to that of the β-1,3-glucanase. Likewise, the accumulations of salicylic acid increased at 24 hours after inoculation of 69.95 µg g-1 fresh weight. Moreover, cassava treated with Zacha11 showed the biochemical components of lignin and pectin in the cassava cell wall. Thus, it is possible that elicitors could be used to reduce Fusarium root rot disease severity by inducing signals mediating defense responses in cassava.

P6.1-045

USE OF AGRICULTURAL WASTE AND INDIGENOUS ANTAGONISTIC FUNGI FOR CONTROLLING ROOT KNOT NEMATODES (MELOIDOGYNE SPP.) ON POTATO IN NORTH SUMATRA, INDONESIA

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Text

Root knot nematode (Meloidogyne spp.) is one of the important pathogen on potato in North Sumatra, especially in Karo District as a center for potato production in North Sumatra, Indonesia. Lack of information and knowledge about this nematode has resulted in no effective control methods to control this nematode in North Sumatra. The research aims to obtain an effective control method based agricultural waste and indigenous antagonistic fungi for improving plant health towards sustainable agriculture. 14 indigenous antagonistic fungi from the potato rhizosphere consisting of the genera Aspergillus (28.6%), Mucor (21.4%), Trichoderma (21.4%), Gliocladium (14.3%), and Penicillium (14.3%) was obtained from exploration results at 4 potato planting locations in Karo District. Further tests were carried out on the 14 indigenous antagonistic fungi isolates using agricultural waste, each of the Solanaceae, Leguminoceae, and Brassicaceae singly and in combination in suppressing the

population of Meloidogyne spp. in the greenhouse. The results showed that the combination of Leguminoceae waste with Trichoderma 1 isolate gave the best results in increasing plant growth, number of leaves, number of branches of potato plants, and decreasing number of galls. The same result got in the demonstration plot test in the field. Molecular identification of Trichoderma 1 isolate was Trichoderma viride.

P6.1-046

EUGENOL, ISOEUGENOL, THYMOL, CARVACROL, AND ESTER DERIVATIVES AS AN ECOFRIENDLY OPTION TO CONTROL COLLETOTRICHUM CHRYSOPHILLUM AND COLLETOTRICHUM NYMPHAEAE

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Text
Glomerella Leaf Spot (GLS) and Bitter Rot are severe diseases of apple and *Colletotrichum nymphaeae and Colletotrichum chrysophillum* are the main species in Brazil. To control GLS in Brazilian apple orchards, mancozeb and thiophanate-methyl fungicides are still largely used. In recent years Colletotrichum resistance to these active ingredients has been reported and, in addition, mancozeb has been banned by some apple-importing countries. So, this study aimed to search for alternatives to control the diseases. We assessed the antifungal activity of eugenol, isoeugenol, thymol, carvacrol, and some of their ester derivatives formulated in DMSO. The best results obtained in two in vitro assays were using thymol, thymol butyrate, and carvacrol. These compounds completely inhibited mycelial growth at 125 mg·L-1 and conidial germination at 100 mg·L-1. In ex vivo assay, carried out with detached leaves of apple trees, all tested compounds significantly reduced GLS symptoms but do not differ from DMSO. In detached apple fruit, eugenol significantly reduced Bitter Rot symptoms caused by *C. nymphaeae* but the effect varied with *C chrysophillum.* Eugenol is a promising alternative to replace fungicides to control *C. nymphaeae* on apple fruit, but new formulations are needed for GLS control.

P6.1-047

HETEROMURUS NITIDUS (COLLEMBOLA) GRAZES THE WHEAT PATHOGENIC FUNGUS ZYMOSEPTORIA TRITICI ON INFECTED TISSUES: OPPORTUNITIES AND LIMITATIONS FOR BIOREGULATION

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Text

Septoria tritici blotch is a worldwide wheat disease caused by the fungal pathogen *Zymoseptoria tritici* that damages leaves through multiple infection asexual cycles and survive through sexual reproduction in crop residues left on the ground during the interepidemic period. Springtails (Collembola) are cosmopolitan soil arthropods and mycophagous species largely contribute to structure fungal communities and could interact with pathogens present on crop residues. In this study, we investigated the interaction between *Heteromurus nitidus*, a common Collembola species, and wheat tissues (leaves and plant residues) infected or not infected by *Z. tritici*. A short time of interaction allowed to test the preferences of springtails among the two types of wheat tissues, while a longer time (for two to ten weeks) allowed to test spore grazing in fungal fruiting bodies by counting springtails and remaining spores. *H. nitidus* was attracted by infected leaves and reduced tenfold the number of pycnidiospores compared to controls (absence of springtail). Results obtained on wheat residues consisting in older dried plant tissues were more contrasted as no spore emission was observed, although springtail populations thrived on them. Our results show the attraction of *H. nitidus* towards *Z. tritici* infecting leaves and suggest that springtails could contribute to reduce the pathogen inoculum. Microcosm experiments should be conducted to further assert this bioregulation potential.

P6.1-048

THE OCCURRENCE OF ALTERNARIA SPECIES INVOLVED IN DIEBACK OF PINUS PINEA IN TUNISIA.

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Text

Fungal pathogens are among the main causes of forest trees decline including *Pinus pinea* due to dynamic interactions between the plant host and several abiotic and biotic factors. Recently during our surveys, symptoms of branch canker and canopy transparency have been monitored on *P. pinea* trees in Tunisian Nabeul forest. Nevertheless, this phenomenon has been less well-studied in Tunisia. The aim of this study was to identify the causal agent of *P. pinea* trees dieback and to evaluate the factors influencing its occurrence. Ecological factors (rainfall, temperature, and altitude), dendrometric parameters (crown width, total height, and trunk circumference) of pine trees, and the incidence of the pathogen were detected. For diagnosis, 10 symptomatic branches were collected from each declining tree and transferred to the laboratory for fungal isolation. Two *Alternaria* species were identified: *Alternaria alternata* and *A. infectoria* by means of morphological features and phylogenetic analysis of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA. The incidence of *Alternaria* species on *P. pinea* trees appeared to be significantly correlated to the trunk parameters and ecological factors. Our findings show that the decline in stone pine trees could be mainly linked to the environmental factors. To our knowledge, data from the present work provide the first record of the species of *Alternaria* associated with *P. pinea* branch dieback in Tunisia.

P6.1-049

MODELING MULTIPLE PESTS FOR AGROECOLOGICAL PROTECTION OF RICE IN CAMBODIA

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Text

Damages caused by various pathogens, animal pests, and weeds in rice crops can result in important yield losses (Oerke, 2006). In order to limit these damages, farmers use large amounts of pesticides, especially in Cambodia (Flor et al., 2019). This leads to high health and ecological risks. Two major challenges must be completed to support farmers in the agroecological transition, and to limit the use of pesticides on rice crops: i) to link pest populations and pest injuries with the associated risks of crop losses (yield and/or quality) in a given production situation and for given cropping practices; ii) to understand which agroecological levers can prevent pest development and damages. We calibrated the Decision Support System DSSAT using the CERES-Rice model to simulate the impact of different cropping systems (e.g. number of cycles per year, cultivars, fertilization practices) representing various farming systems (e.g. conservation agriculture) on rice growth and yield in Cambodia. The simulated growth dynamics represent potential crop growths in the considered agro-environmental conditions. The impacts of multiple pests are then integrated using coupling points and damage functions. This presentation introduces the structure, parameterization, model's goodness of fit, and potential use of the model to help adapt crop management strategies in different environments.

P6.1-050

RETHINKING STRATEGIES FOR MONITORING PLANT PATHOGENS VIRULENCE DIVERSITY AND THEIR CORRESPONDING SOURCES OF RESISTANCE TO MOVE TOWARDS A MORE EFFECTIVE DISEASE CONTROL

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Text

Diseases are still a major constraint to crop production despite the great efforts made by multiple stakeholders interested in sustainable food production. Specialists in breeding, pathology, agronomy, plant genetics, among others, are working towards the same goal of improved varieties for effective on-farm disease management. This effort could advance faster if a strategy involving multiple actors in the same production chain is designed. Currently, under the new research scheme in the CG centers, we have defined a work plan that integrates researchers not only from the system but also strategic partners from national programs in the regions where diseases are limiting production. Here we present our strategy that will enable us to 1) know the diversity of races of Anthracnose and Angular Leaf

Spot affecting the cultivation of beans in Southeast Africa, 2) highlight a key component of the strategy which, is the vast genetic diversity held by advanced lines from breeding programs, and 3) showcase the capacity and expertise of regional partners. These components will convene on sentinel trials deployed at multiple sites representative of the producing areas to generate data that will be used to identify genotypes resistant to local races of different diseases, identify resistance genes associated with resistance, and their corresponding molecular markers to facilitate the improvement of market-class varieties.

P6.1-051

A PLANT-BASED COMPOUND AS A NON-CHEMICAL NEMATICIDE OF CITRUS NEMATODE TYLENCHULUS SEMIPENETRANS

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Text
Different types of citrus were planted in the north and south of Iran. The citrus nematode Tylenchulus semipenetrans, the cause of the slow decline of citrus can cause serious damage to the roots of citrus trees alone or together with other soil-borne pathogens. The efficacy of Nemakob® compound with the active ingredient obtained from several types of wood vinegar at the rate of 60 and 80 liters per hectare in two orange orchards in Fars province, Darab city, in two sections of Jannat and Forg were investigated. Nemakob treatments considered in this experiment included simple Nemakob 60 and 80 liters per hectare, and Nemakob plus 80 liters per hectare. The Fosthiasate and Bio-nematon were considered as reference nematicides and the treatment without nematicide was considered as control. In terms of the efficacy of Nemakob treatments compared to Fosthiasate reference nematicide, an increase of 7.9%, 8.7%, and 13.5% efficiency was observed in Jannat section, and 62%, 87.8%, and 62.8% increase in Forg section respectively. In terms of comparing the efficacy of different Nemakob treatments with Bio-nematon biological nematicide, an increase in efficacy of 19.5, 20.4, and 25.7 percent was observed in Jannat section and 23, 42.3, and 23.4 percent in Forg sector. Therefore, taking into account all the dependent variables investigated, Nemakob simple and plus 80 liters per hectare treatments are recommended to control the citrus nematode population, especially in early infections.

P6.1-052

ENCAPSULATED FUNGUS-BASED COMPOUND OF POCHONIA CHLAMYDOSPORIA AGAINST ON ROOT-KNOT NEMATODE OF OLIVE

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Text
The olive root-knot nematode Meloidogyne javanica is considered one of the most important soil-born pathogens of olive trees in Iran. The use of non-chemical methods, especially in the control of plant parasitic nematodes, plays an important role in managing the population and

the damage caused by the pathogen in a sustainable and environmentally friendly manner. Among the infected trees of the Aliabad Mother Garden, a number of olive trees infected with M. javanica, the efficiency of encapsulated fungus-based (Pochonia chlamydospora) suspension, SP2, (5 x 1023 CFU) in the amount of 5 l/ha were compared to Trevigo SC 1.8% 10 l/ha, Nemakob 80 l/ha, and Bio-nematon powder 10 kg/ha in controlling root-knot nematode population. Unsprayed trees were used as controls. Based on the reproduction factor and efficiency percentage, SP2 contains spores and mycelium of P. chlamydosporia with an efficiency percentage of 63.78 and a reproduction factor of 0.58. Bionmaton, Nemakob, and Trevigo were ranked with an efficacy of 27.4, 22.7, and zero, and in terms of reproduction factor of 1.7, 5.3, and 4.7 respectively. Therefore fungus-based compound (SP2) was the best treatment for controlling the root-knot nematode population of olive trees.

P6.1-053

CHANGES IN THE ACTIVITY OF SOME ANTIOXIDANT ENZYMES IN WHEAT PLANTS UNDER THE INFLUENCE OF VIRUS INFECTION AND LIPOSOMAL FORMS OF GLYCANS

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Text

Liposomal forms of glycans (termed TRG) containing rhamnolipids and methylthiosulfanilate as structural elements of liposomes were investigated as plant resistance regulators in wheat infected with wheat streak mosaic virus (WSMV) at different developmental stages: 1) stalk-shooting; 2) ear emergence from the upper leaf boot; 3) earing; 4) flowering; 5) grain formation; and 6) milky ripeness. To assess the modulating properties of TRG, the activity of enzymes involved in the detoxification of reactive oxygen species (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (ARX)) was examined. Enzyme-linked immunosorbent assay (ELISA) was used to confirm WSMV infection in wheat samples. ELISA data showed that plants inoculated at the first 4 developmental stages were successfully infected with the virus, while older plants either contained much less virus or have not been infected at all. After pre-sowing treatment of wheat seeds with TRG, the activity of the enzymes was reduced (18-29%) or remained at the control level. SOD activity in plants inoculated with WSMV at stages1-4 was reduced by 43%. All infected plants showed sharp decrease of CAT activity, whereas the activity of POD showed no dependency on TRG. However, TRG reduced ARX activity up to 30% in both infected and intact plants. Obtained data indicate the involvement of the studied enzymes in plant pathogenesis/virus resistance and possible protective effect of TRG in WSMV-infected wheat

P6.1-054

MICROCARBON PLANT-BASED NEMATICIDE PROMAX® AGAINST MELOIDOGYNE JAVANICA ON GREENHOUSE CUCUMBER

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Text

Among the pathogenic factors of vegetable plants, Meloidogyne javanica has great importance. The use of non-chemical nematicides has been considered. In the present study, a broad-spectrum soil-powerful fungicide, and nematicide are made with thyme oil active ingredient, Huma Gro® PROMAX® with micro carbon technology was used to control greenhouse cucumber in two locations, Tehran with "VIOLA cv." and Kohgiluyeh and Boyer Ahmad with "PHYTO cv.". The amounts ??of 5, 10, and 15 l/ha were evaluated three times at 20-day intervals. Fenamiphos granules 10% as control and at 15g/m2. Before the experiment, soil samples were taken from each experimental plot for the second-stage juveniles, and at the end of the experiment, nematode variables including the number of galls, the number of egg masses on the root, the second juvenile population in the soil, and the root were determined. The efficacy of Promax nematode at 5 l/ha in both greenhouses was more than 90%. The difference between the efficacy of this Promax compared to Fenamiphos in Tehran province with a high initial nematode population was more than seventy percent. The reproduction factor was less than zero in both locations. Therefore, to control the population of root-knot nematode of greenhouse cucumber, Promax 5 l/ha hectare alone at least three times, one at the time of transplanting and the others at 20-day intervals is advisable.

P6.1-055

EFFICACY OF FUNGICIDE ROTATION ON POTATO LATE BLIGHT (PHYTOPHTHORA INFESTANS, (MONT.) MANAGEMENT

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Text

Potato late blight caused by *Phytophthora infestans* is major threats for potato production in Nepal.Due to high risk of resistance development and high cost associated with the fungicides pressed to find the alternatives by spray rotation with other fungicides.Field experiments were conducted for two consecutive years (2019 and 2020) cropping seasons to evaluate the effect of fungicides rotations for the management of potato late blight in Western Nepal. Three fungicides: mancozeb, dimethomorph and fenamidone + mancozeb were evaluated in different rotations to suppress late blight. The experiment composed of eight treatments was arranged in a randomized complete block design with three replications.The lowest mean AUDPC (221.67) was obtained from first spray with mancozeb followed by two spray of fenamidone + mancozeb right after first spray with mancozeb followed by two spray of dimethomorph (233.33) whereas, the highest mean AUDPC was obtained from untreated control plot (522.08) right after mancozeb only treated plot (373.33).The highest tuber yield (14.46 mt/ha) was

obtained from first spray with mancozeb followed by two sprays of fenamidone + mancozeb.The study confirmed that the rotation in fungicide application plays a crucial role to reduce the cost of fungicide applications, risk of development of fungicide resistance by late blight even under high late blight pressure.
Keywords: Disease assessment, Fungicides, Late blight, Potato, Spray rotation, Yield

P6.1-056

STUDIES ON THE PATHOGENICITY OF METARHIZIUM ANISOPLIAE VAR. ACRIDUM AGAINST DESERT LOCUST, SCHISTOCERCA GREGARIA (ORTHOPTERA: ACRIDIDAE) NYMPHS AND ADULTS

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Text
The current study was carried out to tested the efficacy of different entomopathogenic fungi (EPF) formulations i.e. Metarhizium anisopliae against the nymphs and adults of the desert locust, Schistocerca gregaria Forskål under field as well as laboratory conditions. Four different concentrations were applied against different instars of the nymphs and adults of desert locust. Susceptibility was found to be greatest in 3rd-instar nymphs, followed by 5th instars, and then adults. Along with lethal effects, sublethal doses of EPF reduced the number of egg pods/female, total eggs/pod, and egg hatching, while extending nymphal developmental time and reducing adult longevity; again, Metarhizium anisopliae performed well. Sublethal doses not only retarded reproduction, but also caused behavioral changes, like reduction in food consumption, fecal production, and weight gain. All EPF formulations not only showed significant mortality in laboratory conditions, but also performed very well under the field conditions. The maximum mortality against 3rd-instar (81.7% and 74.0%), 5th-instar (73.3% and 65.1%), and adult locusts (67.5% and 58.9%) was observed when using Metarhizium anisopliae under greenhouse and field trials, respectively. The current study showed that all of the EPF formulations have the potential to reduce pest populations, and could be used in the integrated pest management program.

P6.1-057

EFFECT OF PLANT EXTRACTS AGAINST SOME SEED-BORN PATHOGENS IN VITRO

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Text
Given the need to apply environmental strategies in agriculture, there is growing interest in studying the possibilities of plant extracts. Alternaria spp. and Fusarium spp. are the most common pathogens on cereal seeds. Objective of this study was to investigate the fungistatic effect of plant extracts against Alternaria tenuissima (Kunze) Wiltshir and Fusarium culmorum (Wm.G. Sm.) Sacc. in vitro. Plant extracts were made on the basis of the raw

materials of medicinal plants: Salvia officinalis L., Macleaya cordata (Willd) R. Br., Echinacea purpurea (L.) Moench, Mentha piperita L., Thymus vulgaris L., Matricaria recutita L., Datura stramonium L., Origanum vulgare L., Datura metel L., Achillea millefolium L., Apocynum cannabinum L., Artemisia annua L., Artemisia absinthium L., Chelidonium majus L. Agar-disk diffusion method was used. The radial growth and the percentage of growth inhibition were determined. According to our results, extracts of Salvia officinalis, Artemisia annua and Macleaya cordata showed fungistatic effect against both pathogens. Higher inhibition of colony growth was observed against A. tenuissima – 84,3-99,5%. For F.culmorum the maximum effect was obtained when extract of Artemisia annua was used – 82,3%. Results obtained suggest that extracts of these plants can be used in the future to develop plant protection products.

P6.1-058

THE PERFORMANCE OF THE BACTERICIDAL AND PLANT DEFENCE ELICITOR PEPTIDE BP178 IN DIFFERENT PLANT HOST PATHOSYSTEMS

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Text

Functional peptides are low-risk and sustainable plant protection products that provide several mechanisms of action against pathogens. Peptide BP178 was engineered for expression in plants, and consists of a BP100 moiety (1-11), an AGPA hinge (12-15), a magainin sequence (16-25) (GIGKFLHSAK), and a terminal end KDEL sequence (26-29) for secretion through the endoplasmic reticulum of the plant cells. The peptide BP100 has a potent bactericidal activity but did not induce plant defense responses, whereas peptide BP178 retained the bactericidal activity of BP100, but in addition induced defenses in tomato, almond, and *Nicotiana benthamiana* and can be produced heterologously in plants. Specifically, it has been assayed against plant pathogenic bacteria such as *Xylella fastidiosa*, *Xanthomonas campestris* pv. *campestris* and *Pseudomonas syringae* pv. *tomato* and the fungus *Botrytis cinerea*, among others. The peptide has been applied in planta following different strategies depending on the pathosystem such as spray, endotherapy, or transient expression. In these different scenarios, BP178 was able to limit infections total or partially due to its dual mechanism of action. Formulation of the peptide are under field testing against diseases caused by fastidious bacteria (e.g. citrus greening) and fire blight of apple and pear. Funding was provided by: 101060593 and 817526 EU projects, RTI2018-099410-B-C21 MCIN/AEI/FEDER, TED2021-130110B-C43 MCIN/AEI/ Unión Europea NextGenerationEU/PRTR.

P6.1-059

ANTIBACTERIAL ACTIVITY OF SOME SUBSTANCES AGAINST CITRUS BACTERIAL CANKER CAUSED BY XANTHOMONAS CITRI PV. CITRI IN BURKINA FASO

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Text

Bacterial canker of citrus is a disease with high social and economic impact. The present study was conducted to evaluate the antibacterial activity of copper hydroxide and sulfate, Bacillus subtilis, Ocimum gratissimum essential oil, aqueous extracts of Azadirachta indica, Cymbopogon citratus and Eucalyptus camaldulensis leaves, and two formulations based on thymol and eugenol against this bacterial disease. Thus, the method of dilution in solid medium and the macromethod of dilution in liquid medium were used in vitro. Through foliar spraying, in vivo greenhouse trials and in vitro trials were conducted respectively on plans and trees of Citrus reticulata×Citrus sinensis species. From the in vivo and in situ trials, it was found that copper hydroxide at 2.5 g/L; Bacillus subtilis at 5 ml/L and Ocimum gratissimum essential oil at 1 ml/L reduced the severity of citrus bacterial canker by 86.39%, 44.71% and 59.13%, respectively, compared to the untreated control.

P6.1-060

SILICON DELAYS THE SPREAD OF VASCULAR STREAK DIEBACK OF COCOA

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Text

Silicon (Si) has been proven to control fungi infections, primarily in monocots, with fewer studies in dicots. Little is therefore known about its effectiveness in controlling vascular streak dieback (VSD) diseases of cocoa (*Theobroma cacao* L.) caused by *Ceratobasidium theobromae*. VSD is a major systemic disease of cocoa, lethal to seedlings under 10 months old, and kills mature tree branches. Often, fungicides and resistant cocoa varieties are used to curb VDS. This study thus observed the growth of cocoa seedlings under different Si concentrations in its bio-available form [silicic acid ($Si(OH)_4$)] to understand its effect on the physical features that promote the plants' defence and inhibit the progression of *C. theobromae* after inoculation. $Si(OH)_4$ was applied weekly as root applications of 0.25% $Si(OH)_4$ (v/v), 0.15% $Si(OH)_4$ (v/v), and 0.10% $Si(OH)_4$ (v/v) for seven months. The thickest cuticle (1.99 µm) and bark, with better growth parameters, were measured in seedlings given 0.15% $Si(OH)_4$ (v/v), whereas the control seedlings' leaf cuticle thickness averaged 1.10 µm. The $Si(OH)_4$ treatments also inhibited the growth of *C. theobromae* in the stems after inoculation. The improved leaf cuticle thickness, coupled with thicker barks and VSD inhibition, positively correlated with the amount of silicon in the leaves after inductively coupled plasma atomic emission spectroscopy (ICP-OES) analysis. Silicon thus has the potential as a viable alternative for controlling VSD of cocoa.

P6.1-061

CHARACTERIZATION OF THE CAPACITY OF YEAST CELL WALL EXTRACTS TO INDUCE GRAPEVINE TOLERANCE TO DROUGHT AND PATHOGENS

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Text

Viticulture is affected by several abiotic and biotic stresses causing huge yield losses and deterioration of berry quality. Due to climate change drought has become one of the major threats to grapevine production. Among biotic stresses, diseases caused by the oomycete *Plasmopara viticola* and the fungal pathogen *Botrytis cinerea*, causal agents of downy mildew and grey mold, respectively, are major concerns for grapevine. The management of these diseases mostly relies on chemical control with the application of copper-based products and/or synthetic fungicides that determine negative effects on the environment and on the health of vine growers and consumers. Therefore, the searching for sustainable alternatives to the use of chemical control is crucial. On this basis, we tested different cell wall extracts from the yeast *Saccharomyces cerevisiae* for the capacity to induce grapevine tolerance to water deficit and resistance to *P. viticola* and *B. cinerea* infection. One of the yeast extracts that proved effective in inducing tolerance to the pathogens was selected to evaluate its efficacy in promoting internode elongation rate and stomatal conductance under well watering and water deficiency conditions. Our results showed that the yeast extract applied to grapevine did not promote internode elongation but determined a positive impact on stomatal conductance after water deprivation during the recovery phase.

P6.1-062

INTEGRATED PEST MANAGEMENT SMART TECHNOLOGIES TO PRECISELY DETECT AND CONTROL PLANT DISEASES

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Text

The ever-increasing demands of international markets for safe food have led to the development of integrated plant protection strategies (IPMs) for a more efficient and sustainable agriculture and to more robust certification and control systems for agricultural products. Novel IPMs of particularly serious plant diseases and mycotoxin contamination of plant products are being developed using innovative smart agricultural systems. The purpose is to: (a) accelerate the prognosis of disease outbreaks through prediction models; (b) develop methods of artificial intelligent diagnosis using imaging techniques or mass spectrometry sensors; (c) evaluate novel biocontrol and chemical PPPs to control effectively the diseases; (d) develop innovative prototype sprayers actuating different nozzle types and adopting variable rate control based on canopy characteristics and disease development.

Decision Support Systems (DSS) are also developed and validated based on computer-based knowledge systems that enable disease prediction and monitoring and determine the critical stages of the various plant protection spray interventions. The ultimate goal of smart technologies is to reduce the European agriculture reliance on agrochemicals resulting in lower residues and reduced impacts on human health.

The presented research has received funding from the European Union's Horizon 2020 research and innovation programmes under grant agreement No 773718 (OPTIMA) and No 778219 (OchraVine Control).

P6.1-063

AN INVESTIGATION OF WAYS TO REDUCE PHYTOTOXICITY CAUSED BY COPPER-BASED FUNGICIDES IN CITRUS

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Text

In Australia, export-quality mandarins, lemons and limes are predominantly grown in Queensland's subtropical regions. Queensland citrus production in 2021/2022 totalled almost 87,440 tonnes (from 4360 ha) with a farmgate value of around $200 million. Since 1920, the management of citrus fungal diseases has always presented challenges for mandarin growers, including 'Emperor' Brown Spot (EBS) and Citrus Black Spot (CBS) caused by Alternaria sp. and Phyllosticta citricarpa, respectively. Queensland's citrus industry routinely exports premium quality mandarins to more than 30 countries, and exporters are required to meet specific markets' Maximum Residue Limits (MRL) to enable international trade. Copper-based products and dithiocarbamates are broadly used fungicides to protect against various citrus diseases, however, the current dithiocarbamate fungicides face an uncertain future globally due to human health concerns. When over-applied, copper-based fungicides can be toxic to citrus trees. Under certain conditions, they may also cause darkening of existing fruit blemishes and stipple marks, which are significant issues for exports. As copper-based fungicides are essential for citrus disease management in the absence of dithiocarbamates, there is a need to investigate ways to reduce phytotoxicity. These complex investigations are currently underway

P6.1-064

EVALUATING HISTONE ACETYLTRANSFERASES IN PARASTAGONOSPORA NODORUM TO DEVELOP NOVEL FUNGICIDES

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Text
Sustainable crop disease management requires an integrated approach, including the use of chemicals. However, the loss of chemicals to both resistance and regulation, combined with no new fungicide modes of action in the market is posing a serious threat to the global food security. This study applies the genomic approaches for identifying the novel targets to potentially develop them into new fungicide mode of actions. We have characterised two histone acetyltransferase encoding genes in a model crop pathogen Parastagonospora nodorum and has demonstrated that they could be targeted for fungal inhibition. Targeted deletion of PnGcn5 resulted significant reduction in the growth. Attempts to delete PnEsa1 were unsuccessful. In contrast, knock down approach using a long hairpin RNAi showed successful silencing with transformants exhibiting reduced sporulation and pathogenicity. Results suggest an essential function for PnEsa1. Further studies aim to determine the processes disrupted by PnGcn5 and PnEsa1 gene deletion in P. nodorum. This will facilitate the identification of fungal inhibition pathways that could be targeted for the control of plant pathogens

P6.1-065

IMPACT OF FUNGICIDE TANK-MIXING ON CERCOSPORA LEAF SPOT OF SUGARBEET AND GENETIC DIVERSITY OF CERCOSPORA BETICOLA

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Text
Cercospora leaf spot (CLS) caused by Cercospora beticola is the most destructive foliar disease of sugar beet worldwide. Use of fungicides remain critical for managing CLS because most of the commercial sugar beet varieties are susceptible to CLS. Currently Demethylation inhibitor (DMI) fungicides are used along with a broad-spectrum tank-mix partner to manage CLS. However, it is not clear how tank-mix partners interact with DMI fungicides for managing CLS under field conditions. A two-year field study was conducted to evaluate the efficacy of DMI fungicides with multiple tank-mix partners for managing CLS. Treatments included DMI fungicides (prothioconazole, tetraconazole, difenoconazole + propiconazole, and mefentrifluconazole) and tank-mix partners (mancozeb, copper oxychloride + copper hydroxide, sodium bicarbonate, sulfur, potassium phosphite, and Bacillus subtilis). Significant differences were observed for the tank-mix partners with mancozeb, copper, potassium phosphite, and sulfur resulting in increased recoverable sucrose compared to DMI fungicides alone. Interactions among DMI fungicides and tank-mix partners for CLS severity were also observed. We also collected 763 C. beticola isolates before and after fungicide applications. Based on 8 microsatellite markers, an estimated 93 MLGs identified before fungicide applications and 250 MLGs identified following exposure to fungicide treatments. Interestingly, only 37 MLGs were observed in both collection periods.

P6.1-066

INFLUENCE OF SOWING TIME ON THE OCCURRENCE OF ALTERNARIA LEAF SPOT AND RUST ON SUNFLOWER

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Text

The occurrence of Alternaria leaf spot (*Alternaria helianthi*) and rust (*Puccinia helianthi*) on six sunflower hybrids were assessed to notice the effect of sowing time (ST). ST was set taking into account the Celsius degrees at the soil depth of 7 cm: ST1 at 5ºC, ST2 at 7ºC and ST3 at 9ºC. The research was performed in the field experiments in Tulcea county in 2021 under rainfed conditions. Observations on the attack intensity (% of the leaves area) have done after the flowering period. For *A. helianthi* similar results were at ST1 and ST2 (46,7% and 48,7%) while at ST3 the intensity was lower (33,1%). Only one hybrid was attacked by *P. helianthi* at SD1 and four hybrids were attacked at SD2. The attack intensity increased upon the sowing delay from 0,5% (SD1) to 7,9% (SD2) and to 16,1% (SD3).

P6.1-067

ANTAGONISTIC PROPERTIES OF RHAMNOLIPID BIOSURFACTANT OF PSEUDOMONAS AERUGINOSA RS6AGAINST PATHOGENIC FUNGI OF CUCUMBER AND MELON

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Text

The application of chemical pesticides in vegetable and fruit crops has been commonly practiced over the years in combating pests, insects, weeds and diseases. Rhamnolipids (RLs) biosurfactant produced from biodiesel side stream-waste glycerol and extracted from P. aeruginosa RS6 are known to possess antifungal effect against several phytopathogens and has the potential to be used as biopesticides for crop protection in the agriculture industry. The efficacy of RLs as a biopesticide in controlling widespread airborne and soilborne diseases was studied. The field planting study of cucumber and melons was conducted. There was no significant difference observed on the plant's height and leaves when using different treatments, however, a significant difference in fruit weight was observed. It shows that the application of RLs had a significant effect on plant growth and therefore suggested that RLs are an efficient control towards fungi pathogen as the observation of the diseases infections during the field trial showed that the time taken to the diseases infected plant with the RLs treatment took long time compare the plant with other treatments with optimal concentration of RLs at 0.5 g/L. Therefore, the efficiency of RLs as a biopesticide in controlling fungal pathogens widespread especially soilborne and airborne diseases also equally good to control. Hence, RLs are the possible environmentally friendly alternatives to partially substitute commercial chemical pesticides.

P6.1-068

HOW TO IMPROVE THE PERFORMANCE OF MINERAL OIL TO CONTROL POTATO VIRUS Y IN SEED POTATO PRODUCTION?

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Text

Mineral oil is the main alternative treatment identified to limit the spread of Potato Y virus (PVY) in seed potato production, this plant protection product is a part of the biocontrol list according to the French plant health authorities. Because there are currently no exact recommendations for its use, the mineral oil is commonly spread by farmers on potato plants every 2-3 days during the active plant growth and every 7 days when the vegetation is stabilized.
Our main objective is to better understand the duration of the oil protection in order to give recommendations to farmers with regard to the frequency of applications for optimal performance against the virus transmission and being consistent with the environmental recommendation of the French Ecophyto II+ program.
High resolution mass spectrometry using an electrospray ionization source (ESI-HRMS) was selected to detect the mineral oil with a high sensitivity and an experimental protocol was developed to distinguish the fraction of oil which penetrates the leaf from the surface fractions.
The results of kinetic study demonstrated that oil persists on treated leaves surface after 7 days. The relationship between this persistence and the oil ability to protect the leaves against virus transmission by aphids will be discussed.

P6.1-069

STRATEGIES FOR COPPER REDUCTION IN GRAPEVINE, APPLE, ROSES AND VEGETABLES BY USING ALTERNATIVE EXPERIMENTAL PRODUCTS

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Text
Reducing the use of copper fungicides with the aim of phasing out has a high priority in

European policy as well as in organic farming. To reach this goal without compromising yields, all preventive strategies have to be implemented and several affordable alternative products need to be brought to the market. Within the EU-funded project RELACS (2018-2022), we investigated alternative compounds that had reached a high technology readiness level. We focused on major copper-relevant crops/pathogens (grapevine/*Plasmopara viticola*, apple/*Venturia inaequalis*, vegetables/downy mildews/late blight, oil-producing roses). As alternatives, two plant extracts including licorice leaf extract (*Glycyrrhiza glabra*) and a larch bark extract (*Larix decidua*), a rare sugar (tagatose) as well as a test product based on fatty acids (NEU 1143F in apple) were evaluated.
Refined strategies with these alternatives were tested, adapted and validated under practical conditions in different European countries, and further aspects of importance (e.g. mode of action, wine fermentation, compatibility with other plant protection products) examined. All alternatives proved to be effective in one or more of the investigated crops. In some cases, their efficacy as stand-alone treatment was comparable to that of copper. In other cases, the combination of the alternatives with reduced amounts of copper or in combination with other standard methods provided effective protection of the crops.

P6.1-070

TRANSCRIPTIONAL REPROGRAMMING OF LETTUCE ROOTS IN RESPONSE TO CHITIN SOIL AMENDMENT, EFFECT ON PLANT GROWTH, RHIZOBIOME COMPOSITION AND DISEASE RESISTANCE

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Text
Chitin soil amendment improves soil quality, plant growth and plant stress resilience, but the underlying mechanisms are not well understood. To gain a deeper understanding on the effects of chitin, several omics approaches were applied in a multidisciplinary project.
We studied the growth promoting effects on lettuce upon treatment with chitin in two different soil types (potting and greenhouse). In both soils, lettuce grew bigger with chitin amendment. Lettuce grew generally better in the potting soil compared to the greenhouse soil.
The rhizobiome composition was analyzed using metabarcoding. A decrease in α-diversity was observed upon chitin treatment in both soils. Based on β-diversity, chitin amendment had a stronger effect on the fungal community compared to the bacterial one. Both soils contained different genera significantly altered upon chitin treatment. In potting soil, a known plant-growth promoting fungus was significantly more abundant and associated with other chitin degraders. Such association was not observed in the greenhouse soil.
The transcriptional reprogramming of lettuce roots in response to chitin treatment was studied using RNA-Seq. Over 300 genes were significant differentially expressed with chitin amendment.

Our results suggest that chitin soil amendment promote plant growth indirectly by changing the rhizobiome, induce transcriptional and metabolomic changes in lettuce roots, and might activate induced resistance by priming lettuce plants.

P6.1-071

MULTICRITERIA ANALYSIS, A POWERFUL TOOL TO SELECT CONTROL METHODS AND DESIGN A CONTAINMENT STRATEGY AGAINST THE PLANE TREE CANKER DISEASE

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Text

In France, *Ceratocystis platani*, the fungus responsible of the plane tree (*Platanus acerifolia*) canker, is a quarantine pest that must be eradicated. In some regions, namely Provence-Alpes-Côte d'Azur and Occitanie, the disease is out of control in some areas. In 2020, Anses (French Agency for Food, Environmental and Occupational Health & Safety) was requested by the French Ministry in charge of Agriculture to identify different strategies to contain the disease by taking account of the specificity of the outbreak locations (urban or landscape environments, proximity with a river).

To address this question, a multicriteria analysis methodology has been used to design containment strategies relevant to these epidemic situations. The approach has been developed in 3 steps: i) the life cycle of a plane tree contaminated by C. platani has been divided in 4 sequences (standing plane tree, tree stump, contaminated residues after the removal of a plane tree and new planting of a plane tree), ii) 21 control methods against C. platani have been selected and considered as single actions that could be positioned on one of 4 sequences of the tree life cycle, and iii) 13 criteria were selected to evaluate the efficacy, cost, innocuity, scale of implementation (spatial and time) and social acceptability of each method.

Eleven ready-to-use methods (among the 21 control methods and the prophylaxy method) have been ranked by considering the specificity of the 3 outbreak environments.

P6.1-072

EVALUATING BIO-PRODUCTS, FUNGICIDES AND SAR CHEMICALS IN INTEGRATED MANAGEMENT OF ALTERNARIA BRANCH ROT OF CARNATION

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Text
Alternaria leaf spot or branch rot of carnation is a serious disease in carnation causing maximum disease severity of 26.25% in Chail of Solan district in H.P. Considering to the heavy losses annually and the adverse effect of the chemicals on environment, the present study was formulated to include alternative strategies in managing this disease . Various bioresources and SAR chemicals were exploited .Out of the bio-products, neemazal, cow urine were found superior and gave 95.37% inhibition at 30% compared to 10, 20% conc .Cloves extract of garlic suppressed the growth upto 60.80% .The latex of agave and fruit rind of the soapnut gave 50.60 and 47.09 per cent inhibition in mycelia growth. Maximum inhibition (91.42%) in mycelia growth was obtained with neem and mint oil (88.52%). Systemic fungicides, score and contaf completely inhibited the growth at all concentrations followed by fungicide, dithane M-45 (mancozeb) a non-systemic in nature under *in vitro* assessments. In field trail, the best treatments along with SAR (systemic acquired resistant) chemicals revealed the minimum extent of the disease severity with four applications of the sprays in score, which was followed by Contaf and combined treatments of BABA (β amino butyric acid)+ neemazal+garlic extract + neem oil and Chitosan+ neemazal+garlic extract + neem oil .The combined treatments also enhanced all the plant growth parameters in addition to lowering of the disease level to minimum threshold.

P6.1-073

COURGETTE GENETIC BACKGROUND MODULATES DEFENSE MECHANISMS INDUCED BY REGALIA® AGAINST THE FUNGAL PATHOGEN PODOSPHAERA XANTHII

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Text
The biotrophic fungus *Podosphaera xanthii* (*Px*) has evolved adaptive mechanisms to various environments causing powdery mildew diseases leading to yield losses. The appearance of resistant to pesticides *Px* isolates and EU goals for fungicide reduction has made crucial the development of alternative control means. Here, we discuss the interaction of courgette hosts, sensitive (S) or with moderate resistance (IR) to *Px*, after the application of Regalia® (RS), a plant inducer of resistance. In depth transcriptomic and metabolomic analyses, gene and protein expression, and chromatin immunoprecipitation assays were combined with physiological and morphological studies to investigate plant's responses after Regalia® application and/or spore artificial inoculation. Our results demonstrated that RS enhances glycerophospholipid accumulation at the plasma membrane and phospholipid signaling especially in S courgette genotype, while the IR genotype showed practically no response due to inherent level of resistance. The water treated IR plants were characterized by accumulation of salicylic acid, defense metabolites and enriched epigenetic H3K4me3 marks on Pm-0 locus genes (a genomic region that confers resistance to Cucurbits against *Px*). Additionally significant enrichment of Gene Ontology terms related to response to fungi, fatty acids and lipids, was observed on S plants after Regalia® application.

P6.1-074

MANAGEMENT OF COTTON LEAF CURL VIRUS THROUGH MEDICINAL PHYTOEXTRACTS AND ITS IMPACT ON AGRONOMIC PARAMETERS

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Text

Cotton Leaf Curl Virus (CLCuV) is the most destructive disease which is responsible for causing great yield loss. The present study was focused on the eco-friendly management of CLCuV through medicinal phytoextracts. Twelve medicinal treatments with three concentrations (3%, 5% and 7.5%) were applied against CLCuVD. Under greenhouse conditiond, Citrullus colocynthis(11.573) at 7.5% expressed minimum disease incidence followed by Eruca vesicaria ssp. Sativa+ Citrullus colocynthis(15.547), Eruca vesicaria ssp. Sativa+ Citrullus colocynthis + Euclyptus citridora(18.410), Eruca vesicaria ssp. Sativa (21.533), Euclyptus citridora(22.597), Nigella sativa(23.877), Black pepper(26.833), Trigonellafoenum graecum(27.723), Cinnamomum tamala(29.870), White pepper(31.200), Syzygium aromaticum(31.490), Cinnamomum verum (31.617) and Citrullus colocynthis also showed highest agronomic traits While under field conditions, maximum disease incidence was expressed by Citrullus colocynthis(15.060) followed by Eruca vesicaria ssp. Sativa+ Citrullus colocynthis + Euclyptus citridora (23.030), Euclyptus citridora (25.90), Eruca vesicaria ssp. Sativa+ Citrullus colocynthis(29.947), Eruca vesicaria ssp. Sativa (29.690), Trigonellafoenum graecum (33.000), Syzygium aromaticum (33.17), White pepper (34.117), Cinnamomum tamala (36.943), Black pepper (37.010), Nigella sativa (38.897) and Cinnamomum verum (39.037) as compared to control and Citrullus colocynthis showed best agronomic trait values.

P6.1-076

MANAGEMENT OF CITRUS GUMOSIS IN VITRO AND UNDER FIELD CONDITIONS THROUGH DIFFERENT CHEMICALS

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Text

Citrus is a high demanding fruit across the globe due to its nutritional and economic values. In Pakistan, it is regarded as an important source of foreign earning and has promising position in country's GDP. But per hectare yield of citrus is reducing from previous two decades due to a number of biotic and abiotic factors. Citrus gummosis (Phytophthora

nicotiana) is one among the biotic factors affect the quality as well as quantity of produce. That's why in current research seven chemical namely Aliette, Score, Topsin-M, Kocide 3000, Diathane M-45, Ridomil gold, Novice ultra were assessed at three concentrations (200, 250 and 300 ppm) under lab conditions through poisoned food technique while two best performed chemicals (Topsin-M and Ridomil gold) their combinations were evaluated in field trial through soil drenching method under RCBD design. Findings of this study showed that Topsin-M gave the best results with min. mycelial growth (5.3mm) followed by Ridomil (7.46mm) during lab experiment while in field conditions combination of (Topsin-M+ Ridomil gold) showed the most promising results with minimum incidence of disease (19 and 23%) followed by Topsin M and Ridomil as compared to control treatment (77%).

P6.1-077

STEAMING CAN DISINFECT SOIL AND ONION WASTE CONTAMINATED WITH STROMATINIA CEPIVORA CAUSAL AGENT FOR WHITE ROT OF ONION AND GARLIC

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Text

Sclerotinia cepivorum (Teleomorph: Stromatinia cepivora) is an economically important pathogen of Allium species worldwide. The sclerotia can survive in the soil for many years, and it is difficult to manage. White rot is a quarantine disease in Norway. As a result, onion waste is not used as a resource for composting because of the risk of spreading the pathogen with compost. Furthermore, the disposal of onion waste is costly in Norway. The objective was to determine the lethal dose (temperature and duration) that kills the pathogen. A stationary soil steaming machine that has several thermocouples were used for the experiments. The temperatures tested were 60, 70, 80, 90, and 98 °C with a duration of 3 minutes, and 60 °C with 3 minutes of steaming followed by storage in Styrofoam boxes for 24 hours. Untreated sclerotia were used as control. The viability of S. cepivorum were tested by surface disinfecting the sclerotia and plating on potato dextrose agar. The germination and sclerotia production were determined after incubating at room temperature. The experiment had four replications and was repeated twice. There was no germination of sclerotia in the steam-treated groups with the exception that one sclerotium germinated in the treatment at 60 °C for 3 minutes, whereas the majority of sclerotia in the control group germinated. Steaming is promising for disinfesting soil and onion waste contaminated with S. cepivorum, so onion waste can be composted and used as a resource.

P6.1-078

DIFFERENTIAL INDUCTION OF DEFENSE MECHANISMS BY COMMERCIAL BIOPESTICIDES IN CARROT VARIETIES AGAINST THE PHYTOPATHOGENIC FUNGUS ALTERNARIA DAUCI

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Text
Plant Resistance Inducers (PRIs) could allow a plant to combat a pathogen infection, by activating the transcription of certain defense genes. As such products are not currently available to control *Alternaria dauci* on carrot, the approach of this study consisted in evaluating the ability of 3 commercial products, *Sonata*® (*Bacillus pumilus* QST 2808), *Helioterpen Soufre*® (Sulphur co-formulated with pine terpenes) and LBG-01F34® (Potassium phosphonates) to induce plant defense response in two carrot varieties, with different resistance response to Alternaria leaf blight disease (Presto: susceptible; Boléro: intermediate resistant). For the analysis of carrot defense induction, a field experiment was carried out that included 5 treatments of the three bio-PPPs and infection of the carrot plants with *A. dauci*. Analysis of plant defense induction was followed by studying overexpression of marker defense genes using the qPFD® platform (Quantitative Low-Density Chip). The qPFD® technology is a molecular diagnostic tool that allows the simultaneous study of 28 different defence genes. The results showed that that not all the varieties are receptive to the three PRIs and that the level of protection can, in precise situations, be related to an over-expression of certain defence genes and at different time points depending on the variety. For some bio-PPPs protection is not due to induction of plant defense but probably due to direct effect on pathogens.

P6.1-079

DEVELOPMENT OF AN INFECTIOUS CLONE OF BLACKBERRY CHLOROTIC RINGSPOT VIRUS

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Text
Blackberry chlorotic ringspot virus (BCRV), genus Ilarvirus, is one of the most prevalent viruses in the blackberry yellow vein disease (BYVD) complex, which currently hinders blackberry production in the southeastern United States. In efforts to study the virus biology, the three genomic RNA were cloned into a PJL89 Agrobacterium binary vector. Additionally, an extra construct coding for the viral coat protein, hypothesized to be required for replication, was developed. Leaf agroinfiltrations of indicator plants with either the genomic RNAs or the genomic RNAs and the coat protein binary vectors in combination with the tomato bushy stunt virus p19 silencing suppressor construct were conducted. RTqPCR assays using systemic tissues of agroinfiltrated plants revealed positive virus infection on Nicotiana benthamiana and N. occidentalis. Mechanical transmission experiments, carried out using sap from BCRV-positive agroinfiltrated plants, resulted in systemic infection of N. benthamiana, N.occidentalis, and Chenopodium quinoa. The generation of a full-length infectious clone of BCRV facilitates future studies on the biology of this virus, particularly those aimed to dissect its contribution in mixed infections with other viruses in the BYVD complex.

P6.1-080

SAFE CHEMICAL USE FOR EFFECTIVE MANAGEMENT OF PSEUDOCERCOSPORA LEAF AND FRUIT SPOT IN UGANDA

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Text

Pseudocercospora leaf and fruit spot is a citrus disease caused by Pseudocercospora angolensis resulting in 50 to 100% yield losses. It also causes fruit quality decline and significantly reduces the juice content. Carbendazim has proven an effective disease management option. However, the safety of chemical control needs investigation to prevent health hazards resulting from chemical poising. Study was undertaken to determine the chemical regimes offering effective disease control with minimal chemical residue accumulation. Four farmers were selected from each district for the studies with seven experimental plots set per farm, representing different chemical treatments. The pseudocercospora fruit and leaf spot scores at different timepoints determined the effectiveness of treatments, while lab analyses determined the effect of chemical regimes on fruit quality and chemical residues. The different chemical regimes reduced disease variably. The brix content of the fruits under different treatments were measured against the national standard of 8 with fruits mostly having Brix of 7 to 8 degrees although chemical regimes didn't significantly influence the Brix content. The detectable carbendazim in the fruits under different treatments was <0.01 ppm below the acceptable limit of 1 ppm. This implies the different regimes of carbendazim deployed are effective in pseudocercospora disease management and safe if deployed within two to three months.

P6.1-081

CHEMO-ENZYMATIC FUNCTIONALIZATION OF PHENOLIC COMPOUNDS AND EVALUATION OF THEIR POTENTIAL FOR BIOCONTROL IN RAPESEED

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Text

In a context of sustainable agriculture aiming to reduce the use of synthetic pesticides, the discovery of new plant defense stimulators or biofungicides, which can be used in the biocontrol of cryptogamic diseases, represents a major research challenge. Plants synthesize many phenolic compounds involved in different metabolisms and in particular in defense responses. Certain phenolic compounds, such as phenylpropanoids derived from p-coumaric acid, have shown direct antifungal activities to fight against pathogens. The p-

coumaric acid was functionalized with fatty acid chains of different lengths to determine whether these new molecules possessed direct antimicrobial activities and were able to stimulate defenses in rapeseed. The production of around twenty functionalized derivatives of p-coumaric acid was developed through chemo-enzymatic synthesis in order to improve their physico-chemical properties. The evaluation of these molecules for their direct antimicrobial activity against *Sclerotinia sclerotiorum* and their ability to stimulate defenses in rapeseed was carried out. The molecules having presented the most important biological activities are those displaying a fatty acid chain of 10 and 12 carbons with a strong hydroxylation, thus increasing their amphiphilic property. The optimization of the functionalization of the molecules has made it possible to improve their biological activities for potential use in biocontrol.

P6.1-082

MONITORING ERWINIA AMYLOVORA SPREAD IN APPLE ORCHARDS: DEVELOPMENT OF A DETECTION PROTOCOL FROM CORBICULAR POLLEN

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Text

Fire blight caused by *Erwinia amylovora* (Ea) is a plant disease threatening apple production worldwide. As honeybees mediate the spread of Ea in apple orchards, a qPCR-based detection protocol was developed for the early detection of Ea from corbicular pollen. To do that, uninfected pollen was inoculated with cell suspension of strain Ea21 (rifampicin-resistant) at different concentrations (from 1×10^8 to 1×10^0 CFU/mL). Pollen not inoculated with Ea21 cells was used as untreated control. One gram of spiked samples was serially diluted and plated on Nutrient Agar amended with rifampicin. Concurrently, 30 g of spiked samples were homogenized in 120 mL of 0.85% (w/v) NaCl and 0.001% (v/v) TWEEN®80 and incubated on ice under orbital shaking (140 rpm). After 1 h, samples were centrifuged (3,000 rpm, 30 s) and the supernatants were collected and centrifuged (5,000 rpm, 10 min). The pellets were resuspended in Tris-HCl pH 8 buffer (2.25 mL) and exposed to thermal shock (95°C for 15 min and ice for 10 min). Products from the extraction were used directly in qPCR according to EPPO standard PM 7/20 using primer pairs hpEaF/hpEaR and the FAM Taq-man® minor-groove-binder (MGB) nonfluorescent quencher probe hpEaP.
In future, the detection protocol developed in this study might be used to monitor the presence of Ea in pollen collected from hives located close to apple orchards.

P6.1-083

CHARACTERIZATION OF THE PHYSICAL MODE OF ACTION OF AN ESSENTIAL OIL-BASED PRODUCT AGAINST GRAPEVINE DOWNY MILDEW CAUSED BY PLASMOPARA VITICOLA

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Text

Despite the advantage of Plant Protection Products application in agriculture, the massive use of chemicals has caused severe damage to human and environmental health. Within 2030 the EU Commission targets to reduce the use of pesticides by 50%, including those in the list of candidates for substitution such as copper-based products. To cope with this goal there is a crucial need to find non-chemical alternatives for disease management. In this study, the Physical Mode of Action (PMoA) of the botanical product EO41106 based on essential oils was characterized, in particular, for its preventative and eradicant efficacy against grapevine downy mildew caused by the Oomycete *Plasmopara viticola*. Trials were carried out under semi-controlled conditions at BBCH stages 51, 71 and 81. For preventative efficacy, different potted grapevines were sprayed with a copper reference, the EO41106 product and bi-distilled water as control. Inoculations with a sporangia suspension of *P. viticola* have regularly performed leaf discs in the lab, until 18 days after treatment. Incidence was evaluated at symptom appearance. For the eradicant efficacy, leaf disks with symptoms were sprayed with the same products on plates from 24 to 72 h after sporulation. Damaged sporangia were counted. Essential oil-based product EO41106 showed preventive protection comparable to copper in pre-flowering and the highest percentage of damaged spores with eradicant application.

P6.1-084

N-ACETYLCYSTEINE USE FOR BACTERIAL WILT OF POTATOES AND BACTERIAL CANKER OF GRAPEVINE

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Text

Plants are subject to many bacterial diseases that affect its productivity. For most of them, no cure is available, and producers use products such as copper-based mixtures and antibiotics to control them. New, sustainable and effective management options for plant bacterioses are urgently needed. Bacterial wilt of potatoes (BWP) and bacterial canker of grapevines (BCG) are examples of such diseases. Producers can only use kasumin and copper, respectively, to control such diseases. We developed different formulations using the modified amino acid N-acetylcysteine (NAC), an antioxidant that can improve plant-responses to microorganisms, helping to control some diseases. Previously, we have obtained good control results for citrus diseases such as CVC, citrus canker and greening. For BWP we obtained similar control results as those obtained for kasumin, in both disease

control and productivity. It is important to highlight that NAC is not an antibiotic, and therefore, is a more sustainable alternative to be used in field. For BCG, our tests conducted in a commercial field showed an increase of 35% in productivity, with no differences observed for fruit quality. Also, the number of infected leaves was reduced in 40%, indicatingdisease control was the main driver for productive increase. We here present other diseases that can be controlled with the NAC, a naturally found modified amino acid that is a promising alternative for large scale control of bacterial diseases in field.

P6.1-085

IDENTIFICATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH LEMON WOOD ROT IN ARIZONA

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Text

Canker and wood rots are economically important preharvest diseases of lemons in south-western Arizona, where commercial lemon production is concentrated. However, the aetiology and epidemiology of canker and wood rots are not well understood. This study comprised a large survey of canker and wood rot incidence and severity in Arizona and the characterization of fungal species associated with the disease. A total of 5431 trees with ages ranging from 1 to 20 years old in 10 lemon orchards were surveyed from 2018 to 2020. Our survey results revealed that canker and wood rot occurred in all 10 lemon orchards studied. Canker and brown rots of twigs, branches, and trunks were the most prevalent symptoms of affected trees ranging from 1 to 20 years old. In contrast, canker and white rots of twigs and branches were observed mostly on 1- to 5-year-old trees. Disease incidence for both diseases was less than 2% on 1- and 2-year-old trees. Brown rot increased significantly in older trees, ranging from 62.9% to 100%. Fungi were isolated from canker and wood rot samples and identified based on morphological characters and DNA sequences. *Fomitopsis meliae* and *Hypoxylon macrocarpum* were the primary canker and wood rot pathogens, at frequencies of 89% and 11%, respectively. In pathogenicity tests, both fungi were capable of causing canker and wood rots on lemon cv. Lisbon branches and the necrotic length caused by *F. meliae* was twofold greater than that caused by *H. macrocarpum*.

P6.1-086

NEW EVIDENCE ON ADVANCED TECHNIQUES FOR THE EARLY DETECTION OF PLANT DISEASES ON SOLANUM LYCOPERSICUM AND CAPSICUM ANNUUM

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Text

Every year agricultural production is affected by losses that can reach 20-40% of the global

harvest due to plant diseases. Reliable and early detection is crucial to minimise economic and field losses. To identify pre-visual or emerging symptoms enable acting earlier with protection treatments or by targeted removing infected plants to prevent the spread of disease. The most widely used detection methods are lab-based assays. Detection of DNA or RNA is reliable and accurate, but also expensive, time-consuming, and destructive. Optical sensing techniques have the potential to alleviate these problems. The aim of this project is to validate a method for the early detection of specific pathogens including TMV WT, TMV gfp-marked, Pseudomonas syringae, on Solanum lycopersicum and Capsicum annuum. Classical lab-methods including DNA/RNA extraction and the evaluation of pathogen concentration and distribution with qPCR were supplemented by the use of hyperspectral imaging, and non-destructive optical estimation of leaf pigments and measurements of chlorophyll fluorescence-related parameters. In addition, the expression of specific infection-related genes over time was evaluated and some tests with resistance inducers were also carried out to evaluate the plant's response against pathogens. These preliminary results show how the use of advanced sensor solutions has potential to increase the sustainability of crop-management systems and ensure plant health protection and food safety.

P6.1-087

BIOCONTROL OF RHIZOCTONIA SOLANI ON STRAWBERRY IN GREENHOUSE BY EXPERIMENTAL ANTAGONISTS

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Text

There are several soil-borne pathogens that affect strawberry crops, such as *Verticillium, Pythium, Phytophthora, Neopestalotiopsis* and *Rhizoctonia*, with production losses that can be huge. As part of the European project Excalibur, experimental trials have been carried out on strawberry plants cv Olimpia, Portola, Clery and Elodiè in greenhouse with the aim to evaluate the efficacy of microbial antagonists, obtained from suppressive soils, substrates and compost, to control *Rhizoctonia solani.* Results showed a good efficacy of some microorganisms such as antagonistic Fusaria and Trichoderma that significantly reduced *R. solani*, while all remaining tested microorganisms provided similar protection to the commercial mixture of *Trichoderma asperellum* and *Trichoderma gamsii* (64-75% efficacy). However, the differences in efficacy varied according to the strawberry cultivar used in the study.

P6.1-088

CRANBERRY FRUIT ROT: CHARACTERIZATION AND NOVEL MANAGEMENT

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Text
Cranberry fruit rot (CFR) complex which is associated with over a dozen taxonomically diverse fungi, has been described as the most yield-limiting and economically significant disease in cranberry production since the late 1800s. To this date, CFR stands as a significant threat to North American cranberry cultivation. CFR could result in 100% losses if not managed with rigorous cultural and fungicide regimes. Even with fungicides, fruit rot in Northeastern (NE) US ((New Jersey (NJ) and Massachusetts (MA)) ranges from 1-15%. Fruit rot infected lots beyond 12% are heavily discounted and lots with >20% are not accepted by many cranberry handlers (processing industry). Growers are also dealing with a new challenge of losing the most commonly used multi-spectrum mode of action fungicides (chlorothalonils and mancozebs) owing to perceived negative impacts on pollinators, human and environmental health. These changes and associated restrictions are prompting the industry to shift towards the only two available single-site mode of action (FRAC Group 3 & 11) fungicides which are now at the risk of fungicide resistance. We would like to take this opportunity to present on the current status of CFR research in MA. We will present data from CFR fungal characterization studies focusing on year-to-year fungal population variations from wild, conventional and organic systems, and multi-year efforts towards novel and integrated CFR management in organic and conventional production systems.

P6.1-089

INTEGRATED AND BIOLOGICAL PROTECTION STRATEGIES AGAINST POWDERY AND DOWNY MILDEWS ON GRAPE: RECENT RESULTS FROM TRIALS CARRIED OUT IN ITALY.

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Text
Powdery (causal agent *Erysiphe necatrix*) and downy (causal agent *Plasmopara viticola*) mildews are the most important grape diseases, causing relevant losses both in terms of quality and quantity. Climate changes may further favour the development of these diseases in the future, in a context where policies are limiting the use of chemical fungicides, copper included. Low environmental impact products, such as oligosaccharides derived from chitin, essential oil from sweet orange, *Ampelomyces quisqualis* and *Bacillus pumilus* showed to be effective in trials carried out on “Arneis” and “Nebbiolo” both on potted plants and in field conditions, demonstrating to be applicable in vineyard to reduce, at least partially, the use of traditional fungicides and to respect recent restrictions in the use of copper in Europe. In the trials on potted plants, acibenzolar-s-methyl was the most effective, among the other tested products, against both pathogens, followed, on downy mildew, by potassium phosphonates.

P6.1-090

CELLULOSE NANOCRYSTALS AS AN INNOVATIVE TOOL TO CONTROL X. PERFORANS

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Text

Xanthomonas perforans (Xp) is one of the main responsible for bacterial leaf spot on tomato, leading to dramatic crop losses. New tools are required to limit its spread in field and its introduction in new areas, since traditional strategies, such as the use of cupric salts, are losing effectiveness. Among the original approaches proposed in crop protection, nanomaterials could represent a sustainable way to control bacterial disease. In this work we evaluated the antimicrobial mechanisms of cellulose nanocrystals (CNC) on Xp. The obtained results suggested that CNC do not damage the bacterial cells, but they are able to decrease in vitro swarming and swimming motility, with a significant reduction of 30% in motility when supplemented in agar media. CNC presence in liquid media prevent cell adhesion to surfaces; as a result lower level of EPS have been recorded. Moreover, comparison with carboxymethylcellulose pointed out that CNC cannot be attacked by bacterial cellulases. All this combined effects played a major role in tomato plants, where foliar treatment with CNC reduced Xp epiphytic survival and leaf ingress after 7 days from spray inoculation. These interactions were confirmed by looking at the molecular expression of the bacterial cells on plants by RT-qPCR, which showed a down-regulation of pilA, rpfG and engXCA genes. These results highlight the potential of cellulose nanomaterials to develop innovative agrochemicals for controlling bacterial plant pathogens.

P6.1-091

STUDY ON EFFICACY OF ETHANEDINITRILE AGAINST PLASMODIOPHORA BRASSICAE, THE CAUSAL AGENT OF CLUBROOT DISEASE OF CRUCIFERS

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Text

Clubroot disease, caused by *Plasmodiophora brassicae* Woronin, an obligate biotrophic protist, has been a global challenge for oilseed rape farmers to control effectively. The current study investigated the potential of ethanedinitrile (EDN), a cyanogen-based fumigant as an effective tool to control clubroot disease at different concentration rates and durations of exposure. Clubroot-susceptible oilseed rape plants were grown in EDN-fumigated soil and evaluated for clubroot disease severity and plant growth parameters in a greenhouse setting. The results demonstrated a reduction in clubroot disease severity by 81.39% as compared to the control, and complete control was achieved at concentration rates of 42 g/m^3 and 50 g/m^3 and in the 35 g/m^3-48 h variant. EDN was also found to improve plant health, with a 58.24% increase in shoot weight. However, higher concentration rates and longer exposure durations had potential adverse residual effects on the plants, including a decline in seedling emergence rate and plant shoot weight. This suggests that there is a need to find a balance between efficacy and sustainability in terms of plant health. Overall, the results of the study show the

potential of EDN as an effective tool to control clubroot, offering hope for minimizing yield loss due to clubroot disease in oilseed rape and other crucifer crops.

P6.1-092

APPLICATION PROSPECTS FOR THREE BACTERIAL ENDOPHYTES AGAINST SOIL BORNE DISEASES AND CROP YIELD IMPROVEMENTS IN ORGANIC PRODUCTION OF VEGETABLES.

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Text

Naturally abundant microorganisms which colonize plants internally without causing harm to their host plants often provide plant protection against pathogens and improvements in plant growth and soil health. However, results from greenhouse studies do not often translate to consistent field efficacy. In our studies, three isolates of bacterial endophytes (PRT, PSL and IMC8) were efficacious against multiple pathogens in vitro, in greenhouse and in repeated fields trials against Phytophthora capsici and Sclerotium rolfsii. Seed treatment allowed early plant colonization prior to plant exposure to pathogen-infested soil. Plastic mulch was used to control weeds and drip irrigation provided water; no fertilizer was used. Data on tomato and sweet pepper plant vigor, number of fruits, fruit size, disease scores, percent of diseased fruits, and total yield per plant were collected. Analysis of variance displayed significant differences between the bacteria-treated plants and the non-treated control. The three strains performed better than two commercial bio-fungicides, Double Nickel® and Serenade® on disease control and fruit yield following label recommendations. Strain PRT gave the best results for most of the traits followed by PSL. IMC8 was best for plant vigor and larger fruit size, but with a smaller number of fruits per plant. The selected bacterial isolates will be good additions in organic farming disease control and yield improvements.

P6.1-093

MITIGATION OF GREY MOULD DISEASE IN STRAWBERRY PLANTS THROUGH EXOGENOUS APPLICATION OF PHYTOHORMONES

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Text

Grey mould (GM) disease, caused by the necrotrophic fungus *Botrytis cinerea*, is a devastating disease for strawberry plants (*Fragaria* x *ananassa*) and its control requires intensive application of synthetical fungicides. Bio-based products, eliciting plants' defense mechanisms, are promising alternatives for disease mitigation. To combat fungal infections,

plants rely on their innate immune responses, where phytohormones play a critical role. Although some previous studies have evaluated the effect of exogenous application of a given phytohormone on strawberry resilience to GM, a more comprehensive analysis is still missing. This study aimed to evaluate and understand the impact of salicylic acid (SA) and jasmonic acid (JA) and their methylated forms (MeSA and MeJA) on the progression of GM. Six treatments (Control-water, Control-EtOH, SA, MeSA, JA and MeJA) were applied four times (every two weeks) to strawberry plants cv. '*San Andreas*' grown in a greenhouse. Our results demonstrated that JA significantly inhibited *B. cinerea* infection in leaves, as well as MeJA and MeSA. Moreover, it was found that SA and JA inhibited *B. cinerea* mycelial growth when tested *in vitro*. These results suggest that both phytohormones may play a key role on the activation of plant defense mechanisms and/or the inhibition of *B. cinerea* pathogenicity. Understanding these mechanisms may be critical for integrating these compounds for GM management.

P6.1-094

BURKHOLDERIA SP. SSG – A POWERFUL NEW MANAGEMENT TOOL FOR CROP HEALTH AND PRODUCTION

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Text

Burkholderia sp. SSG was an endophyte isolated from boxwood blight-reverted leaves of *Buxus sempervirens* 'Suffruticosa'. This endophyte provided excellent control of boxwood blight caused by *Calonectria pseudonaviculata* (*Cps*). Specifically, SSG at 10^7 cfu/mL lysed all conidia in mixed broth culture. SSG at 10^8 cfu/mL reduced blight of container-grown Justin Brouwers boxwood by >98% when applied 1 day before or 3 hours after plants were inoculated with *Cps* under controlled environments. Its blight control decreased with decreasing bacterial concentration and increasing lead time. When applied on diseased leaf litter under boxwood plants, SSG reduced *Cps* sporulation and consequently mitigated blight incidence by 90%. When applied monthly onto boxwood plants in production fields, SSG provided a similar level of blight control as the fungicide standard – Concert II. SSG also was effective against a variety of other diseases caused by five fungi (*Alternaria tenuissima, Botrytis cinerea, Colletotrichum fructicola, C. gloeosporioides, Pseudonectria rouselliana*), three Oomycetes (*Phytophthora capsici, P. nicotianae, P. ramorum*), plus *Xanthomonas campestris* and tomato spotted wilt virus on eight crops. Additionally, SSG promoted boxwood growth. These properties together make this endophyte a powerful management tool for crop health and production. SSG was identified as a new member of the *Burkholderia cepacia* complex with distinct characters from known clinical strains.

Raising awareness of plants and ways of teaching plant pathology

C8.7-1

TEACHING PLANT PATHOLOGY

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Text

In the past forty years teaching plant pathology changed significantly, both from a student and professor perspectives. Which have been the driving forces? The agricultural sector did change during the past few decades: environmental protection and food safety became more relevant and different needs of the stakeholders emerged. Unfortunately, not always departments and colleges have been able to keep the pace, adapting the content of the courses to societal needs. In the period 1975-1990 plant pathology was taught in Agricultural Sciences as a main course plus several others: physiological plant pathology, plant disease management, generally followed by most students. Others were offered as optional courses. In the early 2000, plant pathology remained as the only mandatory course for students in the agricultural science *curriculum*, and specific courses disappeared. During 2001-2020 the number of *curricula* offered increased, with a growing importance of the agro-food sector. The Internet in the early 2000 permitted much faster access to information and images for both students and instructors, resulting useful also for life-long programmes. Instructional technology, providing tools tools for classroom presentations and communication with students, became crucial during the Covid-19 pandemics. Nowadays, hybrid forms of teaching help reaching a broader audience. The many changes encountered in teaching is described as a kind of journey and critically discussed.

C8.7-2

TEACHING PLANT PATHOLOGY IN THE LARGER CONTEXT OF SCIENCE AND THE HUMANITIES

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Text

In 2013 a class was first developed at the University of Minnesota called “Plague, Famine, and Beer”. This class has gone on to achieve great success in attracting a large and consistent pool of students (100-200 each year) by linking plant pathology to the historical perspective liberal education requirement. The course discusses the history development of the science of microbiology including the study of plant pathogens in the larger context of the history of science and medicine and the development of germ theory. Additionally, both negative and positive effects of microbes on the course of human civilization are covered. In the middle “famine” portion of the class, historically important plant disease epidemics such as the Irish Potato Famine, Ergot of Rye, Chestnut Blight are also discussed considering their respective impacts on human history. Students also gain experience doing the work of historians by directly analyzing primary sources from these events. By including plant pathogens with human pathogens and innovations such as microbial food and drink fermentation, antibiotics, biotechnology, etc., students gain an additional context of the overall importance of the discipline of plant pathology to their lives.

C8.7-3

GRADUATE EDUCATION IN PLANT PATHOLOGY: PREPARING SCHOLARS TO ADVANCE THE DISCIPLINE

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Text

Over the past few decades, we've experienced a rapid expansion in knowledge fueled in part, by the rapid evolution of the technologies that underpin science. Growth in disciplines and subdisciplines coincident with the rapid expansion in scientific journals over this same period leads to important questions regarding the education of the next generation of plant pathologists: what is enough for a student to know and achieve in-order to fulfill the requirements for a graduate degree? Have we become too focused on the acquisition of technological skills at the expense of developing a broad and deep knowledge base essential to advancing plant pathology? Is the steady decline in the number of plant pathology departments an indication of the evolution of our discipline through integration into other disciplines or a reflection of the fragmentation of disciplines into more specialized areas of study? Is the decline in taxon-based courses a result of careful deliberation as to the best approach to graduate education or a consequence of the fragmentation of the discipline? There have been periodic discussions as to whether a Ph.D. program should require coursework or be solely focused on research. The increase in knowledge in all areas of science affords the opportunity for deeper understanding but, is it at the expense of breadth of knowledge? Our goal should be to generate scholars to advance our discipline. The challenge is to determine the best way to achieve that goal.

C8.7-4

A STEP FORWARD IN THE APPLICATION AND TEACHING OF MODERN STATISTICAL METHODS FOR PLANT PATHOLOGY

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Text

Ex vivo and in vivo assays are routinely employed by plant pathologists to evaluate the effects of a certain treatment on Plant-Pathogen Interactions (PPI). Unfortunately, PPI is a complex phenomenon affected by many factors, which dramatically increases the variability associated with experiments. Therefore, the use of appropriate statistical analyses is essential for the positive exploitation of data, thus limiting the risk of misinterpretation of results. For this reason, appropriate handling of statistics is crucial for phytopathologists. However, traditional techniques, such as ANOVA or non-parametric tests, are often

inappropriate or even deleterious for PPI datasets but are still common in scientific publications. The increasing availability of open-source tools for statistical analyses represents an opportunity for researchers, professionals, and students; However, the adoption of uncommon techniques still represents an obstacle because of limited knowledge from potential users. Teachers must address these needs during their lessons. Exploiting real datasets from recent publications, easy-to-interpret and appropriate analyses and instruments (e.g., generalized linear models, beta regressions, machine learning, and effective data visualization) should be considered for plant pathology classes, thus shaping a new generation of plant pathologists that can efficiently exploit information derived from laboratory and field trials.

C8.7-6

A CONVENIENT BIBLIOMETRIC PIPELINE TO SNAP-SHOT LARGE RESEARCH FIELDS - AN EXAMPLE OF APPLICATION WITH THE FIELD OF MOLECULAR PLANT IMMUNITY

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Text
To quickly gain an analytical and conceptual knowledge of a large research field is complex and daunting. Yet, academics of all levels critically need such knowledge to make educational- and career-related strategic decisions, to develop creative and sound research projects, to train students, or to mentor colleagues. Over the last three years, our group developed, used, and published a bibliometric pipeline that allows to snap-shot large research fields in an objective, data-driven manner (Petre et al., 2022; doi.org/10.1094/MPMI-05-22-0112-CR). On the one hand, junior academics, such as early-career scientists and graduate students, can use this pipeline to quickly and effectively apprehend a given research field and to build solid dissertations, thesis introduction, literature reviews, or context parts of grant proposals; and to ultimately make strategic career-related decisions. On the other hand, senior scientists, such as higher education teachers and mentors, can use this pipeline to prepare comprehensive courses and lessons, to explore fields that are outside of their area of expertise, and to effectively advise students and colleagues. At the ICPP, we propose to present an example of application of our bibliometric pipeline to analyze the field of molecular plant immunity, as well as a follow-up study focused on the research community in France. The presentation will highlight the value of our approach for training and mentoring graduate students.

P8.7-001

FARMER FIELD SCHOOLS AS A MEANS OF ENHANCING FARMER KNOWLEDGE IN COMMON BEAN PRODUCTION AND DISEASES MANAGEMENT IN UGANDA

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Text

The Farmer Field School (FFS) is a Farmer Participatory Research approach that can be used to determine end-user acceptance of new technologies. In 2019, we initiated a program of FFSs in 5 districts of Uganda to promote biofortified beans and crop management practices including chemical disease management, use of NPK, row planting and weed management. We established six 1-acre FFSs in each district run by pre-existing Farmer Groups. Key FFS activities included land preparation, variety selection, row planting, fertilizer application, seedling and foliar diseases assessment, application of Ridomil Gold and harvest of beans. Farmer knowledge of introduced technologies was assessed at the onset of the FFSs and at the end of the season Thirty-five, 15 and 36% of the participants said they understood fertilizer application, row planting and disease identification and control using Ridmil Gold, respectively. Better still, 29, 64 and 18% said they got full knowledge of the practices above, respectively. Similarly, 40, 29, and 11% participants reported to have acquired new knowledge on proper chemical dosages, use of protective clothing and proper disposal of used chemical containers, respectively. Participants acknowledged positive impact of FFSs on pre-existing Farmer Groups as FFSs offered access to new knowledge and technologies, motivated participation and unity, provided access to information and increased their visibility and chances of benefiting from Government programs.

P8.7-002

ADDRESSING THE GLOBAL GAP IN SEED PATHOLOGY EDUCATION

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Text

Seeds are foundational to production of most plant species. Efficient cultivar development, seed production, and seed distribution require a global effort, representing a $50+ billion industry annually. As a result, regional and global movement of seeds is a critical component in the seed supply. Because seeds, in some cases, can be a pathway for dissemination of plant pathogens, phytosanitary restrictions on seed movement are enforced increasingly in most countries. This fuels the demand for education and training in seed science and seed pathology, including knowledge needed to address rapid technological advances, phytosanitary concerns, and evolution of seed trade policies. However, very few university programs are dedicated to seed pathology. Many seed companies have had to create in-house training programs to develop expertise in seed pathology that is essential to seed production and management, and government agencies have difficulty hiring staff with relevant seed pathology expertise. To help address the global gap in educational resources and training in seed pathology, a 12-week, 1 h/week, online, introductory course is being developed by seed pathologists from academia, industry, and government agencies in various countries to provide a high-level perspective on seeds and seed pathology. We will discuss this course as the first in a series of seed pathology educational events being developed to address the global demand for seed pathology education and training.

P8.7-003

DON’T RISK IT! - AN EPPO COMMUNICATION CAMPAIGN TO RAISE AWARENESS OF INTERNATIONAL TRAVELLERS ABOUT THE RISKS OF CARRYING PLANTS IN THEIR LUGGAGE

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Text

Travelling long distances with plants or plant products of unknown phytosanitary status can present serious risks to agriculture, forestry and the environment, as these plants may carry pests and diseases. International travellers are generally unaware of these risks, in particular when travelling between continents which have quite distinct floras and faunas. In order to raise public awareness about the risks of moving plants during international travels, EPPO launched a communication campaign in 2013 with the slogan 'Don't Risk It!'. A poster and its accompanying leaflet were provided to National Plant Protection Organizations of EPPO countries (in Eurasia and the Mediterranean Basin). They were designed as templates so that they could be easily translated and adapted. The main objective was that these documents should be displayed in airports or any other sites where travellers would easily see them. So far, more than 20 EPPO countries have used this communication material in airports, train stations, border inspection points, travel agencies, websites, international fairs, etc. Countries have also been quite creative and used the slogan or the visual material to prepare videos, luggage tags, bookmarks and other goodies. It is hoped that the ‘Don’t Risk It!’ campaign will continue to contribute to a better understanding of the general public about the importance of protecting plant health and ultimately reduce the risks of introducing dangerous diseases into new areas.

P8.7-004

ONE HEALTH PERSPECTIVES ON SMALLHOLDER COCOA PRODUCTION AND POVERTY

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Text

Yields of cocoa have stagnated despite decades and millions of dollars spent on research and farmer training. In Indonesia and Papua New Guinea we have shown that labour availability and productivity is the major constraint to cocoa production, due to poor education, aging and poor health of farming families, narrow income diversification and increasing dependency on off-farm and remittance income. Conventional farmer training programs focus on technical aspects of cocoa farming and ignore the capacity of individual households to invest in improved technologies, usually requiring available healthy, educated and productive labour. On the cocoa growing island of Bougainville we addressed labour

productivity through transdisciplinary training on family nutrition and health, gender equity, income diversification, local leadership and goal setting to complement conventional farmer training. Dietary diversity and food security improved, food crop and cocoa production increased. The importance of educated, healthy labour was confirmed by when the local demand for food in remote rural communities stimulated innovation and the adoption of new technologies and increased local food and cocoa production when relatively young, healthy and well-educated labour returned from urban centres during the COVID-19 pandemic. Healthy crops require healthy farmers

Re-emergence of tobamoviruses threatening global vegetable production

C8.4-1

GLOBAL EMERGENCE OF TOMATO BROWN RUGOSE FRUIT VIRUS IN TOMATO AND PEPPER AND ITS MANAGEMENT

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Text

Tomato brown rugose fruit virus (ToBRFV) is an emerging tobamovirus that was first identified in 2014 infecting greenhouse tomato in the Middle East (Jordan and Israel). Due to its ability to break the popular resistance gene (*Tm-2*^{2}), this seed-borne and mechanically transmitted virus quickly spread around the world, causing disease outbreaks on tomato and pepper in over 30 countries in just few years. Most of the outbreaks occurred on tomato in a greenhouse, field-grown tomato and pepper are also susceptible. ToBRFV can be transmitted through hands-on activities during transplanting, pruning, de-leafing or fruit harvesting. With increasing activities in global seed production and trade, seed health testing will ensure a seed lot free of ToBRFV to be used for planting. In nursery propagation, it is necessary to monitor seedlings for symptoms, followed by a confirmation test. In greenhouse production, it is important to establish a strong cleaning, disinfecting and sanitizing program. Several effective disinfectants (Clorox, Virkon S, and Virocid) capable of inactivating virus infectivity have been identified. However, the most effective means of management would be to develop a new tomato cultivar with resistance to the virus. In screening the USDA tomato germplasm, a new source of resistance to ToBRFV was identified in a tomato relative, *Solanum pimpinellifolium*. QTL analysis, SNPs identification and their applications for marker-assisted selection will be discussed.

C8.4-2

WATER AND SOIL CONTAMINATED WITH EMERGING TOBAMOVIRUSES ARE THE SOURCE OF PLANT INFECTIONS

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Text

The recent emergence of tomato brown rugose fruit virus and tomato mottle mosaic virus (ToBRFV and ToMMV, *Tobamovirus, Virgaviridae*) poses a significant threat to global tomato and pepper production. Tobamoviruses have highly stable virions and are mainly transmitted through infected seeds, planting material and mechanically, but other transmission routes should not be neglected. Our objective was to determine the possible transmission routes of emerging tobamoviruses via infested water or soil. Results of our study indicate that ToBRFV can remain infectious in water stored at room temperature for up to four weeks, while its RNA can be detected in water for at least four months. We have shown that ToBRFV-contaminated water used for irrigation in hydroponic or soil-based cropping systems can infect tomato plants through the roots after one to six months of exposure. In addition, soil infested with ToBRFV or ToMMV was confirmed to be an inoculum source for planted tomato seedlings and seedlings grown from seeds. These results indicate new epidemiological pathway of ToBRFV and ToMMV via water and soil. The results fill existing knowledge gaps and point to the need to control the presence of emerging tobamoviruses and to disinfect irrigation water and soil in tomato and pepper production if contamination occurs.

C8.4-3

IMPROVING STRATEGIES FOR TOMATO BROWN RUGOSE FRUIT VIRUS SURVEILLANCE IN TOMATO FRUIT PRODUCTION

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Text

Tomato brown rugose fruit virus (ToBRFV) is a member of the genus Tobamovirus. Since it was first detected in Jordan and Israel in 2014/15 it has spread to the major tomato production regions of the world. It has become a major constraint on commercial tomato production in the Northern Hemisphere impacting yield and marketability of tomato fruit from

affected crops. Since 2018, National Plant Protection Organisations have focused on stringent biosecurity measures, such as seed testing, to minimise the risk of transnational spread. Strategies for containment at the national level have focused on crop surveillance, however, visual inspection is inadequate for detection of the virus. Laboratory diagnostic protocols are limited in being able to handle adequate numbers of plant samples, especially for testing young plants.
Studies have been conducted to investigate the optimum sampling strategy to detect the virus from plants including both inoculation trials and outbreak sampling exercises. These have identified that sepals and young leaves give the most reliable detection irrespective of plant age. Additionally, non-invasive, non-crop based monitoring approaches have been investigated including swab and irrigation water testing, using both laboratory and onsite methods. These approaches could offer early detection of the virus from an infected glasshouse. Approaches for integrating these approaches into monitoring both pre- and post-outbreak will be discussed.

C8.4-4

THE EMERGING TOMATO BROWN RUGOSE FRUIT VIRUS REVEALS A CROSSROAD BETWEEN VIRAL MOVEMENT AND PLANT IMMUNITY

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Text

The tomato *Tm-2*^{2} gene was considered one of the most durable resistance genes in agriculture, protecting against viruses of the *Tobamovirus* genus. However, an emerging tobamovirus, tomato brown rugose fruit virus (ToBRFV), has overcome *Tm-2*^{2}, damaging tomato production worldwide. *Tm-2*^{2} encodes a plant immune receptor that recognizes its effector, the tobamovirus movement protein (MP). Recently, we have established that ToBRFV MP (MP^{ToBRFV}) enabled the virus to overcome *Tm-2*^{2}-mediated resistance. Yet, it was unknown how *Tm-2*^{2} remained durable against other tobamoviruses (e.g. TMV and ToMV) for over 60 years. Here, we show that a conserved cysteine (C68) in the TMV MP (MP^{TMV}) is both sufficient to trigger *Tm-2*^{2} resistance and essential for intercellular movement of the virus. Substitution of MP^{ToBRFV} amino acid H67 with the corresponding amino acid in MP^{TMV} (C68) activated *Tm-2*^{2}-medited resistance. Phylogenetic and structural prediction analysis revealed that C68 is conserved among all Solanaceae-infecting tobamoviruses except ToBRFV, and localizes to a predicted β-barrel fold common to various viral MPs. Cell-to-cell and systemic movement analysis showed that C68 is required for the movement of TMV and ToMV, by determining the MP subcellular localization and targeting it to plasmodesmata. The double role of C68 in viral movement and *Tm-2*^{2} immune activation could explain how TMV and ToMV were unable to overcome *Tm-2*^{2} for such a long period.

C8.4-5

CUCUMBER GREEN MOTTLE MOSAIC VIRUS SYMPTOMATOLOGY IN CUCUMBER PLANTS EXPOSED TO FLUCTUATING TEMPERATURES AND SOIL PATHOGENS

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Text

Studies of early stages of cucumber green mottle mosaic virus (CGMMV) disease could be affected by extreme diurnal temperatures. Our studies disclosed a new phenotypic description of CGMMV disease initiation, characteristic of cucumbers grown under extreme environmental temperature fluctuations. We have revealed two new phenotypes that developed gradually, preceding severe symptoms of post-recovery CGMMV infection. 'Early post-recovery stage' bright yellow islands ('early BYIs') with defined boundaries amid asymptomatic leaf blades were first emerging followed by 'late post-recovery stage' ('late BYIs') with diffused boundaries. Profiling ontology of cucumber differentially expressed genes in BYIs vs the associated dark-green surrounding tissue disclosed activation of jasmonic acid (JA) pathway in 'early BYIs'. JA signaling was inactivated in 'late BYIs' concomitant with increasing expressions of JA signaling inhibitors and downregulation of the phenylpropanoid pathway. Regarding RNA silencing we have found that specifically at the 'early BYIs', RDR1c was significantly upregulated. Knockdown of cucumber rdr1c1 and c2 genes using CRISPR/Cas9 system increased viral accumulation. Plants with a homozygous mutation in rdr1c were highly susceptible to coinfection of soil-mediated CGMMV and necrotrophic pathogen Pythium spp. leading to plant collapse.

C8.4-6

THE ROLE OF THE WEED SPECIES AMARANTHUS VIRIDIS IN THE EPIDEMIOLOGY AND SURVIVAL OF CUCUMBER GREEN MOTTLE MOSAIC VIRUS

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Text

Amaranthus viridis is a weed host of cucumber green mottle mosaic virus (CGMMV) that can occur in and around Australian cucurbit crops. Experimentally, it has been shown to be a viable host for CGMMV and mechanical inoculation with infected A. viridis was used to successfully transmit the virus to susceptible cucurbit species. CGMMV can be difficult to detect in field collected A. viridis, which might be associated with virus titre and distribution

within the plant. It is not known if CGMMV reaches the seeds and if it can be successfully vertically transmitted in this weed host. This raises questions about the importance of A. viridis as virus reservoir and its contribution to the CGMMV disease cycle. Using a series of pot trials this study examined the systemic movement and virus titre of CGMMV across the life cycle of A. viridis. Seeds collected from virus positive plants were tested for presence of virus using quantitative RT-PCR. Seed transmission of CGMMV was analysed by testing second-generation plants propagated from collected seeds. Preliminary results indicate systemic movement of the virus within A. viridis. The results of this study will inform CGMMV management in Australian cucurbit crops.

P8.4-001

AFRICAN EGGPLANT-ASSOCIATED VIRUS: CHARACTERIZATION OF A NOVEL TOBAMOVIRUS IDENTIFIED FROM SOLANUM MACROCARPON AND ASSESSMENT OF ITS POTENTIAL IMPACT ON TOMATO AND PEPPER CROPS

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Text

A novel tobamovirus was identified in a fruit of *Solanum macrocarpon* imported into the Netherlands in 2018. This virus was further characterized in terms of host range, pathotype and genomic properties, because many tobamoviruses have the potential to cause severe damage in important crops. In the original fruit, two different genotypes of the novel virus were present. The virus was able to infect multiple plant species from the *Solanaceae* family after mechanical inoculation, as well as a member of the *Apiaceae* family. These species included economically important crops such as tomato and pepper, as well as eggplant and petunia. Both tomato and pepper germplasm were shown to harbor resistance against the novel virus. Since most commercial tomato and pepper varieties grown in European greenhouses harbor these relevant resistances, the risk of infection and subsequent impact on these crops is likely to be low in Europe. Assessment of the potential threat to eggplant, petunia, and other susceptible species needs further work. In conclusion, this study provides a first assessment of the potential phytosanitary risks of a newly discovered tobamovirus, which was tentatively named African eggplant-associated virus.

P8.4-002

DIAGNOSIS AND CHARACTERIZATION OF SEED BORNE VIRUSES IN CUCURBITACEOUS CROPS: HIDDEN THREAT FOR GLOBAL CROP PRODUCTIONS AND SEED INDUSTRIES

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Text
Cucurbitaceous crops are one of the important groups in the vegetable crops that are rich in neutraceuticals and pharmaceutical value. However, investigations during 2017-2023 revealed that seed borne viruses are most significant because of its survivability, persistent nature, transmission potentiality, contagiousness, severity, loss of yield and quality of the produce. In the current investigations, collected the virus infected cucurbit host plants viz., ashgourd, bitter gourd, bottle gourd, chow-chow, cucumber, gherkin, muskmelon, pumpkin, ridge gourd, snake gourd, spiny gourd, squash, teasle gourd and watermelon and seeds; further, developed the diagnostics through molecular, serological, and biological techniques. The sequencing results concluded that Cucumber green mottle mosaic virus and whiteflies transmitted Tomato leaf curl New Delhi virus is predominantly detected in bottle gourd and ridge gourd seed coat, respectively. Further, confirmed the CGMMV and ToLCNDV easy transmission through sap on bottlegourd and ridgegourd, respectively. Finally, developed and demonstrated the integrated virus disease management strategies for ridgegourd, cucumber and muskmelon. Indeed, seed borne viruses might be entered to India through escapes while importing from other countries through commercial seed industries; hence, vigilance and stringent seed quarantine measures to be implemented globally.

P8.4-003

REMOTE REAL-TIME RT-PCR FOR MONITORING AND EARLY DETECTION OF TOMATO BROWN RUGOSE FRUIT VIRUS IN TOMATO CROPS IN SICILY

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Text
Tomato brown rugose fruit virus causes destructive disease on tomato. Following its first outbreak occurred in Italy, it has become a serious threat to tomato crop.
For that purpose, 600 tomato samples were directly analyzed in field in 3 Sicilian different areas with the bCUBE® system (Hyris Ltd). The bCUBE® was integrated within a network of mini-lab, established and monitored in real time by the Plant Virology Lab located at the University of Palermo.
A quick in-field sample preparation procedure was used. In detail, ~100 mg of tissue were homogenized in a sample bag with 3mL of extraction buffer. Five µL of extract was spotted on a 1 cm2 hybridization membrane, dried at RT for 5 min, and placed in a 2 mL tube with 250 µL of glycine buffer. After manual mixing of the tubes for 20 sec, 3 µL of the extract were used for the RT-qPCR assay according to the procedure developed by Panno and co-workers (2019). The results were visualized on a smartphone directly in the field and at the University of Palermo using the webApp developed by Hyris.
To confirm the RT-qPCR analyses in field, 125 samples were also analyzed at the Plant Virology laboratory of ISPP-CNR (Turin) using routine procedures such as RNA extraction and a RT-qPCR. All samples resulted positive in-field for ToBRFV were confirmed by the laboratory analyses. The developed mini-laboratories network, data visualization, and

analysis confirmation allowed remote and continuous virus monitoring and in-field ToBRFV detection.

P8.4-005

LATENT INFECTION BY TOBAMOVIRUSES

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Text

Tobamoviruses are among the most well-studied plant viruses and yet there is still a lot to uncover about them. On one side of the spectrum, there are damage-causing members of this genus: such as the tobacco mosaic virus (TMV), tomato brown rugose fruit virus (ToBRFV) and cucumber green mottle mosaic virus (CGMMV), on the other side, there are members which cause latent infection in host plants. New technologies, such as high-throughput sequencing (HTS), have enabled us to discover viruses from asymptomatic plants, viruses in mixed infections where the disease etiology cannot be attributed to a single entity and more and more researchers are looking at non-crop plants to identify alternative virus reservoirs, leading to new virus discoveries. However, the diversity of these interactions in the virosphere and the involvement of multiple viruses in a single host is still relatively unclear. For such host–virus interactions in wild plants, symptoms are not always linked with the virus titer. Although latency has been observed for many tobamoviruses, the mechanisms underlying this phenomenon are still elusive. While there are multiple definitions of latent infection in the literature, there are even more explanations that use this umbrella term. It is important to study new and emerging tobamoviruses that are threatening horticulturally important crop plants, as well as those that cause a latent infection, which can go unchecked and potentially cause diseases on other hosts.

P8.4-006

SEARCHING FOR A MILD ISOLATE TO USE IN CROSS-PROTECTION STUDIES FOR TOBRFV

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Text

Tomatoes and other solanaceae are among the most important food crops in the EU and worldwide. The tomato brown rugose fruit virus (ToBRFV) is a recently emerged tobamovirus that has quickly spread since its discovery in 2016 and can be a severe threat to pepper and tomato cultivation. ToBRFV can overcome established resistances against tobamoviruses, such as the Tm-2^2 gene in tomato (Luria et al. 2017) and L genes in pepper plants (Fidan et al. 2022).
The use of cross-protection presents the possibility to establish resistance without the necessity for time-consuming breeding approaches and thus without affecting established

cultivar traits.
Different approaches are applied in the VIRTIGATION project to screen for mild isolates that can be used in cross-protection studies. Here we employed the application of nitrous acid and we investigate mutagenesis in tissue culture for its suitability to generate ToBRFV mutant isolates. This method has been recently established for potato virus Y, where attenuated mutants were found after regeneration of shoots from leaf discs of infected plants (Ogawa et al. 2013).
Using these methods, we look for candidates with attenuated ToBRFV disease phenotypes. The virus isolates present in the plants will be sequenced and evaluated for their potential to be used as cross-protection agents against infection of tomato with the severe wild-type ToBRFV.

P8.4-007

THE ABILITY OF ELISA, REAL-TIME RT-PCR, AND BIOASSAY IN THE QUANTIFICATION OF THE VIRUCIDAL EFFICACY USING MENNO FLORADES DISINFECTANT AND TOMATO BROWN RUGOSE FRUIT VIRUS AS AN EXAMPLE

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Text
Tomato brown rugose fruit virus (ToBRFV) is a new emerging tobamovirus considered to be one of the biggest threats to tomato production. It is a regulated/quarantined plant pathogen in many countries including Germany. The virus has been reported in more than 35 countries in tomato, pepper, and herbaceous plants. It overcomes the Tm-2 resistance genes in tomatoes and outbreaks caused by ToBRFV have been reported in several countries since the first outbreak in 2014. Tobamoviruses are easily transmitted by mechanical contact and remain infectious for serval years outside of the host cells making their eradication from infected areas very difficult. Therefore, the application of an effective disinfectant is mandatory. MENNO Florades is an authorized plant protectant in Germany showing very promising results in the deactivation of ToBRFV on different surfaces. We compared three different quantification methods including quantitative ELISA, RT-qPCR, and bioassays as methods based on virus proteins, viral genomic nucleic acids, and virus pathogenicity, respectively, for their suitability to quantify the virucidal efficacy of MENNO Florades. Finally, only the bioassay could estimate the efficacy of the disinfectant with satisfying accuracy.

Resilience in soil health and disease suppression

C3.4-1

AN INTERNATIONAL COLLABORATION FOR THE DEVELOPMENT OF A BIOLOGICAL NEMATICIDE FOR USE IN SUB-SAHARAN AFRICA

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Text

Farmers of root and tuber crops, such as yam (Dioscorea spp.), are particularly affected by plant-parasitic nematodes; due to economic losses caused by decreased crop yields and crop quality. The lack of nematicide availability and selection across Sub-Saharan Africa (SSA) severely limits farmers' productivity and profitability. Backed with grant funding from the Bill and Melinda Gates Foundation, AgBiome is leading a multinational team of researchers to discover, develop, and commercialize a bacteria-based nematicide for use across SSA on root and tuber crops.This team of researchers, formed in 2018, includes scientists from national research organizations (Tanzania Agricultural Research Institute - TARI), academic institutions (University of Abomey-Calavi and University of Ibadan), and non-governmental organizations (International Institute for Tropical Agriculture -IITA). AgBiome and the research and development team will partner with local commercial organizations to deliver a safe, effective, and affordable tool for farmers; enabling them to better reach their full economic potential.

C3.4-2

THE CHANGES OF RHIZOSPHERE MICROBIOME AND TRANSCRIPTOME IN ARABIDOPSIS GROWN UNDER MESO-PLASTIC CONTAMINATED SOIL

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Text

Agricultural mulching films made of polyethylene, a petrochemical product, are widely used in agricultural fields to increase crop yields. However, removal and disposal of the Agricultural mulching has become a challenge, the plastic pollution in farming soil is increased, similar to that seen in the ocean. Accumulated agricultural plastics in soil affect the physicochemical properties of soil, soil microbiota, and plant growth. Moreover, recent study indicated that nano-plastics in the soil can be taken up by plants. However, knowledge about tripartite interactions among plastic, microorganism, and plant remains unclear. Here we showed their interaction at the transcriptome level in the model plant, *Arabidopsis thaliana*. Plants were incubated in the 0 and 5 % meso-plastics (mechanically pulverized forms of low-density

polyethylene films)-contaminated soils that had been pre-incubated for 120 days under dark conditions (25? and 80% relative humidity). The transcriptome analysis revealed that certain genes were down-regulated or up-regulated when plants were exposed to high concentrations of meso-plastics. On the other hand, plastic contamination also affected the community structure of the rhizosphere microbiome. These results showed that agricultural plastic remnants caused physiological changes in plants and the change of microbiota in soil and propose the potential impact of agricultural plastic pollution on phytobiome.

C3.4-3

A CULTUROMICS APPROACH IDENTIFIES RHIZOSPHERIC BACTERIAL STRAINS INVOLVED IN LEGUMES PROTECTION AGAINST THE ROOT ROT AGENT APHANOMYCES EUTEICHES

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Text
In recent years, using a consortium of selected bacteria from the root microbiota, also known as a "synthetic community" (SynCOM), to promote plant health has become increasingly common. The objective of this study is to elaborate a model SynCOM from the rhizosphere of Medicago truncatula (Mt) to unravel the role of root microbiota in mediating plant-microbe interactions in the context of biotic stress caused by Aphanomyces euteiches, a devastating oomycete that causes root rot in legume plants. A high throughput culturomics protocol was used to obtain 1364 isolates from the rhizosphere of Mt. The collection of isolated bacteria was genotyped using Illumina 16S metabarcoding sequencing. The UCLAST algorithm was employed with a 97% identity to select 812 pure isolates with 79 unique OTUs. The relative abundance analysis of the collection showed that the uppermost taxa were similar to those observed in molecular identification obtained from soil DNA. Among the selected 812 pure isolate 12 were found to inhibit A. euteiches growth in a dual culture assay. In planta testing, only one strain of Pseudomonas sp. showed significant difference from the untreated control. This collection of 79 unique OTUs was used to constitute a synthetic community as a model of the root microbiota of Medicago plants. A multi-omics approach will be used to analyze the behavior of this SynCOM in a gnotobiotic system to study its role in plant microbe interactions.

C3.4-4

SHIFT OF BACTERIAL COMMUNITIES IS INVOLVED IN THE STIMULATION OF PLASMODIOPHORA BRASSICAE RESTING SPORE GERMINATION

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Text

Clubroot, caused by *Plasmodiophora brassicae*, is a severe soil-borne disease in cruciferous crops worldwide. *P. brassicae* can survive in the soil as resting spores for many years. The germination of resting spores is a key process for successful infection leading to disease and thus identifying germination stimuli is imperative. Previous studies reported that root exudates of host and non-host plants were able to stimulate the germination of *P. brassicae* resting spores. In contrast, our studies showed that pure root exudates collected under sterile conditions cannot stimulate resting spore germination. Instead, various bioassay results demonstrated that *P. brassicae* resting spore germination is likely influenced by the microbial communities rather than being simply induced by root exudates. 16s rRNA gene amplicon sequencing results showed clear shifts in the abundance and composition of bacterial communities in the samples with higher germination rates compared to the initial community. The initial microbial community can be regulated to one that induces resting spore germination under suitable conditions (e.g. soil moisture, nutrients and carbon sources). Root exudates could be one of the carbon sources modulating the microbial community. This study presents novel insights significantly improving our understanding about the interaction of soil microbiota with *P. brassicae* and its host plant, which may enable to develop novel strategies of clubroot disease control.

C3.4-5

THE INTEGRATION LEVEL OF MICROBIOME STUDIES DETERMINES POTENTIAL DETECTION OF GLYPHOSATE-ASSOCIATED DYSBIOSES ON/IN PLANTS AND ANIMALS AND SUBSEQUENT DISEASE DEVELOPMENT.

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Text

Glyphosate is the most used pesticide worldwide. It interferes with the shikimate pathway in plants and, depending on the genetic make-up, in many microorganisms, impeding the production of aromatic amino acids. Research results of glyphosate effects on microbial communities have been contradictory. We show that contrasting results are mostly due to differences in the integration levels studied. Thus, short-term treatment effects on microbial biomass or composition at higher taxonomic levels usually are minimal. In contrast, when using more detailed DNA sequencing techniques it was shown that especially chronic glyphosate treatments resulted in changes in the prevalence of specific genera or species as well as biological processes. Plant growth promoting rhizobacteria and beneficial intestinal bacteria often are negatively affected, while pathogenic bacteria and fungi are enhanced. Thus, by selectively weakening bacteria, fungi and protozoa, repeated exposure to glyphosate may change microbiome compositions in rhizo- and endospheres of plants, as well as animal guts. Such shifts in microbial composition have been implicated in enhanced susceptibility of plants to *Fusarium* and *Rhizoctonia*, of birds and mammals to toxic *Clostridium* and *Salmonella* species, and of bees to *Serratia* and Deformed Wing Virus. For the determination of health effects via dysbiosis there is a need to define the correct level of integration needed in microbial community analyses.

C3.4-6

EXPLORING SUSTAINABLE SOILBORNE DISEASE MANAGEMENT SOLUTIONS FOR SPECIALTY CUT FLOWER PRODUCTION

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Text

Specialty cut flowers are produced in diverse systems, ranging from crates, raised and in-ground beds in high tunnels, or open fields. Agricultural soil and soilless substrates are often repurposed for production, introducing the risk of many soilborne diseases. Commercial growers have historically relied on soil fumigants for disease control, but the global phase-out of methyl bromide has left them with limited chemical options. At the same time, an increasing number of growers choose to adopt more sustainable practices due to concerns for human and environmental health. In this study, we explored the use of a non-pesticide-based soil disinfestation technique known as anaerobic soil disinfestation (ASD) for its potential to reduce incidence and severity of Rhizoctonia stem rot in zinnia. Wheat bran, soybean meal, and tomato pomace were incorporated as carbon sources in soilless and soil-based substrates, which were then irrigated to saturation, covered, and incubated for 4 weeks. Zinnia plugs were then transplanted in the substrates and monitored for disease development. Incidence and severity of stem rot were significantly reduced in all carbon source treatments when soil-based substrate was used, and in the tomato pomace and wheat bran treatments when soilless substrate was used. These results indicate that ASD is a suitable option for control of *Rhizoctonia solani* in specialty cut flowers and should be further investigated under a variety of production conditions.

F3.4-1

PHYTOPATHOGENIC FUNGI MODIFY THE BACTERIAL DIVERSITY OF THE WHEAT RHIZOSPHERE GROWN IN CONVENTIONAL AND ORGANIC AGRICULTURAL SOILS UNDER AMBIENT AND FUTURE CLIMATE SCENARIOS

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Text

Due to their specific indigenous microbial communities, soils vary in suppressiveness towards pathogens. To explore the effects of climate change and agricultural management on the ability of soil microbiota to suppress phytopathogenic fungi, we collected soils from conventional and organic farming (CF and OF, respectively) plots exposed to ambient (A) or future (F) climatic conditions at the "Global Change Experimental Facility" (Bad Lauchstädt, Germany). The preconditioned soils were cultivated with wheat at the greenhouse and tested

for their suppressiveness towards Fusarium graminearum (Fg-1) and Gaeumannomyces tritici (Ggt). The soils inoculated with Fg-1 or Ggt presented a significantly lower microbial diversity ($p<0.05$) in the rhizosphere than the respective control soils. The preconditioning under ambient or future climatic conditions caused community differences in the conventional farming soil (CF-A vs. CF-F; $p<0.001$) for both inoculated treatments, while no such differences were observed for the organic farming soil. Overall, taxa affiliated with Actinobacteria and Proteobacteria were most abundant in all treatments and the most differentially abundant between CF-A and CF-F after inoculation with Fg-1. These taxa may contribute to disease suppressiveness and consequently support crop health. The study further revealed that soils preconditioned under different climatic conditions can help understand the impact of climate change on the functioning of soil microbiota.

P3.4-001

THE ROLE OF SOIL BACTERIA AND ROOT EXUDATES IN REGULATING DORMANCY AND GERMINATION OF MICROSCLEROTIA OF VERTICILLIUM LONGISPORUM

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Text

Verticillium longisporum is a soil-borne, vascular pathogen on many brassicaceous plants. It produces dormant structures, microsclerotia, on the stems of host plants at crop maturity, which are very persistent in the soil. Previous reports speculated on two potential causes of soil suppressiveness to soil-borne pathogens, competition between microorganisms for nutrients or antagonistic interactions among them. Our study showed that compared to water control, none of the tested nutrient solutions showed any significant enhancement of microsclerotia germination. Hence, to reveal possible antifungal compounds, bacteria were isolated from soil and tested for their effects on microsclerotia. The results confirmed that bacterial volatiles have significant suppressive effects on microsclerotia, and volatile fatty acids are likely to be important factors determining dormancy of microsclerotia. Earlier research indicated an effect of plant root exudates on soil-borne pathogens, but this has been tested without considering the impact of soil microorganisms. In this study, microsclerotia were first suppressed by treatment with soil bacteria and exposed to plant root exudates. The results indicate that root exudates of both host and non-host plants can off-set the suppressive effect of soil bacteria on microsclerotia. Furthermore, our study indicated that the rescuing effect of root exudates is not owing to the reduction in bacterial viability but to changes in system metabolites.

P3.4-002

ORGANIC AMENDMENTS TRANSFORMED THE APPLE (MALUS DOMESTICA BORKH.) RHIZOSPHERE FUNGAL, BUT NOT BACTERIAL, COMMUNITY IN AN ORCHARD REPLANT SOIL.

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Text
Apple replant disease (ARD) is caused by a complex of soilborne pathogens and suppresses yields in apple orchards. Long-term applied organic soil amendments have potential for sustainable disease management. Our study investigated changes in the composition of the apple rhizosphere microbiome under glasshouse conditions in response to the long-term application of organic soil amendments (mulch, mulch+compost, or untreated) in an ARD apple orchard. All treatments possessed a highly similar bacterial assemblage dominated in relative abundance by the phylum Proteobacteria (32- 33%) and genus *Conexibacter* (7%). The ascomycota was dominated by fungal phyla within the apple rhizosphere with the control having a lower relative abundance (67%) than the mulch (84%) and mulch+compost (79%). Differential abundance was also observed for the phylum chytridiomycota which had the highest relative abundance in the control (14%), followed by the mulch+compost (4%) and mulch (3%). As expected, the cellulolytic genera *Coniochaeta* and *Zopfiella* occurred at higher abundance in the mulch (8% and 5%) and mulch+compost (5% and 3%) than in the control (1% and 0.3%). The composition of the fungal communities of the soil treatments were significantly different (R^2 = 0.16, $P_{PERMANOVA}$ = 0.001) based on a beta diversity analysis. The study has increased our knowledge on transformation of the apple rhizosphere microbiome induced by long-term application of organic amendments.

P3.4-003

BIOLOGICAL SUPPRESSION OF SOILBORNE FUNGAL PATHOGEN VERTICILLIUM DAHLIAE IN COTTON SOILS

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Text
Verticillium dahliae Kleb. is causes wilt diseases in >200 plant species worldwide and verticillium wilt has a significant impact on cotton production in Australia. The control of soilborne plant diseases involves management of the pathogen at different microsites in soil at different time periods (pre-season or in-crop) and the control of infection and incidence. We demonstrate efficacy of two short-term soil-based lab-assays using a GFP-transformed non-defoliating (ND) strain of V. dahliae or qPCR technique (ND and defoliating strains, D) to quantify pathogen suppression capacity (PSP) of Australian cotton soils. Surface 10cm soil samples collected from established field experiments at ACRI, Narrabri and NorthStar in NSW were used. GFP assays indicated the biological nature of PSP capacity in soils from ACRI fields with varying disease incidence history. PSP estimates from qPCR assays showed significant differences in the growth pattern of both the D and ND strains in soils under different rotation treatments. Pathogen growth was higher in soils from continuous cotton and fallow rotations & lowest in Corn-Cotton rotation. These assays also detected changes in PSP upon addition of C and N (crop residues, chitin, N fertilizer), and bacterial amendments to soils, and distinguish between suppressive and conducive soils. A rapid soil

diagnostic tool would not only help understand the soil microbiome role in PSP, but how it can be enhanced to increase cotton production.

P3.4-004

WOODCHIP AMENDMENT CAN ALTER BANANA SOIL MICROBIAL ABUNDANCE AND DISEASE SUPPRESSIVE POTENTIAL

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Text

Long-term banana cultivation alters soil microbial communities compared with areas of native vegetation. Specifically, the fungal microbial communities in banana soils are less abundant and less diverse, allowing potentially pathogenic groups of fungi, such as *Fusarium oxysporum* complexes, to dominate. Woodchip amendments could potentially increase soil fungi, leading to greater disease suppression in banana soil. We tested three rates equivalent to 2, 20 and 100 t/ha of woodchips from leguminous trees, *Erythrina* sp. and *Leucaena* sp., incorporated into the soil used for commercial banana production. One month after adding woodchips, Ducasse (Musa ABB) was planted into the soil. One month later, the plants were inoculated with a spore suspension of *Fusarium oxysporum* f. sp. *cubense* (Foc) Race 1 (VCG 0124). Results indicated that the incorporation of woodchips did not significantly impact the growth of banana plants. Woodchip amendment significantly increased the abundance of bacteria, archaebacteria and fungi in the soil compared to non-amended soil. Fungal biomass was significantly increased following the application of *Leucaena* sp., whereas bacterial biomass was enhanced following the incorporation of *Erythrina* sp. An increase in the rate of woodchip application increased the proportion of omnivorous nematodes and decreased the proportion of bacterivores. Woodchip amendment can increase the soil microbial biomass, which reduces FocR1 colonisation of the banana corm.

P3.4-005

EFFECTS OF SOIL PHYSICOCHEMICAL PROPERTIES ON FUSARIUM OXYSPORUM F. SP. ELAEIDIS DISEASE INCIDENCE AND SEVERITY IN OIL PALM PLANTATIONS OF CAMEROON DEVELOPMENT CORPORATION (CDC).

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Text

Oil palm is one of the tree crops that utilize a high amount of soil nutrients for optimum growth and development and is one of the leading cash crops in the economy of Cameroon. This study was carried out to evaluate the effect of soil physiochemical properties of Foe disease incidence and severity on oil palm estates of CDC (Bota, Debundscha, Idenau and Mondoni). Twelve bulked soil samples were collected from each estate and analysed using standard procedures. Tolerant oil palm hybrid seedlings were planted in soils collected from CDC estates and some in sterilized soil (control experiment). All soil physicochemical parameters negatively influenced the disease incidence and severity of Foe on the seedlings in prenursery. Soil samples from Idenau had highest pH value 6.3±0.03 while Debundscha had lowest pH of 5.5±0.1. Mondoni recorded high amount of total nitrogen; carbon/nitrogen ratio (40.8±12.8), organic matter (OM) (5.3±0.08), organic carbon (3.0%) and cation exchange capacity (CEC) (18.0±0.7). Soils from Idenau were the opposite. After planting disease free seedlings, soils from Mondoni had the lowest disease severity index (4.55%) meanwhile that of Idenau was the highest (12.02%). Therefore, appropriate nutrient management such as the application of organic and inorganic fertilizers should be used for soils that are deficient and low like that of Idenau.

P3.4-006

CROPPING SEQUENCES THAT REDUCE PATHOGEN INOCULUM AND MAINTAIN OVERALL SOIL BIOLOGICAL HEALTH REDUCE DISEASE INCIDENCE OF VERTICILLIUM WILT OF COTTON

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Text

Verticillium wilt (VW) is a major economic constraint to cotton production in Australia. The causal agent, *Verticillium dahliae* Kleb (Vd), is a soilborne fungus that has a wide host range and survives long-term in soil by producing microsclerotia. Management of VW requires an integrated approach that reduces soil inoculum and maintains soil biological health.

A field trial was conducted to examine the impact of crop rotation on suppression of VW and overall soil biological health. Four treatments (sorghum, corn, fallow, and cotton) were examined. Disease incidence was assessed late season. Surface soil samples collected pre-plant and late season were analysed for Vd population and microbial abundance and catabolic diversity.

Disease incidence and soil inoculum levels were reduced following 2-year rotations with non-hosts or fallow compared to continuous cotton. The fallow-fallow (FF) rotation had the lowest abundance of fungal populations and overall catabolic diversity of soil microbial communities, whereas crop sequence that included sorghum had the highest values. Although FF rotation reduced Vd level and disease incidence, the decline in overall microbial populations and activities in the long-term could potentially make soils more conducive to soilborne diseases. Results suggest that management of VW in cotton through crop sequences that include other crops, may be a better option as they not only reduce disease incidence but also maintain overall soil biological health.

P3.4-007

THE RELATIONSHIP BETWEEN SHIFTS IN THE RHIZOSPHERE MICROBIAL COMMUNITY AND ROOT ROT DISEASE IN A CONTINUOUS CROPPING AMERICAN GINSENG SYSTEM

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Text

The root rot disease causes a great economic loss, and the severity usually increases as ginseng ages. However, it is still unclear whether the disease is related to changes in microorganisms during the entire growing stage of American ginseng. The present study examined microbial community in the rhizosphere and chemical properties of the soil in 1–4-year-old ginseng plants grown in different seasons . Additionally, the study investigated ginseng root rot disease index (DI). Results showed that the DI of ginseng increased 2.2 times in one sampling site and 4.7 times in another during the 4 years. As for the microbial community, bacterial diversity increased with seasons in the first, third, and fourth years but remained steady in the second year. The seasonal changing of relative abundances of bacteria and fungi showed the same trend in the first, third, and fourth years but not in the second year. The Mantel test showed that soil chemical properties, including available nitrogen, phosphorus, potassium, calcium, magnesium, organic matter, and pH, were significantly correlated to microbial composition. The contents of available potassium and nitrogen were positively correlated with DI, while pH and organic matter were negatively correlated with DI. In summary, we can deduce the second year is the key period for the shift of the ginseng rhizosphere microbial community. Disease aggravation after the third year is related to the deterioration of the rhizosphere microecosystem.

P3.4-008

IMPACTS OF ELEVATED CO2 ON INTERACTIONS WITH ROOT MICROBES FOR ENHANCED OAK GROWTH AND DEFENCE AGAINST PATHOGENS

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Text

Symbiosis between plants and beneficial soil microorganisms like mycorrhizae are known to promote plant growth and defence against (a)biotic stresses in plants. Climate change (CC) is increasing the number of plant stress factors, such as increasing atmospheric CO2 (eCO2). Therefore, understanding the role of mycorrhizal in plant responses facing environmental changes is critical. However, mycorrhizae role is largely underappreciated, and field experiment research is scarce. This project will test the mycorrhizae effects on oak,

the eCO2 impact on mycorrhizal colonization and their role in mediating growth-defence trade-offs. For this, microscopy and untargeted metabolomic analyses were performed in oak seedlings grown under ambient CO2 (aCO2) and eCO2 and then infected with powdery mildew at the Free Air CO2 Enrichment (FACE) facilities of the Birmingham Institute of Forest Research (BIFoR). Powdery mildew infection was monitored and mycorrhizae staining was performed by ink-acid method in 20 seedlings/CO2 level. Root-extracted metabolites were subjected to LC-MS/MS analysis. Spectra were filtered by using XCMS R scripts. Statistical analysis, using METABOANALYST, showed statistical differences among treatments. Identification and functional pathway analysis was performed using MARVIS to select putative biomarkers. Data showed a protective role of mycorrizhae that can be limited by future CO2 atmospheric levels, endangering the forest health under climate change conditions.

P3.4-009

EFFECT OF RESIDUE MANAGEMENT ON CERCOSPORA LEAF SPOT OF TABLE BEET AND SOIL MICROBIOME.

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Text

Cercospora leaf spot (CLS), caused by *Cercospora beticola*, can detrimentally affect foliar health in table beet leading to significant economic loss. CLS management currently relies on fungicides, but the high genetic diversity of *C. beticola* can rapidly develop fungicide resistance. Management of infested crop residue can help to delay the onset of the disease by reducing the primary inoculum in soil. A replicated field experiment was conducted to assess the impact of different residue management techniques on CLS in table beet. The experiment was a completely randomized block design with four replications of each treatment and nontreated controls. Infested beet leaves were used as the source of inoculum. Treatments were applied in fall 2021 and table beet was planted in summer 2022. Heat treatment of residue significantly reduced the CLS severity and AUDPS by 45.3% and 51.8%, respectively, compared to the no residue management control. Treatments with CaCO3 and a combination of chopping and plowing had significantly higher CLS severity and AUDPS than plots with no residue control by 92.8% and 149.3%, respectively. Other treatments were not statistically different from either control. *C. beticola* density and the structure of soil microbiome will be determined by qPCR and next generation sequencing of soil DNA, respectively. Identification of alternative CLS management strategies can help reduce the crop loss and frequency of fungicide applications for enhanced profitability.

P3.4-010

IS THE PLANTING OF PESTICIDE-TREATED SEED PROFITABLE? RESULTS FROM A LARGE-SCALE FARMER-LED ARABLE CROPPING SYSTEM EXPERIMENTS IN NORTH-EAST FRANCE

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Text

Farmers and policy makers rely on crop management data to evaluate socio-economic and environmental sustainability of cropping practices. The use of pesticide-treated seed is a widely used practice worldwide to control seed- and soil-borne pathogens affecting crop establishment and yield. In Europe, farmers raise concern about risks of yield losses due to a lower or no availability of chemicals previously used for seed treatment due to tighter restrictions imposed by the EU pesticide regulation 1107/2009, including the ban of several widely used fungicides (e.g. thirams, Metalaxyl-M). Within this context, research needs to provide a clear answer as to whether the economic viability of arable farms will be jeopardized when there is no access to pesticide-treated seeds. To respond to this concern, a 6-year specific project has been set up within the framework of French national action plan Ecophyto aiming at assessing economic and environmental sustainability of cropping systems without pesticide seed treatment compared to commonly used pesticide-treated seed. Large-scale farmer-led experiments using sequences of key arable crops (wheat, barley, maize, oilseed rape etc.) were put in place across contrasted environmental conditions of NorthEast France. Preliminary results show no significant yield differences between pesticide-treated and non-treated seeds raising questions on the prophylactic use of pesticide-treated seeds to yield gains.

P3.4-011

THE ROOT MICROBIOME OF DRYLAND AND IRRIGATED SPRING WHEAT: AN EIGHT-YEAR STUDY

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Text

Root-associated microbiomes are critical to the growth and health of wheat. Long-term trends in wheat-root microbial community dynamics are poorly understood. We characterized the growing season and long-term population dynamics of bacterial communities in the rhizosphere and endosphere of wheat grown for eight years in dryland and irrigated plots in Washington State, USA with less than 305 mm annual precipitation. The diversity and richness of bacterial communities declined from the bulk soil to the rhizosphere, and to the endosphere. Bacterial richness and diversity were significantly greater in irrigated wheat. Some genera showed a consistent periodicity in their population trends during the growing season from March to August. Over eight growing seasons, Proteobacteria and Bacteroidetes (*Pseudomonas*, *Variovorax*, *Chryseobacterium*) maintained stable rhizosphere

populations in both dryland and irrigated wheat. Populations of some Bacteroidetes and Proteobacteria (*Mucilaginibacter*, *Sphingomonas*, *Massilia*, *Burkholderia*) were dryland adapted and persisted or increased in the dryland rhizosphere whereas others (*Rhizobium*, *Acidovorax*, *Terremonas*, *Hyphomicrobium*, *Bdellovibrio*) increased on irrigated wheat. In contrast, some taxa, including many Actinobacteria, declined in abundance over eight years in both the endosphere and rhizosphere regardless of irrigation. Our results provide insight into how water and monoculture impact the population dynamics of the wheat-root microbiome.

P3.4-012

EXAMINING THE IMPACT OF SOIL ON TREE HEALTH AND DISEASE PROGRESSION

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Text
Forests are globally important ecosystems that provide goods and services to the planet. They have a crucial role in supporting biodiversity, storing carbon, and regulating the climate. The forests around the world, including temperate European forests, are under threat by the global increase in temperature and unpredictable climate conditions caused by climate change. These climatic changes are also causing the spread of new pests and diseases northwards as the planet warms. In recent years, some devastating diseases have affected trees and forests in continental Europe and the UK. To understand the role of soil, both its biotic and abiotic components, in tree health and disease development, we are analysing the effects of soil treatment on diseased and healthy trees. Using the study system of Oaks affected by Acute Oak Decline and Horse Chestnuts affected by Bleeding Canker, we investigated if the treatments successfully reduce disease or help recover existing diseased trees by analysing phenotypic measurements of the tree, the soil and tree microbiome and the tree metabolome. The initial assessments have revealed differences between diseased and healthy trees in the baseline measurements. So far, no effect of the treatment has been detected, and no difference between the treatments has been observed, but measurements over two further years will continue.

P3.4-013

EFFECTS OF TERMINATION TIME OF LEGUMINOUS COVER CROPS ON ROOT DISEASES OF SUCCEEDING PEAS

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Text
Many long-lived soil borne legume pathogens also persist in leguminous cover crops, making the intensification of legume production for biological N-fixation as well as grain production a

challenge. An open question is if the pathogen load of the cover crops can be influenced by the method and timing of the cover crop termination. In a two-year model crop rotation consisting of overwintering legume cover crops (CC) followed by maize in the first year and grain peas in the second year, the phytopathological risks of legume CC in minimum tillage systems were examined. CC were terminated either very early in spring in March, in late spring (May) or as control not at all (no maize) in the rotation. The pathogen load in the soil after maize was tested with peas as a model crop under field and controlled conditions. In the CC roots, by May, the load of *Didymella pinodella* was usually significantly higher than in March. Vetch favored *Fusarium culmorum* in the following maize, clover *F. avenaceum* independent of termination time. In the field, the following pea crops were mainly symptom free but with moderate incidences of *F. avenaceum* (40%) and *F. redolens* (30%). In the greenhouse, emergence of peas compared to sterile sand was reduced in all field soils but disease symptoms on the emerged peas were lower than in sand. Symptoms were slightly higher in soils where the CCs had been terminated late compared to early terminated CCs. but much less than in sterile sand.

P3.4-014

UNDERLYING MONOCULTURE SOIL CONDITIONS CAUSES OF THE DURIAN PHYTOPHTHORA STEM CANKER ESCALATION

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Text

The increased local demand and international export to China have caused rapid expansion of monoculture durian plantations. Consequently, durian stem canker escalation in Malaysia may occur without proper disease management strategies comprising both biotic and abiotic risk factors. This study aims to obtain the current disease status in Malaysia and to know soil conditions at various infected orchards. Each sampling plot (2500m^2) had been evaluated for stem canker disease incidence (DI) and disease severity index (DSI). Composite soil samples were collected for pH, texture (particle size) and nutrient availability. All eleven durian orchards were infected and most of the old orchards (≥10 years) DI was 77%. The orchard less than 10 years had the greatest DI (71%) and DSI almost 50% whereas 4 years orchard; 40% stem canker incidence and 16% DSI. The soil pH of both orchards 6.47 (<10 years) and 4.85 (4 years) and at the old orchards between 3.46 to 6.70. Most of the orchards were 80% of clay and silt that retain water. Also, a 2 years old durian orchard showed 2% DSI and 7% DI may risk of stem canker with 87% clay and silt. New durian orchards were relatively richer in nutrients than old orchards especially Ca, Mn and Zn. These soil conditions favour by Phytophthora pathogen during wet seasons could lead to escalation of stem canker particularly new durian area. Thus, this study will aid the agronomic practices i.e. irrigation and fertiliser to reduce stem canker infection.

P3.4-015

ASSESSMENT OF DISEASE THREATS AND BIOFUNGICIDE EFFICACY IN WOOD SUBSTRATES

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Text

In greenhouse production, plants are grown in soilless growth substrates. For decades, peat moss has been the primary substrate for container-grown ornamental and some vegetable crops. Substrate manufacturers have identified wood byproducts to be some of the most promising alternative sources of raw materials for use in substrate formulations. Unfortunately, a change in substrate can be very disruptive to a grower's production system affecting everything from water to pest control. Our objective was to evaluate how wood components (1) affect severity of soilborne disease and (2) affect biopesticide efficacy. Wood components are manufactured in multiple ways with the three most common being hammer milled, twin-disc refined, and single or twin-screw extruded. In this study we evaluated the three differently processed WFs for natural suppression and biopesticide efficacy against damping-off on radish and crown and root rot on chrysanthemum caused by *Rhizoctonia solani*. Our findings provide evidence that the inclusion of wood components, regardless of blend ratio or type, does not impact severity of damping-off disease on radish and may lessen the effect of crown and root rot on chrysanthemum. We also found that blending of wood components with peat did not affect efficacy of *Trichoderma harzianum* T-22. Additional research is needed to determine if the trends we observed hold true for other plant species or other pathogens.

P3.4-016

RESILIENT CIRCULAR GROWING MEDIA FOR THE FUTURE

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Text

Growing media used in horticulture currently largely consist of peat. Due to environmental concerns associated with peat use, there is a rising demand for alternative growing media. Alternatives often contain high proportions of organic materials such as coconut coir, wood fibers and compost, which are associated with increased microbial activity. However, it is unclear what the effect of such increased activity is on the growth and health of the plant. We are developing a reference method for assessing the quality of alternative growing media. To this end, we cultured birch, lavender, Pelargonium and chrysanthemum on one conventional and five alternative mixes of growing media components. We measured changes in physico-chemical properties, such as EC and elemental composition, of the growing media over time. We also determined changes in the bacterial and fungal microbiome using a combination of amplicon sequencing and metagenomics. Finally, we determined plant weight and asked which of the above properties (physical, chemical and/or microbiological) drove this measure. The developed method will be further optimized and used to determine the quality of

alternative growing media. Research was supported by Glastuinbouw NL (via foundation KIJK), RHP, LTO Vakgroep Bomen, Vaste Planten en Zomerbloeiers, Tree Centre Opheusden, Kekkilä BVB, Lensli, Legro, Jiffy and BVOR.

P3.4-018

EFFICIENCY OF BIOCOMPOST ON THE GROWTH OF TOMATO PLANTS AND INCIDENCE OF FUSARIUM WILT IN NURSERY

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Text

Diseases affect productivity and quality of tomato, of which Fusarium wilt caused by Fusarium solani is considered one of important diseases of tomato in greenhouseand field. Syntheticpesticides used for disease management have known negative impacts and risks. In this regard, efforts are directed to ecofriendly, efficient sustainable tools. This study was conducted in a completely randomized block design in the nursery of Agriculture College, University of Bahri, Alkadaro. The objectives of this study are; To evaluate the effect of compost enriched with combinations of compatible isolates of Trichoderma harzianum Th and T. viride, TV with Bacillus thuringiensis Bt Wh-5 JX674041; B t St-6 JX841104, Paenibacillus papillae Pp Om-4 JX841101; Pp Ab-4 KC107788l and Lactobacillus sp. (R4) on seeds germination and other growth parameters. and on the incidence of Fusarium wilt disease of tomato. Tomato Seeds were sown directly into the treated soils and the control ones in plastic buckets in the nursery arranged in a randomized block design with 4 replications. Significant differences (P≤0.05) were existed between treatments in seeds germination, plant height, leaves, flowers and fruits numbers and disease incidence. It is concluded that, combination of microbial antagonist with compost is better solution for enhancing tomato growth and suppressing Fusarium wilt disease than using the compost alone or using chemical fertilizers. QFurthers studies are mandatory.

P3.4-019

IMPACT OF STEAMING ON SOIL HEALTH INDICATORS AND BIOCONTROL AGENTS

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Text
Steaming of the soil is proposed as an alternative to synthetic chemical fumigants, but little is known concerning its effect on soil health and how to combine it with biocontrol agents (BCAs). We tested the effect of steaming on soilborne pathogens, soil health indicators and the colonization of the soil with BCAs. The pathogen systems under study were *Rhizoctonia solani* on lettuce, *Sclerotinia sclerotiorum* on chicory and *Pythium sylvaticum* on lamb's lettuce. Greenhouse experiments were set-up and soil samples were taken at several time points to measure following soil health indicators: (i) phospholipid-(derived) fatty acids to monitor the microbial biomass, (ii) hot-water extractable carbon to monitor the easily available carbon as a proxy for microbial biomass-C and (iii) DNA metabarcoding to monitor relative changes in microbial community. The BCAs were commercially available products based on *Trichoderma* spp. Steaming was highly efficient in killing the survival structures of the three pathogens. A similar or higher yield and plant health were observed for the following crop cultivations in the steam-treated plots as compared to the control. The effect of steaming on the soil health indicators and the survival of BCAs was depending on the experiment and the duration of the steaming. Moreover, steaming seems to improve the establishment of BCAs in the soil, probably due to the release of easily available carbon immediately after steaming.

P3.4-020

WILLOW CHIPS SUPPLEMENTATION IN SOIL ENHANCES RESISTANCE AGAINST POTATO LATE BLIGHT

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Text
Willows (Salix) have bioactive components, salicylates being the most characteristic compouds. Willow extracts are used as biostimulants and willow bark infusion is also listed as a basic substance in EU pesticide legislation against fungal diseases. Also, use of wood as soil amendments has been shown to promote the growth of saprophytic fungi and retain excess nitrogen. The aim of the study was to test the ability of willow chip supplementation in soil to increase the resistance against potato late blight pathogen *Phytophtora infestans*. The trial was conducted in greenhouse conditions. Fresh willow chips or dried willow bark was added to peat 6 % v/v. Infection rate was tested by inoculating leaf discs (cv. King Edward and Melody) with sporangia of *P. infestans.* Willow chip supplementation in soil caused no adverse effects on the crops in visual observation. With King Edward, after five weeks growing period, the average decrease in the infection rate was 7 % with fresh willow chips and 9 % with dry willow chips. With Melody, there were no differences between the treatments. When the growing period was seven weeks, the effect of the treatment was only minimal for both cultivars. It seems that, in the trial conditions, soil supplementation with willow chips increased resistance against potato late blight for a susceptible variety, but for a moderately susceptible variety Melody, there were no considerable effect.

P3.4-021

USING RNA-SEQ TO ASSESS THE META-TRANSCRIPTOME OF MICROBIOME OF FIELD CROPS

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Text

A better understanding of microbes living in the phyllosphere and rhizosphere of crops is important as they regulate aspects of plant performance, and can significantly improve crop yield and quality. The structure and diversity of the microbiota is influenced by the host as well as the growth environment.
Since there are many influencing factors determining the microbiome, a large amount of data needs to be analysed to determine the set of common microorganisms of certain crops have. A large body of data that remains relatively unexplored, and which could help us better understand plant-microbe interactions, are RNA-seq data obtained from different crops and environments.
In this study, the metatranscriptome was explored by analysing RNA-seq obtained from crops grown in field conditions, with potato and cassava as examples. After the removal of transcripts related to the crop genomes, meta-transcriptomics was performed using either unassembled short reads or assembled contigs.
We identified both previously characterised and novel microbes interacting with potato and cassava. For potato, there was a clear difference in the fungal microbiome based on growth site, irrespectively of genotype. In cassava roots, we found microbes varying depending on the carotenoid content of the root. In conclusion, we show that RNA-seq data provides an untapped resource to identify and better understand the microbiome of crops and that the microbiome is both site and genotype dependent.

P3.4-022

MONITORING THE PRESENCE OF ENTOMOPATHOGENIC NEMATODES IN MAIZE FIELDS: TOWARDS BIOLOGICAL CONTROL BY CONSERVATION BIOCONTROL

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Text

Biological control by conservation is based on the use of native organisms to manage the population of pests in fields, and contrast with biological control by augmentation (inoculation of exogenous organisms). However, the conservative approach in biological control requires a good description of the agents present. Entomopathogenic nematodes (EPNs) are ubiquitous since they are distributed in all continents except in Antarctica. In agricultural soils, they offer a high potential for crop protection against insect pest. The classical method used to detect EPNs in soils is the Galleria mellonella-baiting method. But this method allows to detect only 20-40% of EPNs present in soils.

The objective of this study is to compare several methods of EPN detection in order to describe the distribution of EPNs in agricultural soils. In this goal, we assumed that multi-method approach would allow to obtain the most accurate picture of the presence and diversity of EPNs in agricultural soils. We used two techniques of detection: multiple G. mellonella-baiting cycles to increase the efficiency of capture of EPNs in soils and an extraction of soil nematodes followed by morphological identification. Using these methods, we obtained 35% of positive plots instead of 14% with only the classical baiting method. We showed that the depletion trapping method and the extraction of soil nematodes increase both the number of EPN isolates and the EPN species diversity.

P3.4-023

RESPONSE OF THE SOIL MICROBIAL COMMUNITY, DRY ROOT ROT AND COMMON BEAN YIELD TO COVER CROPS IN A NO-TILLAGE FIELD.

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Text

No-tillage and cover crops have been recommended to improve soil health in Brazilian intensive farming systems, but their effects on the soil microbiome need to be better understood. This work investigated in 2021 the metagenome of soil fungi and bacteria in a field experiment and their relation with common bean root rot severity and yield. Fall cover crops were grown from 2017 to 2021 after the summer cropping of soybean or maize and were succeeded by common beans in the winter, under center pivot irrigation. The cover crops consisted of millet, oat, Congo grass, oat, crotalaria and a mix of millet + crotalaria + fodder radish + buckwheat, while fallow plots served as a control. All plantings had the support of GPS RTK equipment, ensuring plot overlap and cumulative results. Rhizospheric 0-10 cm topsoil was sampled in triplicates during common bean flowering. The extracted DNA was subjected to metagenomic analysis according to the 16S and ITS gene sequencing, respectively, to identify which bacteria and fungi responded to the treatments. The cover crops affected 67 taxonomic groups ($p<0.05$). Oat, Congo grass and the cover crop mix recruited arbuscular mycorrhizal fungi (*Glomus* sp.), most Actinomycetes and plant growth-promoting bacteria. These favored common beans with higher yields, despite higher root lesions. Regardless of a lower root disease severity, fallow resulted in the lowest yields throughout the experiment and a poor environment for beneficial microorganisms.

P3.4-024

FORTIFICATION OF COMPOST WITH BIOAGENTS FOR GROWTH IMPROVEMENT AND SUPPRESSION OF WILT DISEASE ON CHICKPEA PLANTS

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Text

Fusarium wilt caused by *Fusarium solani* is the major constraints to Chickpea (*Cicer areitinum*) productivity in the Sudan. Fortifying compost with bioagents can manage pests and diseases, improve soil fertility and sustain productivity. Bioagents-fortified compost was assessed for its ability to control *F. solani* and increase chickpea. A greenhouse experiment was conducted using a randomized complete block design with four replications. Shandi cultivar was used for its susceptibility to Fusarium wilt and root rot disease. Treatments consist of not infected, infected controls, standalone compost and Trichoderma harzianum (Th) or T. viride (Tv) each solo mixed with compatible strains of *Bacillus thuringiensis* (OM4, Wh5, St6, and Ab4 strains) or Lactobacilli strain rb4. Well-matured compost was fortified with each microbial consortium before application. Control plants were given the recommended doses of fertilizers (urea and superphosphate). In the presence of *F. solani* as soil infection, treatments significantly (*P<0.05*) reduced the disease index (DI). The fortified compost with Tv mixed with *B. thuringiensis* strain Wh5 and Th mixed with *B. thuringiensis* strain St6, OM4 and Wh5 each alone, were the best in improving plant growth. Fortified compost with Th mixed with B. thuringiensis strain St6 suppressed disease development by 96% compared to control uninfected and standalone compost. Integrated disease management strategies may benefit from bioagents-fortified compost.

P3.4-025

BIOCHAR AMENDMENT WITH ALTERNATE WETTING-DRYING IN RICE FIELD: AN UNIQUE COMBINATION OF CROP DISEASE MANAGEMENT AND QUALITY ENHANCEMENT

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Text

Over a decade, sustainable and resilient soil amendments are proposed and biochar stands high in global acceptance (**1**). Several research has been carried out to show its potential in plant disease management and tolerance (**2, 3**), although its combination with another sustainable practice, alternate wetting and drying, has not been researched in terms of plant disease management and tolerance. Here we tried this unique combination to show how

efficient it can be in rice cultivation. The dry-wet cycle and biochar alone can reduce waterlog conditions and thus minimize water-borne pathogens, root-knot nematodes and other harmful microbes in the soil (**4, 5**). To suppress these disease-causing microbes furthermore, biochar amendment in the field at 1% (w/w) is highly efficient as proved by the rice plant stress-responsive enzyme assessments. Further metagenomics and high throughput sequencing showed the prominent difference in the microbial diversity where the disease-causing microbial community and root-knot nematode have been substantially minimized in the biochar-amended dry-wet fields with greater nutrients to promote plant health and tolerance. **1.** Majumdar, A, et al, 2023. Chemosphere, 312, 37117. **2.** de Medeiros, E.V, et al, 2021. Phytoparasitica, 49, 713-726. **3.** Mondal, S, et al, 2021. Journal of Plant Diseases and Protection, 128, 819-829. **4.** Majumdar, A, et al, 2021. Journal of Hazardous Materials, 409, 124443. **5.** Poveda, J, et al, 2021. Phytopathology, 111, 1490-1499.

P3.4-026

IMPACT OF DIFFERENT TILLAGE SYSTEMS ON NET CARBON EXCHANGE RATES (NCER) AND WINTER WHEAT INFECTION A LONG-TERM STUDY

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Text

The research was conducted during the years 2021–2023, on the base of a long-term study established in 1998, at the Experimental Station Brody (52°26′ N; 16°18′ E), belon ging to the Pozna? University of Life Sciences. The purpose was to evaluate the impact of different tillage systems on net carbon exchange rates (NCER) and severity of plant infection by pathogenic fungi in whinter wheat. A randomized complete block design was set up with four replicates per treatment (conventional, strip till and no-tillage systems). The results demonstrated higher net carbon exchange rates (NCER), in flag leaf phase and no-tillage systems. Fusarium spp. and Gaeumannomyces graminis occurring on stem base s and roots were the main pathogens found in winter wheat. The incidence of stem base and roots was shown to increase under no-tillage in comparison with phloughing tillage system. Puccinia recondita and Septoria nodorum predominant on leaves. The conven-tional tillage increased the incidence of leaf diseases of winter wheat as related to theploughless tillage systems.

P3.4-027

IMPACT OF SOIL MANAGEMENT ON DISEASE SUPPRESSION OF SOIL BORNE PATHOGENS IN ARABLE FIELDS

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Text

Enhanced soil suppressiveness against plant pathogens is a promising strategy to control diseases and crop losses. Improved management practices are developed, however, the

effect of soil management treatments on the level of suppressiveness is mostly unknown. To acquire this knowledge, samples from several field experiments comparing different soil treatments have been evaluated for soil suppressiveness.
Field soils were tested in pot experiments with garden cress and sugar beet by scoring the disease rate after artificial infection of the soils with respectively *Pythium ultimum* and *Rhizoctonia solani* AG2-2IIIB. These two pathogens are known to react differently on the biotic and abiotic factors in soil, being more or less conducive to general and specific suppressiveness. Pythium suppressiveness was in general enhanced by reduced tillage and the addition of several organic products. Rhizoctonia suppressiveness was not consistently influenced by tillage. And although chitin- and keratin-rich products stimulated Rhizoctonia suppressiveness in pot experiments, this effect could not be attained in field trials up to now. Nevertheless, Rhizoctonia suppressive soils did occur among arable fields of farmers, but how to create such suppressiveness is unclear. One of the factors involved could be the presence of the pathogen itself in the field being a precondition to evoke disease suppression, since Rhizoctonia decline is a well-documented phenomenon for several crops.

P3.4-028

CHITIN-FORTIFIED BLACK SOLDIER FLY COMPOSTED ORGANIC FERTILIZER AS AN EFFECTIVE TOOL FOR MANAGING POTATO CYST NEMATODES AND IMPROVING POTATO YIELDS

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Text

Soil degradation and nematode infestation are major challenges to potato production. Synthetic fertilizers and nematicides are costly, less effective, and harmful to the environment. This study explored the potential for use of chitin-fortified black soldier fly composted organic fertilizer (BSFCOF) as a multipurpose organic fertilizer for improved potato yield, and suppression of potato cyst nematodes under greenhouse conditions. The BSFCOF was applied at a rate equivalent to 150 kg N ha-1 and fortified with chitin from black soldier fly pupa exuviae at inclusion rates of 0, 0.5, 1, 2, 3, 4, and 5%. Potato growth, yield, cyst population, number of eggs/J2 g soil-1, and potato cyt nematode (PCN) reproduction rate were monitored. Results revealed that soil amendment with chitin-fortified BSFCOF significantly increased potato growth and yield compared to the control. The number of marketable tuber yields achieved using chitin-fortified BSFCOF was 63 – 169% higher than the control. Chitin-fortified BSFCOF caused a significant reduction in the cyst nematode population (37 – 87%) and the number of cyst eggs/J2 g soil-1 (50 – 96%) compared to the control. Potato yield and PCN suppression increased with an increase in chitin inclusion. Our findings demonstrate that chitin-fortified BSFCOF is a high-quality and multipurpose soil booster for improved soil health and PCN management.
Keywords: Potato cyst nematodes, Chitin, Insect frass fertilizer, Soil health, Potato yield

P3.4-029

ENHANCING SOIL PHOSPHORUS AVAILABILITY AND WHEAT BIOPROTECTION AGAINST ZYMOSEPTORIA TRITICI USING THE PSEUDOMONAS-EXUDED ISOPYOVERDINE

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Text

Low phosphorus (P) bioavailability in soils is a major constraint for optimal wheat growth. Because of high fixation rates of phosphates on surface soil minerals, phosphate fertilizer use-efficiency can be very low. Iron (Fe) oxides, including goethite, have a strong adsorption capacity and intensely influence the availability of phosphorus for wheat crop. When phosphorous deficiency occurs, wheat is more susceptible to diseases, such as Septoria tritici blotch caused by the hemibiotrophic fungus *Zymoseptoria tritici*. To acquire Fe, some plant growth promoting rhizobacteria produce siderophores, ligands with a high affinity and specificity for iron. The present study aims at deciphering the effect of goethite dissolution provoked by the siderophore isopyoverdine, from *Pseudomonas putida* BTP1, on the co-solubilization of phosphates, to enhance soil P availability and to activate the plant immune system of wheat against *Z. tritici*. The experiments were performed in the greenhouse using a hydroponic system and modified Hoagland medium containing no P or soluble Fe, but with the addition of goethite sorbed with phosphate ions and supplemented or not with isopyoverdine, inoculated or not with the pathogen. Growth traits, plant vigour, and plant defence makers, were assessed. The expected results will provide new knowledge on the valorisation of bacterial siderophores for crop health and nutrition.

P3.4-030

SORGHUM-SUDANGRASS AS AN ORGANIC AMENDMENT IN ANAEROBIC SOIL DISINFESTATION DECREASES DISEASE SEVERITY AND PROMOTES PLANT VIGOR

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Text

Anaerobic Soil Disinfestation (ASD) is a non-chemical control used against soilborne pathogens. The technique uses organic amendments, soil saturation, and tarping to create an anaerobic environment for soilborne pathogens that reduces their survival. The choice of organic amendment used in the ASD procedure can make a difference in reduction of

Fusarium oxysporum (Fo) populations, and in plant health. Many studies on ASD have used wheat bran as the organic amendment for control of Fo, which has been only partially effective against Fo populations. In this study, we evaluated ASD with sorghum-sudangrass (SoSu) residue as the organic amendment and compared it to wheat bran. Soil treatments were ASD + wheat bran and ASD + SoSu. Controls were wheat bran and SoSu amendments, without ASD. Polyethylene mesh bags with 2 g Fo inocula were buried in 5-cm depth soil in pots. After 3 weeks of ASD treatment, Fo populations were assessed from the mesh bags. Strawberry plug transplants were planted in ASD and non-ASD treated soils in pots and evaluated for disease incidence and severity after 2 months. Fo populations were assessed from soil at 3 weeks after ASD treatment, and at 2 months after transplant with standard dilution plating. Plants in ASD + SoSu-treated soil exhibited greater vigor and less disease severity in comparison to plants in soil treated with ASD + wheat bran and control plants with no ASD. Our results suggest that SoSu as an organic amendment improves ASD efficacy.

P3.4-031

GRAPEVINES WITH ESCA SYMPTOMS HAVE LOWER OR HIGHER COLONIZATION BY NATIVE ARBUSCULAR MYCORRHIZAL FUNGI?

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Text

The native arbuscular mycorrhizal fungi (AMF) community was investigated in vine roots affected by esca, a complex and devastating grapevine trunk disease. The AMF symbiosis was analysed on roots of adjacent plants (symptomatic and asymptomatic to esca) in 14 sites of three vineyards in Marche regions (Italy). The whole AMF community in the roots samples was investigated by light microscopy after non-vital staining and by amplicon sequencing of internal transcribed spacer of nuclear ribosomal DNA (ITS2). The non-vital staining identified higher value of AMF colonization, in all esca symptomatic plants (ranging from 24.6% to 61.3%) than neighbouring asymptomatic plants (from 17.4% to 57.6%). The largest number of operational taxonomic units (OTUs) associated to *Glomeromycota* phylum, related to the AMF species, was detected on roots from symptomatic plants (0.42%), compared to asymptomatic ones (0.29%). The native *Rhizophagus irregularis* and *Funneliformis mosseae* species quantified by droplet digital PCR (ddPCR) technology displayed a higher number of 28S rRNA gene of both fungal species more frequently in symptomatic than asymptomatic vines. This work showed that esca infection affect the AMF microbial community associated with roots and suggest an interaction of canopy symptoms with microorganisms inhabiting the rhizosphere.

P3.4-032

EFFECT OF COMPOST AND BIOCONTROL AGENTS ON LETTUCE AND TOMATO FUSARIUM WILTS AND ON RHIZOSPHERE MICROBIOME

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Text
Suppressive composts and biocontrol agents are considered among the alternatives to soil fumigants for controlling soil-borne pathogens. Different mechanisms of action are involved, including competition for space and nutrients, plant resistance induction, bioactive compounds, and direct parasitism. The objective of the present work is to summarize the results achieved by evaluating green composts and biocontrol agents (*Trichoderma* spp., *Bacillus amyloliquefaciens, Pseudomonas* sp.) efficacy against *Fusarium oxysporum* on lettuce and tomato, as well as the effect on the rhizosphere microbiome. Experimental trials were carried out in field conditions, by transplanting plants previously grown using potting substrate containing compost or applying the biocontrol agents in nursery. RT-qPCR and the next generation amplicon sequencing technologies were applied on rhizosphere samples. Composts and biocontrol agents reduced the diseases by 50-70%, compared to the untreated controls. Moreover, a reduction of the abundance of the soil-borne pathogens was observed in the treated soils. The abundance of beneficial microorganisms, such as *Bacillus* and *Trichoderma*, increased in the rhizosphere of plants treated. However, treatments did not affect the microbial diversity according to NGS. Compost and biocontrol agents can be used to reduce plant diseases caused by soil-borne pathogens, most probably improving the abundance of beneficial microorganisms and reducing that of pathogens.

Risk assessment for plant pathogens, a key tool for biosecurity under global changes

C3.2-1

AN OVERVIEW OF THE IPPC GLOBAL FRAMEWORK ON PEST RISK ANALYSIS INTERNATIONAL STANDARDS

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Text
It is known that the introduction and spread (or outbreak) of plant pests significantly affects food security, biodiversity and economic prosperity. A vast range of plant pests threaten global food production, the productivity and biodiversity of forests and the wild flora of the natural environment. The impacts of transboundary plant pests and diseases vary from region to region and from year to year. In some cases, they result in total loss of the crop. Globally, annual crop losses caused by plant pests are estimated at 20 to 40% of production (IPPC 2020). In terms of economic value, plant diseases alone cost the global economy around US$220 billion annually (Agrios, 2005) and invasive insects around US$70 billion (Bradshaw et al., 2016).

Preventing those pests spreading and establishing in new countries and regions is the aim of national plant protection organizations (NPPOs) and the International Plant Protection Convention (IPPC). And pest risk analysis (PRA) is a core process within the scope of the IPPC. With an increasing and more diversified trade, with structural and operational changes in the way NPPOs work, continuing developments in molecular biology and genetic sequencing, and the mitigation of climate-change related impacts on agriculture and plant health will present a major challenge to NPPOs and international organizations (IPPC Strategic Framework 2020-2030). In this scenario, the IPPC international standards global framework on pest risk analysis plays a more important role in preventing the introduction and spread of plant pests, while facilitating safe international trade.

C3.2-2

PROACTIVE GLOBAL BIOSECURITY STRATEGIES: PRIORITIES BASED ON CROP LANDSCAPES, TRADE NETWORKS, AND THE ECOLOGICAL NICHES OF 930 PATHOGENS

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Text

The emergence and spread of plant pathogens is an increasing problem, creating an important challenge for ensuring sustainable global agriculture. An urgent issue faced by the international plant health community is where to prioritize proactive phytosanitary responses. We address this problem by identifying priority locations based on geographic risk assessments of pathogen spread through cropland and trade networks of twelve crops key to global sustainability. Our analysis identifies locations having or connecting to croplands with large host populations, and countries with high imports or acting as trade intermediaries, which are likely high risks for pathogen spread. Globally, these locations are candidate priorities for epidemic surveillance. We then analyzed the geographic distribution and ecological niche of over 930 pathogen species affecting the twelve crops. Pathogen richness and community composition, which tend to group geographically into hotspots and distinct “epidemiological regions,” are multicausal phenomena, with the most important predictors in this analysis being cropland connectivity and climate variability. The low association between host range, temperature tolerance and mode of transmission indicate how a crop’s pathogen communities occupy diverse ecological niches, a challenge for the development of epidemic management strategies. These findings provide new components for designing global surveillance and mitigation strategies.

C3.2-3

THE APPROACH OF THE EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION (EPPO): FROM EARLY WARNING TO RISK MANAGEMENT

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Text

One of EPPO's main priorities is to prevent the introduction of dangerous pests from other parts of the world, and to limit their spread within the EPPO region should they be introduced. The emergence of new pests has always been a challenge for the National Plant Protection Organizations. Based on horizon scanning, the EPPO Secretariat initiated the EPPO Alert List in 1999 to provide an early warning to its member countries on pests that may present a risk. In the EPPO Alert List, potential emerging risks are identified on a pest-based approach. A Pest Risk Analysis (PRA) is carried out for selected pests to evaluate in detail the risk for the EPPO region and to identify phytosanitary measures which can be taken against these pests. EPPO also has identified potential emerging risks on a commodity-based approach. Dedicated EPPO Expert Working Groups conduct PRAs on specific pests or groups of pests. PRAs are critically reviewed by different groups of experts. National or multi-national PRAs are also reviewed. Formal recommendations are made to EPPO member countries. Sharing information is essential and several databases have been established by EPPO including the Platform on PRAs. The EPPO approach to early warning and risk assessment and management will be illustrated in the context of global changes for plant pathogens (e.g. ToBRFV, Meloidogyne sp.)

C3.2-4

PRIORITIZATION OF INVASIVE ALIEN PESTS WITH THE POTENTIAL TO THREATEN AGRICULTURE, BIODIVERSITY, AND FORESTRY IN AFRICA THROUGH HORIZON SCANNING

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Text

Sub-Saharan Africa (SSA) is at crossroads especially from attack by invasive alien species (IAS). A multitude of invasions that have significantly impacted economies, livelihoods, and biodiversity have been recorded. Although countries in SSA have weak border biosecurity systems, availing adequate and timely information on the highest risk species enables planning and implementation of sustainable management options that can avert some likely invasions. Such options include prevention through early detection, containment and eventual eradication where possible. Prevention is achieved through constricting pathways by reducing and limiting the means of entry, intercepting movements at border points, and assessing risk of planned imports. Horizon scanning provides an opportunity in generating such information. It is the systematic search for potential biological invasions and an assessment of their potential socio-economic impacts and potential impacts on biodiversity, considering possible opportunities for mitigating the impacts. CABI has developed a horizon scanning tool that utilises information from the Crop Protection Compendium to select pests that have been reported in other countries but not the countries at risk. The tool has been used to generate pests lists for assessment in Burundi, Ghana, Kenya, and Zambia. Actions such as detection surveillances, regulation, deregulation, pest-initiated Pest Risk Analysis have been suggested for the prioritised lists.

C3.2-5

COMMODITY RISK ASSESSMENT AS A TOOL TO IDENTIFY NEW PLANT PESTS: CHALLENGES, FUTURE PERSPECTIVES AND LINKS TO HORIZON SCANNING AND PEST RISK ASSESSMENT

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Text

A key component of biosecurity is the early identification of the risks associated with a plant commodity to ensure its safe trade as well as to monitor the emergence of new potential threats. The European Food Safety Authority (EFSA) currently performs commodity risk assessment to support with scientific and technical evidence the phytosanitary decision-making process in the European Union (EU). Commodity risk assessment is conducted based on dossiers submitted by Third Countries for the so- called High Risk Plants commodities but also for derogation requests to particular provisions of the EU plant health law. Many plant pests not included in the EU legislation are identified each time a new commodity is assessed. These pests are evaluated for their presence in the exporting country, their association with the commodity and their potential impact in the EU. Pests for which there is insufficient information are further monitored through the EFSA Horizon Scanning activities. New plant pests identified as potential threats for the EU, by the commodity risk assessment as well as by the horizon scanning processes, undergo pest categorisations or, when needed, quantitative pest risk assessments. This presentation will summarise the challenges and future perspectives related to the early identification of new plant pests by commodity risk assessment and the links to the horizon scanning and pest risk assessment processes.

C3.2-6

INTERDISCIPLINARY ANALYSIS AND MODELLING OF PLANT HEALTH THREATS TO SCOTLAND

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Text

We have taken an interdisciplinary approach combining biophysical modelling with social science methods to analyse the threat from emerging crop pests and pathogens (CPPs) to agriculture in Scotland. By building on horizon scanning research with agricultural stakeholders, we have co-constructed future scenarios of risks from CPPs, climate change, and socioeconomic factors. These scenarios are compared with a comprehensive biophysical modelling analysis based on CPP distribution data, climate projections, and trade

flows. Our results identify emerging CPPs of greatest interest, and highlight areas of agreement and disagreement between biophysical models and expert stakeholder opinion to identify knowledge gaps in focal CPP biology and other key parameters required for risk modelling. Areas of disagreement are highly instructive in improving models and in signposting potential threats which are not currently considered by stakeholders. Recent examples of invasive pathogens which were not expected to become problematic in the UK include the tree pathogen Phytophthora pluvialis and wheat stem rust (Puccinia graminis). We have conducted a multi-species analysis rather than focussing on one or a few CPPs, allowing us to draw wider conclusions regarding the application of interdisciplinary methods to pest risk assessment.

F3.2-1

MAPPING GLOBAL RISK OF FUSARIUM WILT IN A CHANGING CLIMATE WITH REMOTE SENSING AND AEROSOL TRANSPORT MODELING

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Text

Fusarium oxysporum (*Fo*) is a ubiquitous soilborne fungus that can cause Fusarium wilt (FW) in 100+ crops. Uncertainties in aspects of its epidemiology and a lack of global distribution data have historically challenged monitoring and containment efforts. Our NASA Interdisciplinary Sciences project seeks to address this need by integrating remote sensing, aerosol transport modeling, and comparative genomics to build a global disease surveillance system for FW incidence and *Fo* dispersal risk in aerosolized agricultural dust. As foundation, we released an interactive, global web map documenting 4500+ FW incidences reported in peer-reviewed literature. Here, we developed a global susceptibility assessment that integrates all three aspects of the disease triangle. We identified agricultural production zones conducive to FW, noting subsets capable of serving as dust sources, by overlapping the MODIS Deep Blue algorithm with a Landsat-based cropland product. We then restricted this assessment to only regions with reported *Fo* in the past 30 years. Conducive disease environment was modeled using multiple satellite-derived products with species distribution modeling. Results from this assessment along with aerosol transport modeling can inform how related incidence sites on opposite ends of dust events may be. This integrated approach to disease surveillance can provide key insights about drivers for current and future FW distribution and the spread of *Fo* on global dust currents.

P3.2-001

NUTRITIONAL MANAGEMENT AND PREDICTION OF TOMATO LEAF CURL VIRUS DISEASE

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Text

Tomato leaf curl virus (TLCV) is a notable constraint for the cultivation of tomato worldwide. It causes 70-100% crop losses in case of severe attacks. Whitefly Bemisia tabaci (Genn.) transmits TLCV persistently and circulatively. As no viricides are available, different insecticides are commonly used for the management of TLCV disease. Frequent use of insecticides creates serious environmental issues and resistance in insects. In present study, plants were sprayed with aqueous solution of nutrients and salicylic acid as an eco-friendly approach for the management of TLCV. Furthermore, a disease predictive model was developed based upon environmental factors to find out the possibility of disease outbreaks. Predictive modelling is an efficient tool for accurate TLCV disease management. TLCV disease predictive model was formulated on the basis of weather variables through regression analysis. Nutrients and salicylic acid gave significant reduction in TLCV disease incidence i.e. 49% and 51%, respectively. A disease predictive model based on 2 years of ecological and meteorological data was formulated y= 0.541+ 0.049x1+0.91x2-0.079x3+0.11x4 R2= 0.85. This would aid the farmers to identify, assess and select appropriate disease management methods.

P3.2-002

ADAPTING TO THE PROJECTED EPIDEMICS OF FUSARIUM HEAD BLIGHT OF WHEAT IN KOREA UNDER CLIMATE CHANGE

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Text

Fusarium head blight (FHB) of wheat, mainly caused by *Fusarium graminearum*, is an emerging threat to wheat production in Korea under a changing climate. The disease occurrence and accumulation of associated mycotoxins in wheat kernels strongly coincide with warm and wet environments during flowering. In this study, we adopted GIBSIM, an existing mechanistic model developed in Brazil to estimate the infection risk of wheat FHB, to simulate the potential FHB epidemics in Korea using the 6th Coupled Model Intercomparison Project (CMIP6) climate change scenarios. An integrated modeling combining the results of wheat suitability, heading dates, and FHB infection risks from the CMIP6 scenarios showed a gradual increase in FHB epidemics towards 2100, with different temporal and spatial patterns of varying magnitudes depending on the scenarios. These results indicated that proactive management strategies are needed in the near future to minimize the potential impacts of the FHB epidemic in Korea. Therefore, available wheat cultivars with different heading dates, FHB resistance, market preference, and other characteristics were used in the model simulations as a potential and realistic adaptation measure. As a result, wheat cultivars with the combination of specific characteristics showed significant decreases in FHB epidemics in

future periods, emphasizing the importance of effective adaptation measures against the projected increase of FHB epidemics in Korea under climate change.

P3.2-003

A GENERIC PROCESS-BASED SIMULATION MODEL WITH TWO-WAY COUPLING OF EPIDEMICS AND CROP GROWTH

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Text

Plant disease epidemiological models often simply account for the effect of crop growth through change in the carrying capacity of epidemics. Agrophysiological yield loss models incorporate effects of "pests" (pathogens, insect pests, and weeds), represented as time-dependent drivers, on physiological processes involved in crop growth according to damage mechanisms. Explicit two-way couplings of epidemiological and agrophysiological processes in dynamic simulation models are very rare. Here, we describe a generic, transparent, simple, model structure, with emphasis on polycyclic epidemics affecting the canopy of a crop. Simulated crop growth affects epidemics dynamics in altering the carrying capacity for epidemics. Simulated epidemics, in turn, affect crop growth through damage mechanisms incorporated in the agrophysiological model. The coupled model enables comparing dynamics for biotrophic and necrotrophic pathogens. Outputs from sensitivity analyses show the interplay between damage mechanisms (light stealer, accelerated leaf senescence, assimilate diversion) and their impact on crop growth, which in turn determines the carrying capacity for epidemics through a feedback loop. Potential uses and expansions of this modelling approach are outlined, including risk assessment under global and climate changes.

P3.2-004

CONIFER SUSCEPTIBILITY TO THE PINE WILT DISEASE : COMBINING HOST RESPONSE TO THE NEMATODE BURSAPHELENCHUS XYLOPHILUS AND ITS INSECT VECTOR MONOCHAMUS GALLOPROVINCIALIS

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Text

The pine wilt disease (PWD) is the result of the success of three main events, inoculation of the pine wood nematode (PWN) to the host tree by the insect vector, *Monochamus galloprovincialis*, during its maturation feeding; multiplication of the nematode within the host tissues and development of the insect in the infested trees. Within this conceptual framework, we combined literature and expert knowledge to document the interactions between the nematode, its insect vector and 75 coniferous species.
We ended up with 5 categories of risk of development of PWD (1, no risk to 5, high risk). No pine species in Europe seems completely resistant to PWN, although some species are less favorable to the multiplication of the PWN. In case of an epidemic, the risk of spread of the disease is then directly related to the ability of the insect vector to transmit the nematode from tree to tree. It is recommended that priority be given to eliminating the pine species on which *M. galloprovincialis* is able to complete its life cycle, i.e., with certainty those of category 5 (*P. pinaster*, *P. sylvestris*, *P. nigra* and *P. radiata*) and probably those of category 4 (*P. halepensis* and *P. taeda*). However, for *P. taeda*, uncertainties remain in the literature, both regarding the multiplication of the nematode, the expression of wilt symptoms and the ability of the insect to feed and reproduce on this species. In particular, it cannot be excluded that *P. taeda* acts as a 'healthy carrier' of the disease.

P3.2-005

PEST RISK ANALYSIS, AN EFFECTIVE TOOL TO ENHANCE CRISIS PREPAREDNESS TOWARDS EMERGING PESTS: THE CASE OF TOBRFV IN FRANCE

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Text
In 2019, Anses (French Agency for Food, Environmental and Occupational Health & Safety) was aware of an alert concerning an emerging tomato virus: tomato brown rugose fruit virus (ToBRFV) and initiated an express pest risk analysis of ToBRFV for France. The probabilities of entry, establishment and spread of ToBRFV in France were evaluated and scored as high. The magnitude of the impact of ToBRFV in the area of potential establishment in France was also estimated as high, especially in the endangered area. The latter include areas of large-scale production of tomatoes (in protected conditions and open-field), as well as areas of pepper production. Several phytosanitary measures were recommended. If infected plants are reported in a production unit, eradication was recommended by taking immediate action to destroy all the plants and fruits, coupled with strict hygiene measures including a crop-free period. Based on these recommendations, the French Ministry of Agriculture immediately issued technical instructions for professionals to be applied against ToBRFV. This pest risk analysis performed by ANSES, together with the development of a detection method of ToBRFV by the National Reference Laboratory, and the implementation of quick and drastic measures to eradicate the first outbreak in Brittany by the risk manager, allowed a successful management of the epidemic of ToBRFV in 2020. Today, ToBRFV is considered as transient in France, with only one outbreak under eradication.

P3.2-006

THE RISK ASSESSMENT OF SHARP EYESPOT CAUSED BY CERATOBASIDIUM CEREALE ON CEREALS IN KOREA

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Text

Cereals including barley (Hordeum vulgare L.), wheat (Triticum turgidum), and oat (Avena sativa) are major winter crops in Korea. The level of the total production of cereals is not sufficient. Furthermore, climate change is increasing the threat of fungal disease in the cereals as well as newly emerged pathogens. One fungal disease is sharp eyespot disease. The fungal disease is caused by Ceratobasidium cereale. In this study, we collected fungal isolates and assessed the risk of the disease according to the effect of temperature and humidity. To identify fungal disease, the internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using the primer pairs ITS4/5, producing a 686 bp amplicon. Their sequences showed 99.7% identity to that of C. cereale strain WK137-56 (99.7%; KY379365) and Ceratobasidium sp. AG-D (99.8%; KP171639). Maximum likelihood phylogenetic analysis based on the ITS sequences placed the representative isolates within a clade comprising C. cereale. As a result of examining the optimal growth conditions, the fungus can grow in the range of 10 to 25? with pH 6-7 on a PDA medium and showed the highest growth at 20-25?. Moreover, in the temperature-dependent pathogenicity test, C. cereals showed the highest level of pathogenicity in the crops at 20?. Further studies will be displayed. [This work was supported by a grant from the Rural Development Administration (PJ0149952023).]

P3.2-007

THE EPPO PLATFORM ON PEST RISK ANALYSIS, A HUB FOR RISK ASSESSMENTS OF PLANT PATHOGENS

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Text

The EPPO Platform on Pest Risk Analysis (https://pra.eppo.int/) was launched in September 2018. It allows to share work done on the evaluation of pest risk within the EPPO region and beyond. It includes Pest Risk Analyses (PRAs) produced by national plant protection organizations of countries in the European and Mediterranean region. Documents cover all types of organisms harmful to plants including pathogens, as well as commodity risk assessments. PRAs are prepared for different reasons: for example when a pathogen is intercepted on imported commodities or found during official surveillance activities, to assess the risk of a specific traded commodity, or before allowing the import of pathogens for research purpose. Sharing assessments done in one country may help identify emerging plant pathogens. Checking existing PRAs is a useful first step when preparing a new

assessment. It may help reducing the workload e.g. to produce a risk assessment for the same pest in another area.
The Database is regularly updated with new documents being posted. As of February 2023, more than 1900 documents are available. A number of relevant open access scientific articles are also included. Most of the documents are publicly available but registered users may also choose to share documents to a defined group of users to help collaboration at an early stage. Documents are indexed and can be retrieved by pest or commodity of concern, date, country, as well as with keywords.

P3.2-008

THE EPPO ALERT LIST, A TOOL TO RAISE AWARENESS ON EMERGING PESTS IN THE EPPO REGION

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Text
New plant pests and invasive plants with negative impacts on food security and the environment are regularly reported. Some of these emerging species are new to science, while many others have been introduced into new areas where they become damaging. Early warning is crucial to allow rapid action against emerging pests and invasive plants. The emergence of new pests has always been a challenge for the National Plant Protection Organizations (NPPOs). At the request of its member countries, the EPPO Secretariat started to develop the EPPO Alert List in 1999. This list of pests, pathogens and invasive alien plants has two aims:
- to draw the attention of EPPO member countries to certain organisms possibly presenting a risk to them and achieve early warning,
- to identify possible candidates for Pest Risk Analysis.
The poster will present the triggers to support addition to the Alert List, the information gathered to support this addition and the general maintenance to keep the Alert List focused and relevant. The Alert List is available on EPPO website https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list. When pests are added to the Alert List, this is marked by an article in the EPPO Reporting Service (4500 readers), as well as by publications on social media. The objective is to raise awareness among different audiences (e.g. scientists, NPPOs, professional operators, general public).

P3.2-009

DISTRIBUTION AND DIVERSITY OF ABACA BUNCHY TOP VIRUS AND BANANA BUNCHY TOP VIRUS CAUSING BUNCHY TOP OF ABACA IN CARAGA, PHILIPPINES

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Text

The Philippines contributes more than 80% to the world production of abaca (*Musa textilis*) fiber or 'Manila hemp' that is used in various industrial products. However, abaca industry growth is significantly hampered by the bunchy top disease (BTD) caused by abaca bunchy top virus (ABTV) and banana bunchy top virus (BBTV). Herewith, we surveyed major abaca plantations in Caraga region, Philippines using mapping tools complemented with molecular diagnostics, to generate a distribution map for the incidence of abaca BTD. We showed that BTD is present in all Caraga provinces where a total of 395 samples were collected. A subset (n=120) were tested for ABTV/BBTV using duplex PCR tests where 84 samples were positive for BBTV and 66 samples for ABTV. Interestingly, there is a high rate of ABTV/BBTV co-infection, where 49 samples (41%) tested positive for both viruses. Diversity analyses revealed moderate levels of nucleotide diversity for both viruses with evidence of recombination and phylogenetic lineages showed correspondence with the geographic origin of the global isolates. Furthermore, data from PCR tests were used for the MaxEnt analyses that provided predictive insights on the possible spread of the disease in the region. Overall, we contributed novel information on the distribution and diversity of ABTV and BBTV. By using predictive analyses, we advanced of the understanding of the epidemiology of abaca bunchy top disease in a major growing region of the Philippines.

P3.2-011

AUSTRALIAN PLANT BIOSECURITY

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Text

Australia's biosecurity system helps prevent, detect and manage exotic invasive pests that threaten agriculture and natural ecosystems.

Targeted pest lists strengthen agricultural and environmental biosecurity. There are 27 priority plant pathogens targeted by monitoring, surveillance and diagnostic activities across the Australian borders and post-border by ?governments, industry, and the community. Surveillance is conducted by trained officers and aided using innovative methods including remote sensing and drones?, hyperspectral cameras, suction traps, eDNA sampling and lures.

Diagnostic laboratories receive plant samples and insect specimens for testing, from monitoring, surveillance and inspection activities. The diagnosticians in these laboratories identify pests and diagnose diseases though microscopy, high throughput sequencing and spectrophotometry to stop pests of biosecurity concern from entering and establishing in Australia.

The surveillance and diagnostic techniques used by scientists delivering Australian biosecurity and the tools and systems supporting these activities enables our capable workforce to operate competently and consistently while working in an effective, safe, modern and efficient diagnostic system.

This paper demonstrates how continuously enhancing diagnostic capability and capacity has resulted in greater accuracy, identification of intercepted pathogens to higher taxonomic

levels and greater confidence in Australian plant biosecurity outcomes.

P3.2-012

WHEN FINDING A SOLUTION IS NOT SUFFICIENT: ECONOMIC VIABILITY OF CITRUS DISEASE MANAGEMENT AND THE ROLE OF EXTREME ENVIRONMENTAL FACTORS

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Text

Countries located in arid and semi-arid regions in the world do not only face constraints due to the limited availability of arable lands, but major crops in these countries are continuously attacked by destructive diseases like Witches' broom disease of lime (WBDL). In Oman, more than one million lime trees were damaged due to WBDL. Consequently, Omani lime production and cultivated area have declined over the past two decades by 75%. Several studies have been carried out to address the citrus disease in crop science, very few, and non in the case of Oman, have addressed the economic loss associated with the disease risk or economic gain resulting from disease management strategies. Given these and the lack of a detailed and thorough economic analysis of possible environmental factors that may influence the occurrence and spread of WBDL, the first objective of this study is to derive a bioeconomic model to simultaneously determine optimal disease treatment timing, frequency, and level to optimize tree value and inform growers about best practices to mitigate the risk associated with citrus diseases. This study also aims to investigate environmental factors that affect the occurrence and spread of citrus diseases and present comparisons between infected and non-infected trees. This would help government agencies develop more effective educational and treatment programs in order to align governmental interests and farmers' interests to maximize citrus profit and economic value.

P3.2-013

IDENTIFYING PRIORITY QUARANTINE PESTS BASED ON ASSESSING THEIR ECONOMIC, SOCIAL AND ENVIRONMENTAL RISKS

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Text

The world is witnessing an increased number of plant pests' outbreaks driven by both globalization of trade and effects of climate change. With scarce resources, setting up systematic controls for all potential pests would become inefficient and extremely expensive. Therefore, efforts should be prioritised on those pests with higher risk of having economic,

environmental and social impacts in the EU. In this spirit, the new EU Plant Health Law (PHL) [Regulation (EU) 2016/2031] calls for the identification of quarantine pests that qualify as "priority pests". Priority pests identified as such will have to be the of subject of annual surveys, contingency plans, simulation exercises, and designed action plans at national level.
We have developed a composite index that translates the legislative criteria defining a priority pest into measurable indicators. The composite index has 25 indicators combining both quantitative and qualitative data. These are grouped into 3 domains and 10 sub-domains to cover the most important potential economic, social and environmental impacts. We have applied it to an initial list of 28 quarantine pests in a scenario of pests' maximum potential spread across the EU. The methodology adds to the normally neglected aera of research on economic and social risk assessment of pests. The indicator has been used to derive the first official list of EU priorty pests published in 2020.

P3.2-014

FIG MOSAIC DISEASE IN TUSCANY (ITALY): MOLECULAR CHARACTERIZATION AND INVESTIGATION OF VIRUS-HOST INTERACTION

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Text

Fig mosaic disease (FMD) represents the main threat to global fig production. It has been associated to several viruses and viroids, but only *Fig mosaic virus* (FMV) has been identified as etiological agent. Here, 15 fig trees 'Dottato' were investigated in Tuscany (Central Italy). In February 2022, winter branches were sampled. On July, as leaf visible symptoms occurred in 14 trees, fully developed symptomatic and asymptomatic leaves were collected. Among the FMD-associated viruses we tested, only *Fig fleck-associated virus* (FFKaV) was identified in branches (73% of samples). Differently, not only FFKaV, but also FMV was reported in both symptomatic and asymptomatic leaves (93 and 63% of samples, respectively), with all 15 and 14 trees tested positive to FMV and FFKaV, respectively. FMV amplicons provided nucleotide sequences corresponding to partial RdRp genomic region, among which three new variants (GenBank: OQ291242, OQ291243, and OQ291244). The FMV phylogenetic analysis showed low bootstrap values, and the new variants resulted near to their closest counterparts at GenBank. Investigation of phytopathological responses showed an FMV-induced reduction of carbon dioxide assimilation due to stomatal and no-stomatal limitations, as well as of leaf pigment concentrations. Harsher effects were reported in positive symptomatic leaves, but alterations occurred also in positive asymptomatic ones. This study represents the first investigation of FMD in Central Italy.

P3.2-015

A spatio-temporal database of first introductions of plant pests in the EU

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Text

World trade of plants and plant products has highly increased in the last decades, together with a higher risk of introduction of new pests, including bacteria, fungi, viruses and arthropods. Identifying plant pests' introduction points in the European Union (EU) territory may offer some insights for early detection to prevent new plant pest invasions as well as supporting the surveillance and risk assessment activities carried out in the EU. A key challenge in analysing patterns of pests' introductions is the frequently limited historical record with data on the time and location of the appearance of new species in certain countries. As part of the HoPPI (Hotspot for Plant Pest Introduction) project, data were collected on plant pest first introduction records along the EU between 1999 and 2019, drawing upon a range of published literature and online databases, such as EASIN and EPPO, in order to determine pests' introduction trend over the years and spatial aggregation among EU regions. The database contains expert-revised data on 278 pests: it is estimated that on average around 10 new pests are detected within the EU territory each year, and in some years, there are as many as 25 introductions of new pests. Some of the most high-profile cases included, comprehend the insidious bacterium Xylella fastidiosa, causing serious diseases in a wide range of plants around the world, the fungus Fusarium circinatum, considered one of the most important pathogens of conifers worldwide, or the Pepino mosaic virus, which can cause severe yield losses and reduced fruit quality in affected crops.

P3.2-016

DISEASE SYSTEMS ANALYSIS FOR PROTECTING GLOBAL FOOD SECURITY: AN INTRODUCTION TO THE R2M TOOLBOX

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Text

Epidemic modeling and forecasting are key to improving global food systems. As food security is challenged by pest and pathogen invasions, natural and manmade cataclysms, and climate change, digital agriculture can support informed decision making and mitigate the impacts of disasters. Digital agriculture uses farm and disease models to provide decision support about potential epidemic outbreaks. Here we describe three computational tools in the Rapid Risk and Mitigation (R2M) toolbox for agricultural stakeholders and policy makers, to support the mitigation of diseases and pests. i) Impact network analysis (INA) is a scenario analysis framework, designed to simulate epidemic and socioeconomic networks and to identify strategies for regional disease management. ii) GeoPathome is an interactive web data scraper, informing users about the geographic distribution of pests and pathogens

and the potential for invasions through international trade. iii) The R2M meta-tool for expert knowledge elicitation is an interactive interface that generates a survey for experts based on a curated set of disease-risk questions. These three tools use R, an open-source language, to harness observed or speculative data to help agricultural stakeholders streamline data collection and scenario analyses of potential disease outbreaks. Effective use of these disease modeling and risk analysis tools will support disease management for a more secure and sustainable future for food systems.

P3.2-017

ESTIMATION OF TYLENCHULUS SEMIPENETRANS POPULATION ON DIFFERENT CITRUS VARIETIES GROWN IN THE NATIONAL AGRICULTURAL RESEARCH CENTER, ISLAMABAD, PAKISTAN

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Text

Citrus is an important agricultural fruit crop. Pakistan holds a leading position in citrus production, with a production area of 204.4 thousand hectares. Tylenchulus semipenetrans is an important and widespread plant-parasitic nematode that affects citrus worldwide and can cause citrus slow decline disease in all citrus growing areas of Pakistan. A study was conducted to determine the nematode population on six different citrus varieties: Arnold blood (Carrizo), Cara Cara (Troyer), Daisy Manadarin (Troyer), Mc Mahon (Carrizo), Ryan Navel (Carrizo), and Salustiana (Troyer), grown in orchards of the National Agricultural Research Center, Islamabad. Nematodes were extracted from soil and roots of 10-year-old citrus plants using Hemming's Tray method. The quantitative data for nematode population in 100ml of soil sample for each plant was calculated. The study revealed that the majority of the extracted nematodes were T. semipenetrans based on their peculiar morphological characteristics. The maximum nematode population was observed in Arnold blood, followed by Daisy Manadarin, Cara Cara, Ryan Navel, Mc Mahon, and Salustiana, while the minimum nematode population was observed in Harvard blood. This information is important for citrus growers in Pakistan, as citrus slow decline disease can significantly reduce citrus production. Therefore, appropriate measures should be taken to manage the nematode population, prevent the disease, and maintain healthy citrus orchards.

P3.2-020

VIRUS DISEASES OF VEGETABLES IN MALI AND NORTHERN COTE D`IVOIRE, WEST AFRICA

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Text
Virus disease is among the major constraint to vegetables production in sub-Saharan Africa including Mali and cote d`Ivoire. Vegetable diseases caused by geminiviruses and more specifically species under the genera begomovirus are among the major production constraints. To assess what were the predominant and emerging virus diseases of major vegetables in Mali and northern Cote d`Ivoire, a field survey was conducted during the major vegetable growing seasons. Virus like disease samples from tomato, pepper, okra and African eggplant were collected and the virus(es) present in each was diagnosed by ELISA and PCR. The survey and diagnosis analysis using PCR amplification of Begomovirus universal primers and ELISA analysis identified several viruses in both countries. In Mali of the 12 okra samples tested, 11 were positive to Geminiviruses and from 29 pepper samples 16 were positive using PCR amplification. Moreover, ELISA test detected 3 PMV, 1 PMMV, 3 WSMOV and 5 PoTV viruses from 11 plants tested. In the northern Cote d`Ivoire both the ELISA and PCR test identified pepper yellow vein Mali virus, African eggplant yellowing virus, pepper veinal mottle virus, Capsicum chlorosis virus and tomato chlorosis virus. The presence of these viral diseases in vegetables at different levels of frequencies calls an attention to develop virus resistant varieties.

P3.2-021

BUILDING GLOBAL SURVEILLANCE AND MITIGATION STRATEGIES FOR LAUREL WILT

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Text
Laurel wilt, caused by the fungus *Harringtonia lauricola* and vectored by ambrosia beetles, is a devastating disease of avocado and many species in the Lauraceae. Laurel wilt is not yet present in all regions where Lauraceae are present, including some important avocado production regions. We evaluated which locations are particularly useful for surveillance and mitigation to slow the future spread of laurel wilt and to warn areas that are at particular risk to prepare management strategies. We analyze global wood trade, Lauraceae host connectivity, and climate suitability to identify candidate priority locations for early surveillance of laurel wilt. Analysis of the international wood trade network showed that disease-free countries such as Mexico, Brazil, and Australia have multiple trade links to countries with laurel wilt. Potential epidemic networks were based on maps of host landscape connectivity for Lauraceae and cultivated avocado. For avocado, locations in southern Mexico and Central America were important locations to monitor for laurel wilt, while for the Lauraceae, locations in Amazonia, and western Europe were important. Maps of global climate suitability for laurel wilt identify locations with current and future climate suitable for the establishment of laurel wilt, including locations in southern Brazil, southeastern China, and eastern Australia. These results can inform regional, national, and global monitoring and mitigation strategies for laurel wilt.

P3.2-022

RISK ANALYSIS OF THE CUT FLOWER TRADE AND GEOGRAPHIC HOTSPOTS TO PROTECT GLOBAL AGRICULTURE FROM INVASIVE PESTS AND PATHOGENS

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Text

The international trade of cut flowers reached $21.3 billion in 2021, growing 28% in the last decade. Most cut flowers are traded fresh with limited sanitation options available, increasing the risk of pest and pathogen introductions. High rates of invasive pests have been found in inspected cut flowers, and rates are expected to increase with growth in international trade. Strategies for early surveillance and detection for pests and pathogens are needed. Here, we analyzed formal international trade and the geographical distribution of pests and pathogens associated with cut flowers to identify candidate priorities for surveillance locations. Trade networks highlight the risk posed to the USA, the largest importer (20%), and the Netherlands, exporting more than half of global cut flowers, as well as other countries. In the absence of adequate phytosanitary measures, the role of Uganda and the Netherlands in trade networks for rose planting materials could pose a risk for dissemination of associated pests and pathogens. Similarly, in the absence of adequate phytosanitary measures, the role of the Netherlands and Colombia in chrysanthemum flower trade networks could pose a risk for introduction of chrysanthemum white rust, a quarantine pathogen in the USA. These network-based analyses can guide phytosanitary measure prioritization globally for effective use of resources to protect agriculture from invasive pests and pathogens.

P3.2-023

QUICK ASSESSMENTS OF THE POTENTIAL FOR ESTABLISHMENT OF QUARANTINE PESTS IN SWEDEN

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Text

The environmental conditions and availability of plant hosts in a country greatly influence the potential for establishment of new plant pests and have important implications for their management. In the EU, regular surveys for all union quarantine pests shall be carried out on a regular basis in all member states according to the plant health regulation ((EU) 2016/2031)). However, surveys are not required if the ecoclimatic conditions or the absence of hosts prevents the establishment or spread of the pest. For some quarantine pests it is

uncertain whether the ecoclimatic conditions or host availability in Sweden allow an establishment. Quick assessments of the degree to which the conditions are suitable for the establishment were performed for 82 quarantine pests to support management decisions by the Swedish National Plant Protection Organization. Depending on the biology of the pest species, different factors affect the potential for population development and the uncertainties associated with the assessments. The assessments were based on the likelihood of the pests to survive and reproduce in Sweden both outdoors and in protected cultivation. The following factors were considered i) the presence of host plants, ii) the presence, or potential establishment, of vectors if required for transmission of pathogens and iii) the prevailing ecoclimatic conditions. Assessments were done using a four-level scale and the uncertainty was included as plausible min and max options.

P3.2-024

BACTERIAL LEAF STREAK OF MAIZE CAUSED BY XANTHOMONAS VASICOLA PV. VASCULORUM, A DISEASE THAT MIGHT THREATEN EUROPEAN MAIZE PRODUCTION

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Text

Plant diseases caused by *Xanthomonas* species affect many different crops worldwide. The species *Xanthomonas vasicola* currently includes four pathovars: *arecae*, *musacearum*, *vasculorum* (Xvv), and *holcicola* (Xvh). Of these only Xvh has a validly published name, although the others have been proposed.

Maize (*Zea mays*) is the most widely grown cereal in the world. Bacterial leaf streak of maize caused by Xvv was first reported in South Africa in 1948 and was not reported anywhere else until 2014 when it was identified in Nebraska, USA; this disease then spread rapidly and is now present in 10 US states. Recently it has also been reported in some regions of Argentina and Brazil. This disease is not currently present in Europe, but should be considered as a potential theat to maize crops. At Fera, artificial inoculations on a range of maize varieties grown in the UK indicate wide susceptibility to this pathogen. The main host of Xvv is maize, but evidence has shown that other monocot species like oat and rice are also hosts.

The most likely pathway of entry in Europe will be on seed, although there are only a few reports of seedborne infections. By sequencing Xvv isolates from South Africa, the US, Brazil, and Argentina, and comparing sequences with publicly available data, we aim to determine what is the most likely origin of the infections observed in South America and use this to inform a plant protection strategy for the UK.

P3.2-025

IMPACT OF CLIMATE CHANGE ON POTENTIAL DISTRIBUTION OF DICKEYA ZEAE CAUSAL AGENT OF STALK ROT OF MAIZE IN SIALKOT DISTRICT PAKISTAN

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Text

Zea mays L. is the 3rd most influential crop by its bulk production all over the world. Bacterial stalk rot of maize caused by Dickeya zeae result into significant crop yield reduction, thus need to be addressed. Further, to produce the epidemiological evidence for the management of the disease outbreaks in the hot spot region, extensive field surveys during 2021 revealed that out of 266 visited areas, high disease incidence was observed in Bhoopalwala (78.5 %) followed by Bangla Chowk (76 %), Suraj (74 %), and Bakhray wali (68.5 %), while the lowest disease incidence was recorded from Pasrur (20 %), Chawinda (15 %) and Head Marala (9 %). The Maxent algorithm modelling was used to predict areas at risk of disease outbreaks in Sialkot regions and, to predict and forecast the disease outbreaks in coming years. Among the nineteen bioclimatic/environmental variables, four factors including temperature seasonality (standard deviation*100) (bio-4), mean temperature of wettest quarter (bio-8), annual precipitation (bio-12), and precipitation of driest month (bio-14) were found to be the most critical factors influencing the disease distribution in current and coming years. It is predicted that high risk area and disease distribution will increase across the four tehsils of Sialkot Pakistan over the years 2050 and 2070. These results are important for policymakers and researchers to take effective disease control measures against bacterial stalk rot of maize disease outbreak.

P3.2-026

HORIZON SCANNING FOR PLANT HEALTH THREATS: 7 YEARS OF SUPPORT TO EU RISK MANAGERS

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Text

In December 2016 EFSA was mandated by the European Commission to carry out a horizon scanning exercise on emerging plant pests. From that moment, and in close collaboration with the Joint Research Centre, EFSA started its monitoring and extraction of news and scientific data on plant pests using the Medisys platform (https://publications.jrc.ec.europa.eu/repository/handle/JRC53155).
Medisys retrieves continuously relevant items from the web by searching on more than 21,000 media and scientific literature sources from around 200 countries for keywords (more than 13,000) from the pre-defined ontology and classifies them according categories. The retrieved items are then reviewed and filtered by a group of experts who selects the most relevant ones. The result of this activity is compiled and presented in freely available monthly

newsletters (https://efsa.onlinelibrary.wiley.com/doi/toc/10.2903/(ISSN)1831-4732.Horizon-scanning-for-plant-health).
In case of non-regulated pests, further analysis of potential risks is carried out by applying a fast-track procedure that allows identifying the most relevant “emerging pests of the month”. The results of this analysis are also included in the newsletter, whose content is presented and discussed each month by the EU Commission and Member State representatives. During these seven years of activity the process and quality of outputs have been refined and the contribution of this activity to the timely reaction to emerging threats by EU risk managers and risk assessors is fully acknowledged.

P3.2-027

EFSA ACTIVITIES ON RISK ASSESSMENT AND PREPAREDNESS FOR INVASIVE ALIEN PLANT PATHOGENS IN EUROPE

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Text
The European Food Safety Authority (EFSA) is the Agency of the European Union responsible for risk assessment in food safety, animal health and welfare and plant health. EFSA conducts risk assessment for individual plant pests with a two-phase fit for purpose approach, a simpler and narrative pest categorisation first, followed by a quantitative probabilistic pest risk assessment for more complex questions, where quantification of the risk allows the comparison of outputs under different scenarios, including risk mitigation options and climate change scenarios. EFSA is also funding research projects to reduce key uncertainties and knowledge gaps by generation and collection of observational and experimental evidence. Trends and examples from risk assessment of invasive alien plant pathogens are presented.

Keywords: pest categorisation, pest risk assessment, quantitative, probability

Social and cultural dimensions of international forest health

C7.6-1

THE GLOBAL FOREST HEALTH CRISIS: A PUBLIC GOOD SOCIAL DILEMMA IN NEED OF INTERNATIONAL COLLECTIVE ACTION

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Text

Society is confronted by interconnected threats to ecological sustainability. Among these is the devastation of forests by destructive non-native pathogens and insects introduced through global trade, leading to the loss of critical ecosystem services and a global forest health crisis. We argue that the forest health crisis is a public good social dilemma and propose a response framework that incorporates principles of collective action. This framework will enable scientists to better engage policymakers and empower the public to advocate for proactive biosecurity and forest health management. Collective action in forest health will feature broadly inclusive stakeholder engagement to build trust and set goals; accountability for destructive pest introductions; pooled support for weakest-link partners; and inclusion of intrinsic and non-market values of forest ecosystems in risk assessment. We provide short-term and longer-term measures that incorporate the above principles to shift the societal and ecological forest health paradigm to a more resilient state.

C7.6-2

NGA RAKAU TAKETAKE - CONTROL, PROTECT, CURE NOVEL. TOOLS AND TECHNOLOGIES FOR DETECTION AND MANAGEMENT.

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Text

Aotearoa New Zealand’s BioHeritage Challenge is leading the development and implementation of a programme, known as Ngã Rãkau Taketake (NRT) – Saving our Iconic Trees. The name Ngã Rãkau reflects the historical connections that Mãori and other New Zealanders have with our kauri and myrtaceae trees, whilst Taketake refers to the permanence of that relationship. This programme aims to protect and restore that relationship and connection. Kauri dieback is threatening Aotearoa New Zealand’s taonga (treasured) kauri with extinction, whilst myrtle rust (and its associated offshore strains) is threatening many iconic native species as well as plants important to primary industries. New Zealanders have strong emotional and cultural attachments to kauri and the native estate,

the loss of which is having a major impact on our communities. The myrtle rust and kauri dieback spaces desperately need a suite of fully integrated management tools and approaches to saving our ngahere (forest). To address this, Ngã Rãkau Taketake is investing in research and related activities in the field of 'Tools and Technologies for Detection and Management'. Our team has been using an innovative co-development, co-design outreach process with local indigenous tribes to socialise new technologies, assess important values and concerns, and ensure research, operations and case studies are fully integrated, of which examples and learnings will be presented.

C7.6-4

INDIGENOUS SEED BANKING TO PROTECT AGAINST PLANT PATHOGENS IN AOTEAROA NEW ZEALAND

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Text

This paper considers the dangers that plant pathogens represent for native plants in Aotearoa New Zealand, and how Indigenous M?ori seed banking can help to save them. The growing impacts of climate change and environmental instability increase the risk of the introduction, adaptation, and spread of plant pathogens in Aotearoa. As Indigenous people of the land, M?ori have a special relationship with and responsibility for the natural environment including native plant species which can be endangered by pathogens. For example, austropuccinia psidii is one such pathogen which affects many native trees including p?hutukawa, manuka, and r?t? all species of cultural significance. While the introduction of such pathogens is not easy to control, the preservation of native plants through methods such as seed banking can act as insurance against incursions. Preserving these plants through seed banking is essential to M?ori, but it is complicated by colonisation and the Crown's (illegal) ownership of native flora and fauna which is perpetuated through government-owned seed banks both in Aotearoa and overseas. Te Tira Whakam?taki, home of Aotearoa's M?ori Biosecurity Network, is seeking to provide a much-needed alternative; an Indigenous-owned M?ori seed bank programme. The programme already has provided training and equipment to iwi/hap? to start their own seed banking at-place, but an Indigenous Seed Banking Declaration and a national M?ori seed bank are the next steps.

C7.6-5

MEDIA COVERAGE OF FOREST SURVIVAL IN TURKEY

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Text

Public opinion plays a significant role in shaping policies that impact forest and tree health directly or in the long run. This paper explores media coverage of news related to tree and forest health in the years 2021-2023 in two daily newspapers that have the widest reading

coverage in Turkey: “Sözcü” with an opposition and “Hürriyet” with a pro-government stand. Unprecedentally widespread forest fires witnessed in the summer of 2021 in Turkey have once again opened debates related to forest survival and the role of human action in shaping nature. News related to forests have been firstly about forest fires and secondly about the transfer of state-owned forest lands for development and tourism. We see that the survival of forests is reflected in relation to the survival and the well-being of wildlife. Ecological concerns are openly voiced or implied as overriding priorities and decisions to designate forest lands for other purposes is deemed to be detrimental. News is given of local protests. The disastrous earthquakes that took place in February 2023 in Turkey have accelerated the media coverage and debates over the country-wide planning of land designated for agriculture, forestry and urban dwelling usage. Amidst such agonized debates we perceive a novel sensitivity about land use that also embraces concerns over forest health and survival.

C7.6-6

AN INDIGENOUS SOLUTION TO A PLANT PATHOGEN IMPACTING AN ICONIC TREE SPECIES IN AOTEAROA-NEW ZEALAND

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Text
The pathogen Phytophthora agathidicida is having a devastating impact on the kauri (Agathis australis), a tree species that is a paramount symbol of M?ori, Indigenous peoples of Aotearoa, cultural identity. M?ori across regions have mobilised, motivated by deep cultural responsibilities to restore and protect kauri and surrounding environments, encapsulated in the proverb “Ko au ko te kauri – ko te kauri ko au, I am the kauri and the kauri is me”. In 2019 M?ori knowledge and practitioner experts were afforded a rare opportunity to employ their own epistemological frameworks, implement their experience and apply their customary practice to conceptualise, develop and then trial treatment and holistic management systems to combat the impacts of this pathogen. Termed the ‘rongoa approach’ this uniquely M?ori approach employs M?ori knowledge and M?ori traditional practices and has proven to offer a credible pathway forward in reversing the physical manifestations of this disease in infected trees and provide ongoing protection against reappearance of symptoms. We will present an overview of the epistemological construct that has been a unique collaboration between knowledge holders from different tribal areas that informed the ‘rongoa’ approach to improve tree health, and how this work was conducted without compromising M?ori cultural norms and expectations. Results will be discussed within the context of tree and wider biodiversity health, and the empowering outcomes for M?ori.

P7.6-001

M?TAURANGA — AN INDIGENOUS RESPONSE TO MYRTLE RUST.

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Text

M?ori (Indigenous peoples of New Zealand) have traditionally used ‘m?tauranga (traditional knowledge), to articulate a knowledge system that encompasses inherent and acquired knowledge. While western science has demonstrated an appetite to identify opportunities to align or integrate m?tauranga of the Ngahere (native forests), wai (water) and moana (sea), M?ori have been reluctant to share their knowledge, and the protection of m?tauranga and ‘knowledge holders’ has become fundamental in any engagement with western science, agents and/or enterprise.

M?tauranga M?ori, used to describe a whole M?ori system, is not western science and research . M?ori knowledge systems are made up of observations and intimacy with our world over millennia and these systems inform the way we make decisions.There are obvious distinctions between the two systems. Western science doesn’t require that you do anything with knowledge, while M?ori knowledge requires that you take action and are accountable for that action. Western scientists seek expertise in singular fields, while M?ori knowledge is acquired across a range of domains which allows M?ori to better respond to the needs of the natural environment and their people. Using the myrtle rust incursion, we explored Maori expectation of the inclusion of m?tauranga in the response and how science and research has enabled kaitiakitanga (guardianship) and rangatiratanga.(decision making) solutions and approaches in the biosecurity system.

P7.6-002

INDIGENOUS FRAMEWORK TO RECOGNISE AND GIVE EFFECT TO CULTURAL AND DATA SOVEREIGNTY TO ELEVATE INDIGENOUS PEOPLES INTO SCIENCE AND BIOSECURITY SYSTEMS

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Text

Surveillance in Aotearoa New Zealand is part of a biosecurity response that protects our borders and landscapes and our natural biological heritage and economy. Prioritisation of investment and resourcing in surveillance is determined by an ‘ecosystem approach’ that values nature as much as it contributes to the needs or values of people. The Matauranga Maori Framework (for surveillance of for plant pathogens) informs research in the Biological Heritage, National Science Challenge in New Zealand that addresses the impact of plant pathogens, phytophthora agathidicida and austropuccinia psidii on native species. Both pathogens are destroying native species culturally significant to Maori. The Framework aims to enable better engagement of Hapu/Iwi (indigenous tribes) across central and local

government agencies, including the Ministry for Primary Industry (MPI), Department of Conservation (DOC), regional councils, stakeholders and communities engaged in a surveillance effort. Seeking to normalise 'cultural licence' and to inform systemic change, the Framework encourages early engagement with indigenous tribal authorities and knowledge holders in the development and design of research, and recognises the importance of indigenous peoples relationship with their lands and possessions.

Soil-borne plant viruses

C6.5-1

VIRUSES INTERACT WITH SOIL AND PLANT MICROBIOMES

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Text

Plants depend on the soil microbiome for mineralization of nutrients. Soil viruses outnumber the soil microbiome 10-1000-fold. Therefore, they can serve as a lineage-specific top-down control on the soil microbiome. While largely understudied, we know that soil viruses can affect soil carbon sequestration, the rhizosphere environment and plant health. Many soil viruses carry horizontally transferred genes that code for plant polysaccharide degradation or methanotrophy, which may resolve metabolic bottlenecks experienced by their microbial hosts during infection.

Advances in sequencing technology combined with methods for enrichment of viruses and tracing isotope flow through the microbial community allow us to probe the diversity and effects of viruses on their hosts over different stages in the life cycles of plants and soil. Recent work suggests that up to half of the mortality of soil bacteria in the week following wet-up is due to viral lysis. During the winter there is a positive correlation between virus activity and host activity, implying continued effect of viruses in wet soil. Presence of plant litter in soil during the growing season propels viruses of fungi. Finally, there is evidence for consistent presence of viruses in legume nodules, where they may control population size of nitrogen fixing symbionts.

Soil viral ecology is a nascent field highly relevant to plant health and carbon storage under global climate change

C6.5-2

NATURALLY OCCURRING VIRAL CHIMERIC RNA – A NOVEL CLASS OF FUNCTIONAL SUB-VIRAL AGENTS ASSOCIATED WITH VIRUS INFECTIONS IN PLANTS

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Text

Benyviruses have multipartite RNA genome. Several exoribonuclease-resistant RNAs (xrRNAs) are associated with benyvirus infections. Recently it has become evident that xrRNAs are produced by many viruses. Whereas xrRNAs contribute to pathogenicity of these viruses, the role of xrRNAs in virus infectious cycle remains elusive. We will present evidence that xrRNAs produced by a benyvirus are involved in formation of monocistronic coat protein-encoding chimeric RNAs (chRNAs). Naturally occurring chRNAs, we discovered, are composed of 5´-end of RNA 2 and 3'-end of either RNA 3 or RNA 4. Using computational tools and site-directed mutagenesis we addressed the mechanism chRNAs biogenesis. Moreover, knock-down of the expression of exoribonuclease-encoding gene *XRN4* inhibited biogenesis of both xrRNAs and chRNAs. We will present a model to explain the mechanism of the chRNAs biogenesis. This model integrates our findings and data into a broader picture on the role of intermolecular RNA-RNA base-pairing interactions of xrRNAs with genomic RNAs in template switching during virus replication cycles to yield formation of chRNAs. Furthermore, we found that chimeric RNAs are biologically active as the coat protein is translated from chRNAs and they move systemically in the infected plants. Our results suggest an additional level of organization and expression of viral RNA genomes (i.e. formation of functional chimeric RNAs) beyond classical genomic and sub-genomic RNA species.

C6.5-3

VIRUS-NEMATODE–PLANT INTERACTIONS DURING TRANSMISSION OF GRAPEVINE FANLEAF VIRUS

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Text

Many animal and plant viruses rely on vectors for their transmission from host to host. Grapevine fanleaf virus (GFLV) is a picorna-like virus from plants responsible of a severe grapevine degeneration observed in vineyards worldwide. GFLV is specifically transmitted from grape to grape by the ectoparasitic nematode Xiphinema index. Structurally, GFLV is an icosahedral virus of 30 nm of diameter with a pseudo T = 3 symmetry composed of 60 identical coat protein subunits (CP) of 54 kDa. The capsid of GFLV, carries the determinants of this specificity.

Over the past years, structure-function studies have provided important insight in the specificity of transmission. We could show that a GFLV strain poorly transmissible by nematodes possess a single Gly297Asp mutation responsible of the defect in transmission. This mutation maps to an exposed loop at the outer surface of the capsid that does not affect the conformation of the assembled capsid, nor of individual CP molecules. The loop is part of a positively charged pocket with essential function in GFLV transmission by X. index which is also mutated in escape variants isolated from plants that express a Nanobody specifically

recognizing GFLV that are naturally resistant to the virus. Our data suggest that perturbation of the electrostatic landscape of this pocket affects the interaction of the virion with specific receptors of the nematode's feeding apparatus, and thereby severely diminishes its transmission efficiency.

C6.5-4

SOIL-BORNE MULTIPARTITE BNYVV DIFFERENTIALLY CONTROLS ITS SEGMENT COPY NUMBER

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Text

Multipartite virus genomes are composed of two or more segments each packaged into independent viral particles. A potential benefit for multipartitism is the regulation of gene expression through segment copy number variations. The soil-borne beet necrotic yellow vein virus (BNYVV) containing four or five genomic RNAs may be considered as a model of multipartitism among RNA viruses. Here, we investigate the relative frequency of the four genomic RNAs of BNYVV during infection of different host plants by a validated protocol of dual step reverse transcriptase droplet digital PCR. We show that some viral genomic segments accumulate at low frequency, whereas others dominate. BNYVV genome formula reaches a different setpoint in distinct host plant species and organs. Moreover, the vector, Polymyxa betae, seems to affect the accumulation of BNYVV genomic RNAs in its hosts. We characterized as well the BNYVV genome formula within zoospores and resting spores of P. betae taking into account contamination issued from infected Beta vulgaris roots. Results show the virus reaches a dedicated set-point genome formula also in each of the two forms of the protozoan life cycle. Hence, our results indicate that BNYVV can differentially control the copy number of its segments.

C6.5-5

TOWARDS A BETTER UNDERSTANDING OF FUROVIRUS-HOST INTERACTION – IDENTIFICATION OF FACTORS INFLUENCING INFECTION

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Text

The *Polymyxa graminis*-transmitted viruses can cause extensive losses in cereal crops. We are studying the interaction between virus and host plant with the aim to better understand the molecular mechanisms underlying infection, to better characterize the threat virus infection poses in a changing climate, and to derive resistance strategies complementary to resistance breeding approaches.

To obtain insight into the mechanisms underlying virus intercellular transport and transmission, we identify and characterize interaction partners of furovirus CP-RT and movement proteins using protein biochemical and cell biological methods. We obtained evidence that the endoplasmic reticulum may be important for virus transmission and identified a pentatricopeptide repeat-containing protein and a heat shock protein 70 as interacting partners of the Japanese soil-borne wheat mosaic virus (JSBWMV) movement protein.
By investigating furovirus infection under specific environmental conditions, we identify, which parameters specifically influence infection and the stability of resistance. These parameters can then be used to derive strategies for virus control and thus contribute to a sustainable and environment-adapted agriculture.

C6.5-6

ADAPTION OF BNYVV TOWARDS RZ1 RESISTANCE IN SUGAR BEET

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Text
The beet necrotic yellow vein virus (BNYVV) causes rhizomania in sugar beet, which is controlled since more than two decades by the Rz1 resistance gene. The development of resistance-breaking strains has been favoured by a high selection pressure on the populations. Resistance-breaking is associated with mutations at amino acid positions 67-70 in the pathogenicity factor P25 and the presence of an additional RNA component (RNA5). However, natural BNYVV populations are highly diverse with more than 25 known tetrad variants and different RNA5 species making investigations on the resistance-breaking mechanism rather difficult. We refined our previously developed reverse genetic system for BNYVV (A type) to study the Rz1 resistance-breaking mechanism by agroinoculation of seedlings. This allowed a clear discrimination between genotypes. A screen for resistance-breaking revealed that multiple tetrad variants allow BNYVV to overcome Rz1. Furthermore, certain mutations impaired the viral pathogenicity in the susceptible genotype. Finally, the supplementation of an additional RNA5 species, either from the J or P type group, allowed virus accumulation in the Rz1 genotype independent of the P25 tetrad. However, this effect was impaired in a genotype carrying Rz2, which is another resistance gene against BNYVV. Our infection system enables a rapid identification resistance-breaking mutations and highlights the plasticity of the BNYVV genome allowing adaption towards Rz1.

P6.5-001

VIRUSES TRANSMITTED TO RICE BY POLYMYXA GRAMINIS : A TRIPARTITE INTERACTION

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Text

The rice stripe necrosis virus (RSNV, *Benyvirus*) is a bipartite virus, present in West Africa and emerging in South America. It is transmitted to rice by a soil protist, obligate endoparasite of rice roots, *Polymyxa graminis* (*Pg*). The diversity, biology and specificity of this tripartite interaction are poorly known, and no effective means of control are available to date. Our objective is to improve knowledge on the infection process of rice by RNSV. To better control the inoculation, we are developing cDNA infectious clones by homologous recombination in yeast and fusion PCR. Introns are inserted to prevent the toxicity of the Triple Gene Block proteins previously observed for another benyvirus, namely BNYVV. Different inoculation methods of the RSNV clones will be tested in controlled conditions, and compared to *Pg*-mediated inoculation. The symptomatology, multiplication rate and viral movement will be monitored in plants. The specificity of the rice/*Pg*/RSNV interaction will be evaluated using different lineages of both the vector and the virus. Based on the methodologies developed, we will characterize the single resistance source identified so far and screen for others in representative accessions of rice. The results obtained here will contribute to the development of tools for the study of the tripartite interaction as well as the identification of efficient and durable control methods.

P6.5-002

DIVERSITY OF POLYMYXA GRAMINIS ASSOCIATED WITH RICE STRIPE NECROSIS VIRUS INFESTED SOILS, AND HOST RANGE OF POLYMYXA GRAMINIS F. SP COLOMBIANA

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Text

The plasmodiophorid Polymyxa graminis, an obligate endoparasite, transmits several phytoviruses on major crops worldwide. In this study, the diversity of P. graminis associated with Rice stripe necrosis virus (RSNV) infested areas and the host range of a monosporosorus isolate of P. graminis f. sp. colombiana were assessed. Four soil samples from Burkina Faso and Mali, collected in sites where RSNV was detected, have been selected. The diversity of Polymyxa associated with these soils was tested using a set of plant species identified as natural hosts of Polymyxa. In this study, P. graminis was detected in roots of rice, wheat and sorghum grown on the four soils and in roots of barley and millet on two of the four soils. Molecular characterization revealed the effective presence of several formae speciales associated with P. graminis f. sp. tropicalis, P. graminis f. sp. colombiana on rice or with another group associated with the ribotype associated with the isolate BF209 (Legrève et al. 2002)[CB1] .The host range of P. graminis f. sp. colombiana, revealed that these isolates can infect other cereal species grown on P. glaucum, S. bicolor as well as some weed species which are D. horizontalis and A. viridis.

P6.5-003

SOIL TRIPARTITE INTERACTIONS BETWEEN HOST PLANTS, VIRUSES AND THE PROTIST VECTORS POLYMYXA

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Text

Viral diseases transmitted by Polymyxa involve complex soil-borne pathosystems involving three actors: a plant host, a root obligate endoparasitic protist vector (Polymyxa spp.) and viruses. These pathosystems are spread worldwide, in both temperate and tropical areas. They affect numerous host plants (both monocotyledons and dicotyledons) due to the large diversity of the Polymyxa-virus pathosystems. Our research aims to assess how the host plant, Polymyxa and the viruses they vector interact, mainly focusing on two Polymyxa-virus models for which genomic data are available : (1) the Polymyxa betae – Beet necrotic yellow vein virus (BNYVV) pathosystem on sugar beets and (2) the Polymyxa graminis – Rice stripe necrosis virus (RSNV) pathosystem on rice. Recent genomic and transcriptomic data regarding the vector Polymyxa open new opportunities to understand the nature and specialization of the Polymyxa-host interactions. It allows a better understanding of how Polymyxa manage to by-pass host defenses and paves the way for identifying effectors involved in host defenses manipulation. Additionally, the first transcriptomic data of in vivo transmission of virus by Polymyxa clarify how the virus impacts plant defenses and therefore Polymyxa-host interactions. These transcriptomic results also give insight on the specificity Polymyxa-virus interactions.

P6.5-004

POTATO MOP-TOP VIRUS SHAPES A DEFENCE-RELATED TRANSCRIOPTOME THROUGH SUPPRESSION OF CHLOROPLAST-MEDIATED IMMUNITY GENES

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Text

Potato is major non-cereal food crop. Potato mop-top virus (PMTV), the causal agent of ´potato spraing´ disease, severely deteriorates the quality of potato tubers. PMTV is transmitted by spores of a soil-borne protist Spongospora subterranea. Efficient approach for controlling the disease is not available to date. Therefore, new genetic information on disease development is crucial to obtain disease resistant varieties. To reveal the novel genetic regulators of viral defense, we performed an RNA-seq of PMTV infected Nicotiana benthamiana plants. Altogether, 2291 host genes were found to be differentially regulated after PMTV infection. Photosynthesis, enzymatic activity, cell wall organization, and cell-to-cell communication were found to be the major affected gene ontology categories. Interestingly, chloroplast localized protein encoding genes were especially affected. In total,

258 choloroplastic genes were differentially regulated where 145 were downregulated. These suggest a putative viral strategy to suppress the chloroplast-mediated defense to gain advantage in replication and movement. Localization of PMTV RNA and a movement protein to chloroplasts further reinforces the hypothesis Thus, RNA-seq data has provided insight into chloroplast defence genes exploited by PMTV to accelerate systemic infection. Further characterization of these candidate genes can be useful for genetic intervention to develop strategies for sustainable control of PMTV and will be discussed.

Synergism/antagonism between microbial pathogens and disease complexes: implications in epidemiology and management

C9.6-1

TOMATO PITH NECROSIS: A BACTERIAL DISEASE COMPLEX CAUSED BY ENDOPHYTIC PSEUDOMONADS

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Text

The spread of tomato pith necrosis (TPN) in the late 1970s led to the description of a new bacterial species, *Pseudomonas corrugata*, later demonstrated to include strains of another species, *P. mediterranea*. The diagnosis by duplex PCR has made it possible to detect the presence, in some cases, of multiple infections of the two bacterial species. In the past 50 years many pseudomonads have been associated with this syndrome. Also called FPTPN (fluorescent pseudomonads associated with TPN) some of them have been attributed to new genomospecies or identified as *P. chicorii*, *P.marginalis*, *P. viridiflava*, alone or in mixed infections even with *P. corrugata* e *P. mediterranea*. In the latter two species a role in the development of TPN is due to the production of the cyclic lipopeptides (CLP) cormicin A and corpeptins. Genome sequencing of species representatives and transcriptomic analysis have helped to improve the knowledge of CLP production. Regulation mediated by the Quorum Sensing system of CLPs suggests that there are conditions in which bacterial concentration exceeds the quorum leading to the development of symptoms. Bacteria of the genus *Pseudomonas* are among the major constituents of the tomato microbiota's endorhizosphere. The presence and interaction of endophytes, also of species not yet described, could support the hypothesis that endophytes, that are potentially harmless, interact so contributing to the development of the pathobiome which leads to conduct to TPN.

C9.6-2

TWO ARE WORSE THAN ONE, THE CONSEQUENCES OF CO-INFECTION WITH A VIRAL/OOMYCETE COMPLEX CAUSED BY CUCUMBER GREEN MOTTLE MOSAIC VIRUS AND PYTHIUM SPECIES UNDER DIFFERENT ENVIRONMENTAL REGIMES.

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Text

The disease triangle is usually focused on one host/one pathogen model. However, pathosystems can also involve co-infection by several pathogen species. Co-infection may exhibit disease symptoms dissimilar to infections by each pathogen alone and may increase the damage to the host. The current case study describes the synergistic effect of co-infection by the C*ucumber green mottle mosaic virus* (CGMMV) and *Pythium* spp. The late-wilting syndrome has lately increased in cucumber greenhouses during CGMMV outbreaks. As wilting appears in patches and is accompanied by root rot, we hypothesized that the co-infection of soil-borne pathogen/s and CGMMV causes this phenomenon. The Greenhouses survey showed that 69% of the wilting plants were colonized simultaneously by *Pythium* spp. and CGMMV, whereas only 20 and 6.6% of the wilting plants were colonized only with Pythium spp. or CGMMV, respectively. Artificial inoculation of cucumber plants showed that co-infection with *P. spinosum* or *P. aphanidermatum* and CGMMV resulted in a significant synergistic wilting effect and reduced growth parameters. Moreover, the synergistic effect was detected under a wide range of (optimal and suboptimal) temperatures, and P*. spinosum*, which mostly prevails in mild temperatures, caused high mortality incidence at an extended temperature range as high as 32°C. The extended damage was accompanied by a sharp downregulation of genes related to the host defense mechanism against the necrotrophic pathogen.

C9.6-3

LIFE HISTORY TRAITS, COINFECTION AND EPIDEMIOLOGICAL DYNAMICS IN A PARASITIC COMPLEX: THE CASE OF ASCOCHYTA BLIGHT OF PEA

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Text

Any host plant is subjected, throughout its life, to the simultaneous or sequential pressure of infection by various pathogens, whose coexistence leads to competitive interactions able to affect epidemiological dynamics. The main objective of this work is to assess the impact of

the coexistence of Peyronellaea (ex Didymella) pinodes (Dp) and Phoma medicaginis var. pinodella (Pmp), two key agents of pea Ascochyta blight, on their epidemic development. Our results suggest that the coexistence of both fungal species derives from three distinct life history and host exploitation strategies. Most Dp strains are 'pioneer colonizers', allowing them to develop first on young hosts. These early attacks weaken the plant, and favor later infection by Pmp and rare Dp strains boasting a 'scavenger' strategy on vulnerable host tissue. The remaining Pmp and Dp strains display an 'intermediate' strategy, and act as opportunists. We also showed that both direct and indirect competition alter these life strategies. However, for each type of competition, host exploitation strategies vary in the same direction irrespective of the kind of competitor, although the intensity of the variation depends on the genetic relatedness between competitors. We therefore show the strong impact of competitive interactions on coexistence and virulence, so that the management of epidemics would benefit from a full understanding and use of underlying mechanisms.

C9.6-4

INTERACTIONS OF TWO PHLOEM LIMITED VIRUSES IN THEIR HOST PLANT AND VECTOR AND IMPLICATIONS FOR VECTOR FITNESS AND EPIDEMICS

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Text

Mixed-virus infection is common in agricultural settings. Viruses in mixed-infection can synergistically/antagonistically interact, and differentially alter host phenotype and vector fitness than individual viruses. Mixed-virus infection in a vegetable pathosystem in the United States has two facets: 1. Mixed-infection in hosts due to multiple viruses transmitted by the same vector, whitefly *Bemsia tabaci* Gennadius; and 2. Acquisition of multiple viruses from multiple hosts by the vector. Facet 1: Begomovirus (cucurbit leaf crumple virus, CuLCrV) and Crinivirus (cucurbit yellow stunting disorder virus, CYSDV) interactions in squash and on *B. tabaci* fitness was assessed. Mixed-infection of CuLCrV and CYSDV in squash resulted in an antagonistic interaction. This interaction differentially affected virus acquisition by whiteflies and settling than single (CuLCrV or CYSDV) infection. Facet 2: Combined acquisition of tomato infecting -tomato yellow leaf curl virus(TYLCV) and squash-infecting CuLCrV by whiteflies was examined. Combined whitefly acquisition of CuLCRV and TYLCV enhanced settling towards non-infected tomato and squash plants. Viruliferous (CuLCrV and/or TYLCV) whitefly fitness study conducted on a virus non-host (cotton) revealed that the mere presence of virus in the vector influenced its fecundity positively. Overall, mixed-virus infection in hosts and acquisition of multiple viruses by the vector could potentially exacerbate epidemics than a single virus.

C9.6-5

SYNERGISM BETWEEN VIRUSES IN THE WHEAT STREAK MOSAIC DISEASE COMPLEX IN WHEAT

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Text

The wheat streak mosaic disease complex causes significant yield losses in the Great Plains of the US. It consists of three wheat curl mite (Aceria tosichella Keifer)-vectored viruses: Wheat streak mosaic virus (WSMV), Triticum mosaic virus (TriMV), and High Plains wheat mosaic virus (HPWMoV). The majority (91%) of TriMV-positive wheat samples from field surveys were co-infected with WSMV whereas WSMV and HPWMoV were mainly (60-80%) detected singly. Double inoculation of a susceptible winter wheat cultivar with WSMV+TriMV in the field exacerbated symptoms and reduced yield by >87% compared to single virus infections. Double inoculation of susceptible winter wheat seedlings with WSMV+TriMV induced disease synergism that resulted in severe stunting, leaf deformation, and bleaching. In co-infected plants, accumulation of both viruses increased by up to 7.4 fold compared to single infections at 14 days post-inoculation. In TriMV-infected wheat seedlings, WSMV showed accelerated long-distance movement with increased accumulation of genomic RNAs compared to buffer-inoculated wheat, which indicated that TriMV-encoded proteins complemented WSMV for efficient systemic infection. In WSMV-infected wheat seedlings, TriMV exhibited delayed systemic infection, but in the late stages of infection, it accumulated rapidly with accelerated long-distance movement compared to buffer-inoculated wheat, indicating asymmetrical interactions between synergistically interacting WSMV and TriMV.

C9.6-6

SYNERGISTIC RELATIONSHIP OF HARZIA IXTARENSIS WITH COLLETOTRICHUM FRAGARIAE CAUSING ANTHRACNOSE ON ANNONA CHERIMOLA FRUIT IN MÉXICO

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Text

Since 2005 in Íxtaro, Michoacán, symptoms of *Harzia ixtarensis* **sp. nov.** infection have been observed on immature *Annona cherimola* fruit with *Colletotrichum fragariae*-induced anthracnose lesions and mummified fruit. This study aimed to evaluate *H. ixtarensis* pathogenicity and its synergistic behavior with *C. fragariae*. During 2019 and 2020 in an *A. cherimola* tree, two randomized experiments were conducted on previously disinfected

healthy immature fruit and on fruit with 14-day-old anthracnose lesions caused by prior *C. fragariae* inoculations. The conidia inoculations treatments (T) plus a control were: T1, *C. fragariae*; T2, *H. ixtarensis*; T3, *C. fragariae* plus *H. ixtarensis* (simultaneous inoculation); and T4 (derived from T1), *H. ixtarensis* inoculation on fruit with anthracnose lesions. The inoculum (20 µL drop of a conidial suspension from 7-day-old cultures containing a mean of 1×10^6 mL^{-1}) was deposited on the fruit surface (with and without a wound). There were significative differences. In situ, inoculations showed that fruit with wounds developed larger lesions than those without wounds. *H. ixtaresis* inoculation on anthracnose lesions produced larger anthracnose lesions than *C. fragariae* alone. When *C. fragariae* or *H. ixtarensis* was inoculated alone, the lesion size was 51 and 99% smaller, respectively, indicating synergy between these species. Thus, *H. ixtarensis* may have a parasitic-synergistic and necrotrophic lifestyle and exhibited symptoms on anthracnose lesions.

P9.6-001

HOW MULTIPLE INFECTIONS AFFECT THE DYNAMICS OF RICE PATHOGEN POPULATIONS IN BURKINA FASO?

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Text

Pathogens actually share host plants with a myriad of other microbes, some of them being pathogenic. Multiple infections, or ‘co-infection’, occur when a single host plant is infected by various pathogen species, or genotypes. This is known to affect symptom expression and pathogen multiplication in various pathosystems. However, the population-scale consequences remain poorly explored. We consequently aimed at integrating each pathogen in the microbial community of its host, with a particular focus on evolution and epidemiology. Our focus is on rice in Sub-Saharan Africa, a crop of strong agronomic importance because of human population growth and change in food habits, and two major rice pathogens: the Rice yellow mottle virus (RYMV) and the bacteria Xanthomonas oryzae (Xo). These two pathogens were shown to interact reciprocally in experimental co-infection. We monitored these two diseases over an eight-years period in 2-6 sites from western Burkina Faso. We aimed at testing whether multiple infections impact the evolutionary trajectories of pathogen populations and drive epidemiological outcome. We show that the viral population evolved over the period, in terms of genetic and pathogenic diversity. Levels of virus-bacteria co-occurrence and co-infection were estimated in the fields. Evidence of intra-species co-infection is shown as well, both for the virus (multiple viral isolates) and the bacteria (various bacterial strains).

The ecology plant viruses and epidemiology of the disease they

cause: How fundamental ecological research in natural systems can inform and advance plant pathology

C4.4-1

FROM BOOTS ON THE GROUND TO NUCLEOTIDES IN THE SEQUENCER: ADVANCES IN THE STUDY OF PLANT VIRUS ECOLOGY USING PLANT VIRUS METAGENOMICS

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Text

Plant virus ecology began to be explored at the end of the 19th century. Since then, major advances have revealed complex virus–host–vector interactions in a variety of environments. These advances have been accelerated by development of new technologies for virus detection and characterization, the latest of which being high-throughput sequencing (HTS). HTS technologies have proved to be effective for non-targeted characterization of all or nearly all viruses present in a sample without requiring prior information about virus identity, as would be needed for virus-targeted tests. Plant virus metagenomics studies have thus made important advances, including characterization of the complex interactions between phytovirus dynamics and the structure of multi-species host communities, and documentation of the effects of anthropogenic ecosystem simplification on plant virus emergence and diversity. However, such studies must overcome challenges at every stage, from plant sampling to bioinformatics analysis. Results of two recent studies will be presented. While the first study aimed at systematically evaluate plant-associated viromes across broad agro-ecological interfaces, the second study aimed at using a predator-enabled metagenomics strategy to sample the virome of a remote and difficult to access densely forested African tropical region.

C4.4-2

ALTERATION OF PLANT SPECIES MIXTURES BY VIRUS INFECTION

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Text
What occurs when virus infection is spreading within a mixed plant species population? This question is important not only for environmentally significant mixed wild species populations but also for economically significant, mixed species managed systems. This contribution re-interprets examples of past research on mixed species managed pasture done over two decades on three continents that demonstrated plant species balance changes arising from virus infection. These examples showed that plants belonging to susceptible pasture cultivars sensitive to systemic virus infection are sufficiently weakened that their ability to withstand competition from non-host plants of other pasture species, or weed species, was decreased sufficiently to alter the plant species balance. Also, a similar alteration occurred when they were competing with virus-resistant or virus-tolerant host plants of the same or other pasture species, or a virus-resistant weed species. Such competition also diminished seed production, which decreased their ability to regenerate. Notably, when two different pasture species infected by the same virus compete with each other, growth of the more sensitive species is suppressed. Because managed mixed species pastures constitute an important component of regenerative agriculture, retaining an optimal balance of pasture species and delaying pasture decline from weed invasion both require effective management of virus diseases.

C4.4-3

VIROME RELEASE OF AN INVASIVE EXOTIC PLANT SPECIES IN SOUTHERN FRANCE

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Text
The increase in human-mediated introduction of plant species to new regions due to global travel and globalization has resulted in a rise of invasive exotic plants (IEPs) that can have significant effects on biodiversity, ecosystem processes, and food production. The introduction of IEPs to new regions often occurs through seed dispersal, and most pathogens

are not vertically transmitted, leading to low viral loads in these plants. Also, most pathogens are not evenly distributed across the Earth, meaning that IEPs colonizing a new territory are unlikely to encounter pathogens from their native range. This situation, referred to as the "enemy-release hypothesis" suggests that decreased pathogen-mediated selective pressures on IEPs in colonized territories will result in increased IEP populations, densities, and geographical distributions. To test the enemy-release hypothesis, the virome of an invasive cane bluestem (Bothriochloa barbinodis) was compared to that of four or more indigenous grass species in both naturalized and native ranges. The results showed that the IEPs had lower viral infection loads than the native grasses, providing evidence that supports the enemy release hypothesis. Novel viruses associated with Bothriochloa barbinodis were further partially or fully sequenced and the phylogenetic relationships of these viral sequences and representative sequences of corresponding virus families were analyzed.

C4.4-4

OCCURRENCE AND PREVALENCE OF SCHLUMBERGERA VIRUS X IN DRAGON FRUIT CROPS IN ECUADOR

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Text

Dragon fruit is a unique and highly sought-after fruit grown in many parts of the world, including Ecuador. In recent years, its cultivation has grown in popularity among farmers, as it is a highly profitable crop due to its domestic and international demand. Despite the growth in dragon fruit production, Ecuador faces challenges such as emerging pests and diseases and the lack of knowledge on their management and prevention. Emerging viruses are responsible for reductions in yields in many crops if they are not prevented and adequately managed. Cladodes exhibiting symptoms of irregular and ring-shaped chlorotic spots and mild chlorotic yellow spots were collected from farms in three coastal provinces and the main production area in the Amazon region of Ecuador. Schlumbergera virus X (SchVX), a potexvirus, was detected in more than 90% of tested cladodes, including symptomatic and asymptomatic samples of the two major dragon fruit species (Hylocereus undatus and H. megalantus) cultivated in the country. The symptoms presented differed among the species and were more severe on H. undatus. Phylogenetic inferences based on the partial nucleotide sequence of the RNA-dependent-RNA-polymerase (RdRp) showed that SchVX isolates found in H. undatus and H. megalantus, regardless of the field location, share a most recent ancestor with isolates from Spain and Portugal. Additional assays on the mechanical transmission of the virus are underway and will be further discussed.

C4.4-5

FACTORS INFLUENCING EPIDEMIOLOGY AND SPREAD OF WHITEFLY-TRANSMITTED CUCURBIT VIRUSES IN THE UNITED STATES VARY AMONG PRODUCTION REGIONS

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Text
Viruses transmitted by the whitefly, *Bemisia tabaci*, threaten cucurbit production in the United States (US) and throughout the world, reducing yield and fruit quality. This threat increases with elevated whitefly populations, prevalence of mixed infections, and the introduction of new viruses. Large whitefly populations occur in summer and fall cucurbit production throughout much of the southwestern and southeastern US, including in areas that once had limited whitefly pressure. Recent surveys conducted in these regions demonstrated that mixed virus infections, which create challenges for disease management and plant breeding, are now common. These mixed infections often include the criniviruses, cucurbit yellow stunting disorder virus (CYSDV) and cucurbit chlorotic yellows virus (CCYV), and the begomovirus, cucurbit leaf crumple virus (CuLCrV) along with other viruses. Epidemiological research to clarify factors driving the perennial threat these viruses pose to production, including identification of weed and non-cucurbit reservoir hosts, impact of timing of infection, and competitiveness of viruses in mixed infections, varies by region. This research is highly dependent on multiplex RT-PCR assays to identify mixed infections. Additional efforts by the Emerging Viruses in Cucurbits Working Group (ecucurbitviruses.org), established in 2022, are underway to improve communication regarding virus threats throughout the industry with the goal of reducing virus spread and impact.

C4.4-6

EPIDEMIOLOGY OF YAM VIRUSES IN GUADELOUPE: ROLE OF CROPPING PRACTICES AND SEED-TUBER SUPPLY

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Text
Among the 25 viruses recognized officially in yams (*Dioscorea* spp.) worldwide, nine have been reported in yams in Guadeloupe. Since the epidemiology of these viruses remains largely unexplored, we undertook a large-scale epidemiological study of yam viruses in Guadeloupe based on the analysis of 1124 leaf samples collected from yams and weeds. We assessed the prevalence of cucumber mosaic virus (CMV), Cordyline virus 1 (CoV1), Dioscorea mosaic associated virus (DMaV), yam asymptomatic virus 1 (YaV1), yam mosaic virus (YMV), yam mild mosaic virus (YMMV), badnaviruses, macluraviruses and potexviruses, and evaluated the effects of key epidemiological drivers of these viruses. We identified several weed reservoirs of YMMV and provide evidence that YMMV isolates infecting weeds cluster together with those infecting yams, pointing to the role of weeds in the epidemiology of YMMV. We report on the occurrence of yam chlorotic necrosis virus (YCNV) in Guadeloupe, the introduction of YMMV isolates through the importation of yam tubers, and the absence of vertical transmission of YaV1. We identified specific effects of some cropping practices, such as weed management and the use of chemical pesticides, on the occurrence of several yam viruses, but no crop-related factor had a strong or general

effect on the overall epidemiology of the targeted viruses. Overall, our work provides insights into the epidemiology of yam viruses that will help design more efficient control strategies.

F4.4-1

STRIVING TO STAY CLEAN: DETECTION OF SWEETPOTATO VIRUSES ON MULTIPLE SEED GENERATIONS IN NORTH CAROLINA

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Text

Under the National Clean Plant Network (NCPN) economic study, the sweetpotato clean centers started an experiment aiming to assess the value of clean seed in comparison to older generation seed. The goal of this study was to evaluate the performance and quality of foundation seed after it had been integrated into commercial sweetpotato operations. In NC, trials started in 2021 with Covington and Beauregard as evaluated varieties. G1 seed was used as a reference to compare the yield and virus incidence of growers' generation 2 (G2), generation 3 (G3) and generation 4 (G4) seed roots. This experiment was repeated in 2022 with Averre and Bayou Belle added to the initial pool of varieties as well as older generations (G5 and G6). It is known that the accumulation and perpetuation of viruses in sweetpotato is a major constraint for production of seed and the commercial crop. The potyvirus complex is prevalent in North Carolina and comprises Sweet potato feathery mottle virus (SPFMV), Sweet potato virus G (SPVG), Sweet potato virus C (SPVC) and Sweet potato virus 2 (SPV2). In 2021, virus data suggested a low incidence of viruses (mainly SPFMV) on G1 material. Potyviruses (mainly SPVG, SPVC and SPFMV) started to be prevalent on G2 and G3 material. In the older generation evaluated (G4), all potyviruses (SPVG, SPVC, SPFMV and SPV2) were detected. In 2022, the same trend was observed as the prevalence of four potyviruses was associated to higher seed generations.

P4.4-001

CHARACTERIZATION OF A NOVEL ORTHOTOSPOVIRUS FROM MACADAMIA IN SOUTH AFRICA

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Text

South Africa is the largest producer of macadamias in the world. In order to ensure the continuation of the industry, it is important that research focuses on the control and eradication of economically-important pests and diseases. Macadamia trees in Mpumalanga show symptoms of severe chlorosis. This chlorosis coincides with a significant drop in production, with losses of up to 60% being recorded. In an attempt to determine whether the chlorosis may

be associated with a virus, high throughput sequencing analyses was performed on RNA extracted from both diseased and healthy trees collected from six different farms. Subsequent data analyses could not find a specific virus being linked to chlorotic trees, however, reads spanning the full genome of a novel virus belonging to the Orthotospovirus genus were obtained from a number of samples. A RT-PCR assay was optimised for the detection of this virus and subsequent surveys linked the virus to ringspot symptoms which are commonly observed on different macadamia cultivars. The virus has been identified from orchards in three provinces. Viruses described in the genus are known to cause severe crop losses. It is therefore important that the virus, provisionally named macadamia ringspot associated virus (MRSV), be further studied to determine whether association with this virus can lead to yield losses, as well as determine the vector of the virus so that control strategies can be implemented to limit the spread of MRSV.

P4.4-002

INCIDENCE AND OCCURRENCE PATTERN ON KIWIFRUITS IN KOREA

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Text

The occurrence of viruses in Kiwifruits in Jeju Island were investigated between the period of 2020 and 2022. The result showed that 3.3 and 6.7% of trees in Jeju Island were infected with AcVA and ASbLV, respectively. Viruses that specifically infect kiwifruit are mostly from Betaflexiviridae family, such as Actinidia virus A, Actinidia virus B, and the Actinidia seedborne latent virus. Infected kiwifruits usually show bright leaf vein, chlorotic ringspots, and spot. However, the symtoms tend to not be detected during the season of vitality restoration, even when infected. Other kiwifruits viruses include cucmber mosaic virus, apple stem grooving virus, potato virus X, cucumber necrosis virus, alfalfa mosaic virus, and citrus tatter leaf virus. But, It does not occur in Jeju Island. Incidence of major viruses varies from year to year, with most cases occurring in the form of multiple. It was found that the damage to kiwifruits in Jeju Lsland caused by the virus was not significant. However, there is a possibility of damage occurring when new varieties are introduced or developed in the future. To prevent fruit damages, continuous monitoring for fruit infecting viruses and viroids will be necessary.

P4.4-003

FIRST VIRUSES INFECTING COCKSPUR CORAL TREE (ERYTHRINA CRISTA-GALLI L.): DISCOVERY OF A NOVEL CAPILLOVIRUS AND A NEW HOST FOR THE PRUNE DWARF VIRUS

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Text

Erythrina crista-galli L. (*Fabaceae*) or cockspur coral tree is a South American deciduous woody ornamental spread worldwide. One out of three *E. crista-galli* plants from the collection of the Botanical Garden in Zagreb exhibited virus-like symptoms such as stunting, white striations of flowers, leaf asymmetry, bleaching, white speckles and striations. Total nucleic acids were extracted from leaves by CTAB method, DNase treated and subjected to HTS (Illumina, 2x150 nt). Total of 4.2 million reads obtained from ribodepleted RNA-seq were analysed by Geneious Prime software and VirHunter. Complete sequence (2129 nt) of prune dwarf virus (PDV, *Ilarvirus, Bromoviridae*) segment RNA3, encompassing CP and MP genes, was obtained (GenBank Acc.No. OP503942). Moreover, almost complete genome (acc. no. OQ067396) was obtained for an unknown virus (*Betaflexiviridae*) spanning 6,483 nts and containing all expected ORFs. Phylogenetic analyses revealed the maximum nucleotide identity was 41% with ASGV (sequence LC143387), far below the threshold for species delimitation making it a candidate for a new *Capillovirus* genus member. Besides PDV, only this new virus was found and validated by RT-PCR in symptomatic *E. crista-galli*, an unexpected fabaceous host with no viruses recorded so far. As asymptomatic plants were not positive for the two viruses in RT-PCR, this makes them “prime suspects” as the disease etiological agents.

P4.4-004

EFFECTS OF TEN SERIAL PASSAGES OF TOMATO SEVERE RUGOSE VIRUS (TOSRV) BY DIFFERENT HOSTS ON THE EVOLUTIONARY DYNAMICS OF THE VIRAL POPULATION

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Text

Tomato severe rugose virus (ToSRV) is the most important begomovirus in Brazilian tomato crops. Many weeds and other solanaceous are associated with tomatoes, and some are hosts of begomoviruses. Here we tried to access the population dynamics of ToSRV after ten serial passages throughout different hosts. The ToSRV-TF isolate, initially maintained on tomato, was transmitted by *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) for ten successive generations to tomato (control), soybean, and *Nicandra physalodes* plants. The ToSRV-TF isolate could infect the three hosts but with different efficiency. There was a higher infection rate in tomato plants compared to other hosts. There was genetic differentiation in the ToSRV population obtained from the different hosts. The seventh serial passage showed genetic differentiation in the viral population from *N. physalodes* plants. This viral subpopulation is possibly better adapted to the host because the virus’s infection rate increased in these plants. In tomatoes, the infection rate was nearly 100% in all passages. The variation in this viral population was constant, and despite this, two separate lineages emerged. Furthermore, the viral population's “disappearance” scenario was observed in soybean plants during serial passages, but the level of diversity remained constant. Therefore, there are differences in the evolutionary dynamics of the ToSRV population over generations in different hosts.

P4.4-005

A GLIMPSE INTO THE GERMAN HOP VIROME

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Text
In summer 2019, citrus bark cracking viroid (CBCVd) was reported for the first time in Germany. CBCVd is a pathogen of citrus plants that causes mild and often tolerated infections of different citrus species, whereas it causes severe disease in hop plants. The project “HopfenViroid” is addressing practical and scientific questions regarding CBCVd. As a part of this project, high-throughput sequencing (HTS) is being applied to investigate the viro-diversity in different German hop-growing sites. In 2021, we started with a pilot study targeting three fields in Hallertau (southern Germany), where CBCVd was previously detected. The samples were collected from hop, non-hop inside the field, and non-hop outside the field. Samples were pooled, double-stranded RNAs were extracted as a viral and viroid enrichment approach, and followed by Illumina sequencing. The bioinformatic analysis showed that all identified viruses and viroids in hops across the three fields were previously described as hop pathogens. In 2022, this study was extended to cover three different hop-growing sites in Germany. The same sampling and pooling strategies were used in 2022. The HTS-data analysis revealed common hop viruses and a viroid infecting the German hops. CBCVd was identified in hops in one site. A non-hop virus was identified in hops in three fields across the targeted sites. This study is still ongoing in 2023 to get a comprehensive understanding of the viro-diversity in German hops.

P4.4-006

SURVEYS FOR THE RESISTANCE-BREAKING ORTHOTOSPOVIRUSES IN TOMATO FIELDS IN FLORIDA

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Text
Members of the genus Orthotospovirus such as tomato spotted wilt virus (TSWV) and tomato chlorotic spot virus (TCSV), are thrips-transmitted and highly destructive tomato viruses. The *Sw-5* gene is a single dominant resistance gene for TSWV and is also effective for TCSV; thus, the use of Sw-5 tomatoes is a critical IPM component. Amino acid (aa) mutations in the TSWV nonstructural movement protein (NSm) result in *Sw-5* resistance-breaking. In 2019-2023, high incidences of orthotospovirus-like symptoms were observed in Sw-5 tomato fields in south Florida. Symptomatic field samples from resistant and susceptible tomato plants were collected, and the *Sw-5* gene and orthotospovirus infection in all samples was verified by PCR and RT-PCR assays, respectively. Sequences of NSm from 131 Florida orthotospovirus isolates were analyzed for novel and known resistance-breaking aa substitutions (C118Y/T120N/D122G). The known aa substitutions were not present in any Florida isolates. However, novel aa substitutions (D17E, C118S, and I135V) in NSm were observed predominantly in TCSV isolates from resistant tomatoes. Furthermore, infection by TCSV^{C118S} isolate was systemic in an Sw-5-resistant tomato and in *N. tabacum* ‘Turk,’ whereas other isolates remained localized or did not infect inoculated plants. Our ongoing

inoculation experiments will help determine these mutations' association with the emergence of potential resistance-breaking virus isolates.

P4.4-007

CAN YOU RESIST ME? SCREENING HEMP LINES FOR RESISTANCE TO EMERGING VIRUSES AND VIROIDS UTILIZING THE HEMP VIROME

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Text

Cannabis has been an emerging industry worldwide within the past decade with a global market valued at 4.71 billion USD for hemp alone, yet the diversity and distribution of associated pathogens have yet to be adequately studied. As production increases and the crop diversifies, the emergence and spread of these pathogens are imminent. The goal of this study is to describe the diversity and distribution of viruses/viroids infecting hemp in Colorado to help prevent crop loss due to diseases. In this study, four major hemp-producing regions in Colorado were analyzed. Tissue samples were collected from two cultivars of hemp from each farm visited in these regions at three timepoints throughout the growing season. These samples were submitted for Next Generation Sequencing and upon bioinformatic analysis, candidate virus/viroid sequences were validated. With metagenomic data from previous work, 26 different lines of hemp were screened for resistance to the top 2 predominant viruses/viroids found in the hemp virome, beet curly top virus and hop latent viroid. These 26 lines are genetically diverse which will facilitate the discovery of candidate genes involved in virus resistance. This work aims to further integrated pest management strategies in the hemp industry to promote sustainable agriculture.

P4.4-008

INTEGRATED MANAGEMENT STRATEGY FOR THRIPS-BORN DISEASE IN TAIWAN AND IMPLICATIONS

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Text

The insect-born disease management strategy includes using insect vector control, field sanitation, and resistant crops. Thrips transmit Orthotospovirus and Machlomovirus in different manners. However, strategies for viral disease management in Taiwan were rarely adjusted to the intricate virus-thrips interactions, cropping systems, and geographical areas. Due to this fact, the study evaluated the crop management regarding melon yellow spot orthotospovirus (MYSV) transmitted by melon thrips (*Thrips palmi* Karny.) on greenhouse cucumber and maize chlorotic mottle machlomovirus (MCMV) transmitted by maize thrips

(*Frankliniella williamsi* Hood) on field maize for better strategies. Removal of initial inoculum including weeds and plant debris from the greenhouse is recommended rather than removing infected plants for MYSV management on protected crops. Chemical control of thrips was effective to eliminate the thrips population and disease infection rate; spinetoram had less fatal effect on the natural enemy Orius species for MCMV management in open fields. The results implied the importance of sanitation and knowing the effect of chemicals for vector control on its beneficial enemy. For various cropping systems, we attend to clarifying inoculum sources, monitoring viruliferous thrips, and developing resistant traits on crops to adapt to the coming impacts of climate change.

P4.4-009

VIRUSES ARE ASSOCIATED WITH BUFFALO GRASS YELLOWING IN AUSTRALIA

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Text

Stenotaphrum secundatum (Buffalo grass or St Augustinegrass) is a hardwearing and vigorous warm season turfgrass popular in many countries including the USA and Australia. In Australia, buffalo grass ranks first in importance based on farmgate value. Buffalo grass yellowing is an emerging disease syndrome that affects both the appearance and vigour of the turf. We tested the hypothesis that buffalo grass yellowing is caused by one or more viruses. We surveyed turf farms in NSW, QLD and WA and found sugarcane mosaic virus (SCMV) to be widespread, while panicum mosaic virus (PMV) was only detected in cultivar Palmetto and a new cultivar recently imported from the USA. Satellite panicum mosaic virus was not associated with these PMV isolates. Network analyses of the coat protein gene indicated that there are two strains of SCMV on buffalo grass in Australia, one matching that found in panicoid grasses in Florida and the second on *Digitaria didactyla* (blue couch) in Queensland. The Australian PMV isolates from buffalo grass have only 86% nucleotide identity across the entire genome sequence to the exemplar isolate from pearl millet in Kansas, confirming that it is a very distinct strain. A new virus named Stenotaphrum nepovirus was also identified by high-throughput sequencing and it was common in all regions and cultivars but symptoms of infection were not obvious. We conclude that SCMV is a major cause of the buffalo grass yellowing disease syndrome.

P4.4-010

SURVEY OF RASPBERRY VIRUSES AND RECOVERY OF IN VITRO RASPBERRY CULTURES IN THE CZECH REPUBLIC AND NORWAY

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Text

In 2021, an international project on raspberry and strawberry viruses was launched (KAPPA project TO01000295). Twenty-nine and fifteen raspberry plants from the Czech Republic and Norway, respectively, were tested by NGS during 2021. The following known raspberry viruses were detected in the plants: BRNV, RBDV, RLBV, RLMV and RVCV. A DNA virus, RYNV was detected in several samples. Further research is needed to establish whether this is a virus infection, or a plant genome associated DNA sequence. Six viruses were detected in raspberry for the first time. Additionally, two novel viruses were identified, tentatively named raspberry enamovirus and raspberry rubodvirus. More than 400 raspberries samples and 200 insect samples have been collected and successively tested for viruses by RT-PCR. RBDV and BRNV had the highest prevalence in the raspberry samples. The most common aphids infesting raspberries in both countries are Aphis idaei and Amphorophora sp. Promising genotypes and new cultivars were introduced in vitro. Of the 7 in vitro genotypes screened, the ´Tulameen´ cultivar was infected with BRNV. The cryopreservation procedure as a “cryoknife” for virus eradication and for safe backup of selected raspberry genotypes was tested. The plant vitrification solution PVS3 was used as a vitrification mean. The first results indicate successful eradication of BRNV virus from ´Tulameen´.

P4.4-011

CHARACTERISATION OF YELLOW DWARF VIRUSES IN CEREALS AND GRASSES IN AUSTRALIA USING HIGH-THROUGHPUT SEQUENCING

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Text

Yellow dwarf viruses (YDVs) are distributed worldwide and can significantly reduce grain yield in economically important cereal hosts such as wheat, barley and oats. YDVs and their aphid vectors are frequently found in cereals and grasses in south-eastern Australia. The serological detection methods most commonly used to identify YDVs in Australia are unable to differentiate between closely related species and are not available for all YDV species. As a result, information about the diversity and distribution of YDVs in Australia, and their relationships with their aphid vectors and alternative hosts, is limited. To begin addressing

this knowledge gap, we used high-throughput sequencing (HTS) to examine the diversity and distribution of YDVs in cereals and grasses in south-eastern Australia. Our results show that Australian YDV isolates are much more complex and diverse than is currently suggested in the literature, both between and within species. Based on these results, new diagnostic tests are being designed to further examine the distribution of different YDV species in Australia, which will help us better understand this complex group of viruses. The information obtained in this study is critical for the more targeted development of virus-resistant cultivars.

P4.4-012

STRONGER TOGETHER: SYNERGY BETWEEN AN EMERGING MONOPARTITE BEGOMOVIRUS AND A DNA?B COMPONENT

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Text

In recent decades, a legion of monopartite begomoviruses transmitted by the whitefly has emerged as serious threats to vegetable crops in Africa. Recent studies in Burkina Faso reported the predominance of pepper yellow vein Mali virus (PepYVMLV) and its frequent association with a previously unknown DNA?B component. To understand the role of this DNA?B component in the emergence of PepYVMLV, we assessed biological traits related to virulence, virus accumulation, location in the tissue and transmission. We demonstrate that the DNA?B component is not required for systemic movement and symptom development of PepYVMLV, but that its association produces more severe symptoms including growth arrest and plant death. The increased virulence is associated with a higher viral DNA accumulation in plant tissues, an increase in the number of contaminated nuclei of the phloem parenchyma and in the transmission rate by B. tabaci. Our results suggest that the association of a DNA?B component with the otherwise monopartite PepYVMLV is a key factor of its emergence. To assess the impact of this DNA-B component on the structure of the geminivirus community, grid-based sampling and the use of metagenomic protocols allowed obtaining of complex networks. Further analysis of our networks using genome sequences showed the presence of nestedness and modularity. This support the need to continue studies of the virus-plant system.
Keywords: Begomovirus,disease, etiology, Tomato, Burkina Faso.

P4.4-013

IMPACT OF PLANT IMMUNITY ON VIRUS ADAPTATION: WHAT EVOLUTIONARY FORCES SHOULD WE RELY UPON IN PLANT BREEDING?

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Text

Plant breeders generally choose resistance genes based on their immediate efficacy and spectrum of action. One question that remains is which parameters of pathogen evolution should be targeted to promote plant resistance sustainability. Experimental evolutions of potato virus Y (PVY) in pepper (Capsicum annuum) genotypes contrasted in terms of resistance mechanisms allowed us to explore this question. These plant genotypes carried the same major-effect resistance gene encoding an eIF4E (eukaryotic initiation factor 4E) combined with different sets of resistance QTLs (quantitative trait loci).
We showed that the evolutionary trajectories of PVY were highly contrasted across plant genotypes. Some PVY evolutionary lineages showed more or less rapid adaptations linked to parallel nonsynonymous mutations in the VPg cistron, others showed losses of adaptation or even extinctions and some showed no significant change.
These varied trajectories can be explained by the level of resistance of the pepper genotype and by the intensity of the genetic drift imposed by the plant on the viral population. The intensity of selection, on the other hand, explains little of the diversity of evolutionary trajectories. In particular, we show that it is possible for breeders to combine high resistance efficiency and durability, even with strong selection on virus populations by a major-effect resistance gene, by using plant genotypes where viruses undergo strong genetic drift during infection.

P4.4-014

GENETIC DIVERSITY OF BARLEY YELLOW DWARF VIRUS (BYDV) ACROSS THE UK

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Text

Sap-sucking aphids impact plants through direct feeding and the secretion of exudates, promoting saprophytic fungi whilst reducing the photosynthetic ability of a host. The capacity to also transmit plant viruses renders aphids a threat to important crop species. Wheat is one such host affected by aphids and the viruses they transmit, most prominently Barley yellow dwarf virus (BYDV). In terms of both worldwide distribution and economic significance, BYDV is one of the most important viral diseases affecting cereals, reducing yield by up to 80%. Genetic sources of resistance to aphids and/or or BYDV may provide an attractive control solution; this forms the basis for much of our work.

The presence and variation of BYDV strains across the UK has not been thoroughly explored with few key strains reported. This is despite knowledge of BYDV diversity being crucial for robust monitoring, effective disease management, the development of improved diagnostics, and identification of resistance-breaking viral variants. Here, work has explored UK BYDV diversity via viral coat protein sequencing and phylogenetic analyses using viruliferous aphid

samples collected from suctions traps across the nationwide Rothamsted Insect Survey.

Besides those already known to be prevalent across the UK, our results suggest the widespread occurrence of unreported BYDV strain(s) and substrain variation; this has potentially significant ramifications and may be a key finding for improved disease management.

P4.4-015

POTATO VIRUS Y STRAIN CHARACTERIZATION, DETECTION AND ANALYSIS

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Text

Potato Virus Y (PVY) is an economically important virus affecting the potato industry worldwide. PVY belongs to the largest family of RNA plant viruses, Potyviridae. The single-stranded RNA virus has a monopartite genome that encodes for a single polyprotein. The genome properties and organization of PVY are important to its nature of recombination. Recombinant junctions at the functional protein sites allow PVY to recombine easily. A recombinant event can occur if there is more than one strain of PVY present in a host plant. PVY exists as a complex of strains and variants: five non-recombinant genotypes with 36 unique recombinant strains. This work evaluates strains of PVY in San Luis Valley (SLV), Colorado based on current detection methods. Sampling of potato leaf tissue was done in the 2021 and 2022 growing seasons. The field samples were tested for PVY and further analysis was performed using NGS. Bioinformatic analysis revealed new insights into mixed infections, possible recombinants, and current detection methods.

P4.4-016

FACTORS AFFECTING THE POPULATION DYNAMICS AND EPIDEMIOLOGY OF VIRUSES INFECTING POTATO.

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Text

Potato virus Y (PVY), Potato Leafroll virus (PLRV) and Potato virus A (PVA) are the most prevalent species in symptomatic plants of seed potato crops. The molecular diversity of PVYN isolates indicated that ~85% belong to the recombinant European (EU)-NTN group. Overcoming innate host resistance appears to be an essential factor in shaping intra-species virus populations. Transmission studies indicate that PVYEU-NTN has the highest transmission rate, a higher propensity of to infect older plants by out-compete other variants and overcoming host resistance mechanisms [1]. Transmission studies revealed. While far less diverse, PVA genome analysis suggest that polymorphism between severe and mild

isolates define novel pathogenicity determinants. A novel potyvirus Potato yellow blotch virus (PYBV) was identified in breeding lines [2]. PYBV elicits characteristic bleached symptoms on leaves and is closely related to PVA. PYBV is a rare virus and has not been found in commercial seed and ware potato crops in Scotland. Further analysis of potato and alternative hosts are on-going to further study their epidemiology. We are currently implementing nanopore next generation sequencing to study the etiology of potato disease in leaves and tubers and identifying potential emerging virus threats to potato and other Solanaceaous hosts.
[1] Davie K et al . (2017). Virus Res. doi: 10.1016/j.virusres.2017.06.012.
[2] Nisbet C et al (2018). Plant Path. https://doi.org/10.1111/ppa.12943

P4.4-017

EMERGENCE OF CACAO-INFECTING BADNAVIRAL SPECIES IN THE TROPICAL AMERICAS AND WEST AFRICA

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Text
At least eight badnaviral species associated with cacao swollen shoot disease have emerged or re-emerged as damaging pathogens of cacao in West Africa since ~2000. In the neotropics, the endemic Cacao mild mottle virus (CaMMV) and Cacao yellow vein-banding virus (CYVBV), once eradicated from cacao farms in Trinidad where they emerged during the 1940's, have been identified in cacao germplasm repositories and commercial cacao farms in Brazil, Trinidad, USA-Puerto Rico. Although CaMMV and CYVBV are distributed more widely than previously expected, symptomatic trees experience minimal damage. Phylogenomic surveillance of cacao-infecting badnavirus species indicate that the West African badnaviral species are distributed phylogeographically while others are widely distributed, collectively, due to mealybug vector dispersal patterns. For CaMMV and CYVBV, infected germplasm collections are the primary inoculum sources of inoculum, and viruses will not spread in the absence of endemic mealybug vector(s). Recent research has provided new insights into factors driving re-emergence and spread of badnaviruses in West Africa and the tropical Americas in commercial cacao and germplasm collections, respectively, grounded in improved molecular detection assays to enable identification of badnaviral phylodynamic-epidemiological predictors and support plant breeding efforts focused on species-specific disease tolerance in parallel with optimum production traits.

P4.4-018

RISK FACTORS ASSOCIATED WITH CASSAVA BROWN STREAK DISEASE DISSEMINATION THROUGH SEED PATHWAYS IN EASTERN D.R. CONGO

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Text
Vegetatively propagated crops are particularly prone to disease dissemination through their seed systems. Strict phytosanitary measures are important to limit the impact of diseases, as illustrated by the European potato seed system.

Cassava brown streak disease (CBSD) is a devastating disease caused by two viral species (CBSVs). After 100 years of research, a disease's westward and eastward spread is threatening people's livelihood in new areas. A better understanding would be an asset to properly managing it. This study tested the efficiency of a multidisciplinary framework in surveying the epidemiology of CBSVs in Uvira territory, Eastern D.R. Congo, and its drivers. Results revealed that three epidemic clusters, among which one potentially interesting for seed multiplication, could be identified in the study area using the five most significant factors: (i) symptom incidence, (ii) number of whiteflies, (iii) types of foliar symptoms, (iv) cutting pathways and (v) plant age. Through risk assessment, we also identified several key socio-economic determinants of disease epidemy: (i) factors related to farmer's knowledge and awareness (knowledge of cassava pests and diseases, knowledge of management practices, support from extension services and management strategies applied), (ii) factors related to the geographical location of farmer's fields (proximity to borders, proximity to a town, distance to acquire cuttings), as well as (iii) the pathways used to acquire cuttings.

P4.4-019

EFFECTS OF SOWING CMV-INFECTED LENTIL SEED ON GROWTH AND YIELD

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Text
Lentil (*Lens culinaris*) is an important food crop and is considered to be one of the main protein sources in many countries. Lentils are vulnerable to several viral diseases, but cucumber mosaic virus (CMV) is one of the most important. CMV is transmitted through seeds and by aphid vectors. CMV is common in lentils in south-eastern Australia, but data is lacking on its effect on growth and yield. In this study, field trials were conducted to quantify the yield risk of planting CMV-infected lentil seed. In preparation for field trials, 143 seed lots were sown in the greenhouse, then seedlings were tested for CMV using tissue blot immunoassay (TBIA) one month after sowing. CMV was detected in seeds of three lentil varieties SP1333, Eston and Indianhead with infection levels of 0.7%, 2% and 3% respectively. These varieties were sown in field trials in a randomised complete block design at four locations. Field plots were tested for CMV infection during the season to assess virus presence. The mean incidence of CMV across all the sites in SP1333, Eston and Indianhead was 24%, 13% and 8% respectively while the mean incidence in control plots did not exceed 1%. Plots were machine harvested and the grain yield from each plot was measured.

Overall, mean yield across all trial sites was higher in the control plots than the virus-infected treatment plots. In general, in the virus infected SP1333 treatment plots, the higher the incidence of CMV, the lower the yield from that plot.

P4.4-020

MOLECULAR CHARACTERIZATION AND PREVALENCE OF A NOVEL STRAWBERRY CRINIVIRUS IN IRAN

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Text

A new crinivirus was detected in strawberry plant (Fragaria × ananassa Duchesne) using high-throughput sequencing. The consensus genome was validated using RT-PCR, cloning and RACE. The complete genome sequence consists of two single-stranded RNAs of 8.8 and 7.1 Kb. The virus shares 37 – 56% nucleotide identity to previously described criniviruses. The RNA-depended RNA polymerase was most similar to tetterwort vein chlorosis virus with 66% aa identity whereas the heat shock protein 70 homolog showed the highest sequence identity to the lettuce chlorosis virus ortholog with 74% aa identity. Given the low sequence similarities with known criniviruses the virus should be considered a new member of the genus. Phylogenetic analysis of the RdRP placed the virus in Crinivirus Group 2 and supported its assignment to a new species within the genus Crinivirus. The virus was detected at high prevalence in the two main strawberry growing areas of Iran and verified to be whitefly-transmissible.

P4.4-021

STUDYING ROSE ROSETTE EMARAVIRUS REPLICATION IN ITS VECTOR PHYLLOCOPTES FRUCTIPHILUS.

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Text

Genus Emaravirus (Family Fimoviridae; Order Bunyavirales) is an emerging group comprising over 20 classified and putative species with a worldwide distribution and economic impact. Amongst them, rose rosette emaravirus (RRV, virus species Emaravirus rosae) is probably the most devastating due to its lethality on rose in North America. Emaraviruses are assumed to be exclusively transmitted by eriophyoid mites (Acari: Eriophyoidea), the smallest and most elusive among the arthropod vectors. Despite the

significance of emaraviruses, there is no understanding of the mode and mechanisms of their transmission. We used direct RT-qPCR to estimate the number of viral RNA copies within individuals of Phyllocoptes fructiphilus, and P. adalius, a confirmed and non-vector species respectively. After a 24 h acquisition period, mites were transferred daily to new, non-RRV-infected tissue. RRV copies in P. fructiphilus significantly increased with peak of virus titer recorded on day five, whereas it remained stable in P. adalius. Our results suggest that RRV replicate in P. fructiphilus, a finding of significance as it contributes to virus spread. These findings provide essential insights into a better understanding of intricate interactions between emaraviruses and eriophyoid mites.

P4.4-022

A NOVEL AMPELOVIRUS INFECTS BLUEBERRY

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Text
A novel ampelovirus-like sequence was detected from a blueberry selection from the National Clonal Germplasm Repository of USDA-ARS in Corvallis, Oregon, United States using high throughput sequencing (HTS). The authenticity of this ampelovirus-like sequence was validated and subsequently, a near complete genome was assembled using HTS contigs and amplicon sequences from overlapping RT-PCR. Sequence comparisons and phylogenetic analyses of several proteins confirmed distinct speciation of the novel blueberry ampelovirus within the genus Ampelovirus, with pistachio ampelovirus A being the closest relative amongst members of the genus. A triplex RT-PCR that targets two regions of the virus and a host gene was developed. An additional isolate of the virus was detected in Arkansas, USA. The source plant was co-infected with blueberry green mosaic-associated virus and exhibited stunting and yellowing. Grafting onto virus-tested plants verified transmissibility of the novel ampelovirus onto blueberry but also validated the symptomology observed. An extensive survey is underway to determine the distribution of the ampelovirus on major blueberry growing regions, blueberry germplasm, breeding programs, and nurseries.

P4.4-023

IDENTIFICATION OF APPLE LUTEOVIRUS (ALV-1) AND POTENTIAL ASSOCIATION WITH DIEBACK OF APPLE TREES IN NORTHERN ITALY

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Text
Apple tree (*Malus domestica* L.) is the most cultivated fruit crop in Trentino, in northern Italy.

For the last several years, an unusual problem of dieback of young apple trees is normally associated with wood pathogens and has never been associated with the presence of viruses. In the apple tree, commonly viruses include apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), and apple chlorotic leaf spot virus (ACLSV) and apple mosaic virus (ApMV). During 2018-2022 RT-PCR was performed for the detection of apple luteovirus 1 (ALV-1) in symptomatic and asymptomatic apple trees and rootstock collected during spring. Total nucleic acids were extracted from leaf and bark tissues and the presence of a named apple luteovirus 1(ALV-1) was performed using specific primers AluDetF6/R6 in RT-PCR. The obtained amplicon was purified and sequenced. The analyzes confirm the absence of ACLSV, ASPV, ASGV and ApMV both on the plant and on the rootstock. Instead, it confirms the presence of ALV-1 especially in the symptomatic plant and in the rootstocks. These reports suggest a possible association with ALV-1 and apple dieback and that rootstocks may be a source of infection spread in an apple tree. To our knowledge, this is the first report of ALV-1 of apple trees and rootstocks in Italy and can be applied for further epidemiological and diagnostic investigations to evaluate the phytosanitary status of the plant material and to control and prevent the spread of the disease.

P4.4-024

IDENTIFICATION AND DISTRIBUTION OF VIRUSES ASSOCIATED WITH BAMBARA GROUNDNUT PLANTS IN BURKINA FASO.

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Text

Plant viruses are a major constraint to the production of bambara groundnut [Vigna subterranea (L.) Verdc.] and can considerably reduce its production and hamper the nutritional quality of the seeds. Indeed, viral diseases cause yield losses of 15 to 87%. The objective of the study is to identify the viruses responsible for the diseases in the main production areas of bambara groundnut in Burkina Faso.
Thus, surveys and collection of samples of infected plants from the three agro-ecological area of Burkina Faso were carried out and submitted to molecular RT-PCR testing.
A total of 135 samples were collected, including 35 samples from the Sahelian area, 50 from the Sudano-Sahelian area and 50 from the Sudanian area. Molecular RT-PCR tests carried out on the collected samples and sequence analyses revealed the presence of three species of potyvirus, including one already described in Bambara groundnut, Cowpea aphid born mosaic virus (CABMV), and two new species provisionally named Bambara groundnut potyvirus 1 (BGVP1) and Bambara groundnut potyvirus 2 (BGVP2), reported for the first time in the country. The distribution of isolates shows that CABMV isolates were most frequent in the Sudano-Sahelian area (71.42%) compared to the Sudanian area (28.58%). In contrast, most BGPV2 isolates (92.31%) and all BGPV1 isolates were found in the Sudanian area. The Sudanian area was the most infected zone with 75% infection rate followed by the Sudano-Sahelian area (25%).

P4.4-025

EVALUATION OF BAMBARA GROUNDNUT ACCESSIONS FOR RESISTANCE TO COWPEA MOSAIC VIRUS (CABMV) IN BURKINA FASO.

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Text

Cowpea aphid born mosaic (CABMV) is a prevalent virus on legumes and particulary on Bambara groundnut in Burkina Faso. Its impact has been recorded also on Cowpea and caused some significant yield losses. The main of this study is to investigate the sources of resistance to CABMV in order to develop control strategies. Thus, eight accessions of Bambara groundnut were assessed in the greenhouse in pots by mechanical inoculation method in two replicates.
Infected plants showed characteristic symptoms of CABMV virus such yellow mosaic, leaf deformation followed by leaf drop and stunting. The results of yield parameters showed that CABMV had an impact on flowering, pod and seed formation. A1 and A6 accessions were less affected with pods losses estimated between 21.71% to 35.74% and 24.44-32.35% for grain yield. These accessions (A1 and A6) were tolerant to CABMV virus and could be used in Bambara groundnut varietal improvement program in order to control effectively and sustainably CABMV virus.

P4.4-026

PREVALENCE AND SPATIAL DISTRIBUTION OF BADNAVIRUS IN THE BANANA (MUSA SPP) MAJOR GROWING AREAS IN BURKINA FASO

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Text

Banana streak virus (BSV) and Sugarcane bacilliform virus (SCBV) are two badnaviruses commonly found in all banana growing areas of the world. It is a threat to the production and improvement of Musa germplasm. In Burkina Faso, the presence of badnaviruses was reported in banana producing regions. The objective of this study was to determine the prevalence of BSV and SCBV in banana production areas of Burkina Faso. A survey followed by a symptomatologic study was conducted in banana plantations in 27 localities of the nine main banana producing regions from July to October 2018 and September to December 2020. In all, 251 leaf samples were collected and analysed for BSV and SCBV infection by Indirect Antigen Coated Plate Assay-ELISA followed by amplification of the RT/RNase H region using Polymerase chain reaction with Badna FP/RP and SCBV F/R primers, respectively. A variety of symptoms were observed on almost all plant organs which were revealed due to BSV by symptomatologic study. The results of serological and

molecular diagnosis revealed a high overall prevalence of BSV in 80.48% of the samples tested. BSV was distributed in seven survey regions out of nine with prevalence ranging from 10% to 100% in North, Centre, Centre West, Hauts Bassins, Cascades, Centre East and Boucle of Mouhoun regions. Very low prevalence was recorded for SCBV in Cascades and East Centre region with 4.35 and 12.5%, respectively. Species detection using specific primers to each species revealed three main species: Banana streak Obino l'ewaï virus (BSOLV), Goldfinger virus (BSGFV) and Imové virus (BSIMV) in the samples tested, respectively in the proportions of 23%, 8% and 0.8%. Co-infection between BSV species was also detected. Keywords: Banana streak virus, Sugarcan baciliform virus, Indirect Antigen Coated Plate Assay, Polymerase Chain Reaction, Musa spp.

The future of disease surveillance and prediction: Beyond the usual suspects.

C9.4-1

LANDSCAPE ECOLOGY OF RICE YELLOW MOTTLE VIRUS ALONG THE NIGER VALLEY AND IMPLICATIONS FOR DISEASE SURVEILLANCE AND ANTICIPATION

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Text
Rice yellow mottle caused by Rice yellow mottle Sobemovirus (RYMV) is the major rice disease in Africa. RYMV spread was inferred based on regular sampling of virus isolates taking into account the rice growing ecologies and the hydrographic network in the River Niger Valley. RYMV disease outbreaks started with changes in rice agroecosystem particularly when irrigation schemes started in the early 1980s, allowing double cropping per year. The disease underwent a rapid spread over hundreds of kilometers along the River Niger except the outmost northern loop part where the ecology is dominated by floating rice. Spatial and temporal host continuity and inoculum build-up led to the development of severe epidemics. There was no evidence of long-distance dissemination of the virus through natural water. The progressive reduction in host habitat fragmentation may be a key driver in the RYMV spread and need continuous disease surveillance.
Keywords: Rice yellow mottle, virus spread, Niger Valley, ecology

C9.4-2

THE ROLE OF VINEYARD CHARACTERISTICS, TECHNOLOGY AND CULTIVATION IN THE GRAPE DISEASES PREVALENCE: FIRST LECTURES OF A LARGE-SCALE CITIZEN SCIENCE

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Text

The grape production dynamically evolves under the pressure of technological innovation, increasing consumer expectations and regime of global climatic challenges. The role of specific climatic and technological factors in the occurrence and spread of grape diseases might be a well-known issue. However, only a few studies applied a complex methodological approach to the relationship among the vineyards' local properties, cultivation practices and infection severity, although these factors could explain the temporal and spatial variation of the plant diseases. In this citizen science study, we aimed to test the relative importance of the most relevant vineyard characteristics (e.g., inclination, row orientation), crop technology and grape cultivar features on the long-term infection prevalence of primary grapevine pathogens, such as *Botrytis cinerea*, *Plasmopara viticola* and *Erysiphe necator*. We found certain factors that could predict and determine the prevalence of pathogens, which could interfere with the cultivar-specific features. Our results indicated that vineyard characteristics, crop technology, and cultivars features could highly determine the production efficiency via the level of disease control on a long-term basis. In future, these findings may play a key role in plantation establishment and applying precision technologies (i.e., differential spraying), which become increasingly intense due to the general pesticide reduction and global climatic changes.

C9.4-3

COMBINING NETWORK ANALYSIS AND MACHINE LEARNING FOR DESIGNING SURVEILLANCE STRATEGIES

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Text

Surveillance in plant health consists in collecting direct or indirect information on the presence, the distribution and the dynamics of diseases and pests. Surveillance actions are expected to be improved in terms of efficiency if they are designed based on knowledge or assessment of the risks. This talk will illustrate with several case studies (e.g., Xylella fastidiosa and sugarbeet yellowing) how the construction and analysis of networks associated with machine learning may be used to identify new indicators of risks, to build integrated indicators, to assess spatial interconnections and, finally, to design improved spatio-temporal surveillance strategies.

C9.4-4

PATH-FINDER: FINDING THE SPORE BEFORE THE DISEASE

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Text

Airborne plant pathogens pose an existential threat to regions naïve to their impact. There are many examples where such pathogens are detected too late; when entire forests are dying (ash dieback - *Hymenoscyphus fraxineus*), stands have been decimated (chestnut blight - *Cryphonectria parasitica*), or keystone ecosystems all but destroyed (myrtle rust – *Austropuccinia psidii*). Once established, pathogen eradication becomes exceedingly difficult as they continue to spread, and management resources are allocated in a reactive manner. Here, we present a surveillance technique that co-opts existing air pollution monitoring infrastructure for the early detection and monitoring of airborne plant pathogens. For this, we used *A. psidii* as a case study and verified its presence in the air of southeast Queensland, Australia using a new species-specific TaqMan qPCR assay. We also present metagenomic results from the same air filters that extend beyond targeting specific plant pathogens to include other potential biosecurity threats. *Austropuccinia psidii* has not established in Western Australia. Routine monitoring for spores from the eastern Australian states using the technique proposed here, will enable more efficient allocation of resources thereby enhancing our ability to predict, find and contain *A. psidii*, and other pathogens like it, before disease is established. A proactive, rather than reactive response.

C9.4-5

THE PHYTOPATHOLOBOT: AN AUTONOMOUS ROBOT FOR REAL-TIME DISEASE DETECTION AND SEVERITY ESTIMATION IN VINEYARDS

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Text

Interest in agricultural robots for data-driven decision making and improved efficacy has grown significantly in recent years. However, advancements in robotics for disease management have lagged. Labor shortages present a grand challenge for not only agricultural production and disease management, but also breeding programs and academic research. Scouting robots can help ameliorate this challenge by providing accurate disease incidence information at greater temporal and spatial resolutions than humans can feasibly and cost-effectively provide. The PhytoPatholoBot (PPB) is a fully autonomous robotic imaging rover optimized for deployment in trellised crops. PPB autonomously traverses vineyards using an RTK-GPS system and collects side canopy imagery with a custom multispectral camera. PPB employs a semantic segmentation model for near real-time identification and severity estimation of Grapevine Downy Mildew and Grapevine Leafroll

Virus. Experimental results show high correlation between PPB and human ratings for both diseases. PPB ratings correlate well with remote sensing measurements from UAVs, airborne, and spaceborne platforms, which could be further developed into a closed-loop autonomous training system between ground and remote sensing systems. The PPB could one day provide stakeholders and academic users with large-scale disease incidence data to strategically deploy limited mitigation resources and validate remote sensing-based disease warning systems.

C9.4-6

HOW REACHABLE IS EUROPE FOR THE JAPANESE BEETLE: TRACKING PLANES, TRAINS AND TRUCKS TO INFORM SURVEILLANCE STRATEGIES

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Text
The Japanese beetle (*Popillia japonica*) is a polyphagous insect listed as priority pest by the EU phytosanitary legislation. The beetle was first detected in Continental Europe in 2014, in the Italian regions of Piedmont and Lombardy. Since then, it has quickly invaded a large portion of Northwestern Italy and Southern Switzerland, despite the eradication effort of regional phytosanitary services. Furthermore, several interceptions of living adults have occurred in distant locations as a result of unintended passive transport of the beetle. Indeed, it is well established in the literature that *Popillia japonica* is capable of being dispersed over large distances via passive human transportation (hitchhiking behavior). In this work we analyzed how the invaded areas of Northern Italy and Switzerland are connected to the rest of Europe via three main transportation networks: planes, trains and trucks. We built reachability maps from the invaded zone highlighting sites that are likely to act as stepping stones or entry points for the beetle, both close and further away from the currently infested zone. Combined with a suitability map, this allows to prioritize sites for early-detection surveillance based on how likely they are to be reached, as well as their potential for being a future outbreak of infestation. This is crucial, as experiences in North America proved that early detection followed by effective eradication protocols can prevent the establishment of the beetle.

F9.4-3

APPLE SCAB FORECASTING: AN INSIGHT INTO PREVAILING AND PROSPECTIVE METHODS

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Text
Apple scab is the most devastating important disease that has invaded almost all apple-growing regions worldwide, causing economic losses in terms of fruit quality and yield. It is a complex disease that develops following two phases, a monocyclic phase caused by ascospores produced in pseudothecia originating from leaf lesions present the previous fall is usually large and is released over a period of 1 to 2 months following bud break, and a polycyclic phase produced by conidia originating from lesions on leave and fruits. PAD is a useful tool to forecast the total amount of inoculum in an orchard and has been shown to effectively improve apple scab management. In IPM programs, the control of apple scab is based on the use of fungicides, and these programs are developed to improve fungicide efficacy using procedures to estimate potential ascospore dose, weather-based models to estimate ascospore maturity, leaf growth, and risk of infection periods. ? In orchards managed according to advisory information, a significant increase in yield was obtained relative to the common management policy. ?During some years, the complete management of the scab by applying 3-4 fungicides and 2 percent urea spray at the time of leaf fall was obtained. Warnings are issued mainly via a call-in telephone, SMS, WhatsApp, Agriculture Govt. department, and broadcasted through radio stations.

P9.4-001

ASCO DASHBOARD – AN ONLINE INTERACTIVE TOOL FOR EXPLORING AND INVESTIGATING ASCOCHYTA RABIEI IN AUSTRALIA

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Text
Ascochyta blight of chickpea poses a major risk to the chickpea industry in Australia and worldwide and therefore significant efforts are invested to better understand and monitor the populations of *Ascochyta rabiei*, the causal agent. However, the outputs of these efforts often remain within the domain of researchers and plant pathologists and are not made available to the industry and public outside of internal reports and scientific publications.
Here we introduce the **Asco dashboard** (http://bit.ly/asco-dashboard), an online interactive tool that was developed to provide a visual and intuitive spatio-temporal overview of a comprehensive collection of 1,155 *A. rabiei* isolates collected in Australia between 2013-2022. The dashboard consists of an interactive map with pins representing the location of collected isolates, along with collection metadata, aggressiveness assessment and genotypic information (when available). The dashboard provides summary tables and plots highlighting the risk to common chickpea varieties used in Australia. Dashboard users have access to the underlying data, which can be downloaded. Further work is underway to improve the interface and allow users to select isolates out of the map and to expand this work to other worldwide *A. rabiei* collections.
Feedback from the community and early adopters proves that this is a useful tool for researchers, agronomists, and growers trying to understand the risk of Ascochyta blight in their region and nationwide.

P9.4-002

THE FRENCH EPIDEMIOLOGICAL PLANT HEALTH SURVEILLANCE PLATFORM : AN INNOVATIVE APPROACH TO IMPROVE SURVEILLANCE EFFICIENCY

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Text

Climate change and international trade intensification has increased health issues for plants. Governments and representatives of EU citizens have agreed to protect the economic, social and environmental resources related to plant health by adopting the plant health regulation 2016/2031 (EU). A major aspect of these regulations is to strengthen, prioritize and harmonize plant pest surveillance based on common scientific technical principles. Epidemiological surveillance is essential to prevent and control health risks, particularly emerging diseases. In July 2018, France signed an agreement whereby public and private organizations constitute the first dedicated network to plant health surveillance by creating the Epidemiological Plant Health Surveillance Platform (ESV Platform).
Various missions are entrusted to the platform such as international plant health media and scientific monitoring system, the provision of pest recognition sheets, assessments of the territory's health and surveillance systems or the proposal for improvements in surveillance based on the risk. We are working on several pathosystems including Xylella fastidiosa, HLB or the pine wood nematode. From the centralization of surveillance data of the territory as well as research and operational works, we assess and improve the surveillance of the health status of plants. The ESV Platform provides methodological and operational support for public policies and the supervisors of monitoring systems.

P9.4-003

PREDICTION OF SCLEROTINIA SCLEROTIORUM OCCURRENCE USING SMARTPHONES AND AN IMAGE ANALYSIS PROGRAM

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Text

Previous studies have reported that crops infected by phytopathogens can be detected using image analysis techniques. Our studies have shown that image analysis using smartphones can determine the efficacy of soil disinfection techniques and pesticides, but prediction of disease occurrence has not been actively progressed due to difficulty of detection until symptoms or signs appear. The objective of this study was to predict *Sclerotinia sclerotiorum* occurrence based on RGB changes in the image data from pictures taken with two smart phones, Galaxy Note10 and iPhone13 mini. A nitrogen fertilizer and commercial fertilizer for

red lettuces were applied with twice the recommended amount at 10-day intervals. The negative control was applied with no fertilizer. *S. sclerotiorum* was injected 25 days after fertilization and pictures were taken twice a week. RGB proportion was significantly different for each treatment. Signs were detected on 11/28 but the proportion of a black color (RGB 0 0 0) in the infected red lettuces on 11/24 was higher than that of the uninfected red lettuces. The phenomenon appeared in all the treatments regardless of the smartphone models. If more image data is collected, at least, *S. sclerotiorum* occurrence can be predicted using just a smartphone in commercial farms. Furthermore, a combination of the image analysis and drone technologies may contribute to predicting the occurrence in the case of large-scale farms with less time, labor, and cost.

P9.4-004

USING 'SENTINEL' PLANTS TO IMPROVE EARLY DETECTION OF INVASIVE PLANT PATHOGENS: XYLELLA FASTIDIOSA IN OLEA EUROPAEA AS A CASE STUDY

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Text

A key challenge in plant disease management is achieving early detection of invading pathogens, which requires effective surveillance through the implementation of monitoring programmes. However, when monitoring relies on visual inspection as a means of detection, surveillance is often hindered by a long incubation period during which plants may be infectious but not displaying visible symptoms. 'Sentinel' plants - alternative susceptible host species that display visible symptoms of infection more rapidly - could be introduced to at-risk populations and included in monitoring programmes to act as early warning beacons for infection. However, while sentinel hosts exhibit faster disease progression and so allow pathogens to be detected earlier, this often comes at a cost: faster disease progression typically promotes earlier onward transmission. Here, we will construct a computational model of pathogen transmission to explore this trade-off and demonstrate how including sentinel plants in monitoring programmes could facilitate earlier detection of invasive plant pathogens. Using *Xylella fastidiosa* infection in *Olea europaea* (European olive) as a current high profile case study, for which *Catharanthus roseus* (Madagascan periwinkle) is a candidate sentinel host, we will demonstrate that including sentinel plants in monitoring programmes can reduce the expected prevalence of infection upon outbreak detection substantially, increasing the feasibility of local outbreak containment.

P9.4-005

PAIRING HIGH RESOLUTION SATELLITE IMAGERY AND TERRESTRIAL ROBOTICS TO DETECT AND MONITOR GRAPEVINE DOWNY MILDEW EPIDEMICS

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Text
Grapevine downy mildew (GDM), caused by the oomycete *Plasmopara viticola*, plagues humid production regions and can cause 100% yield loss and vine death under conducive conditions. Growers currently rely on frequent fungicide applications for control, but this practice has led to widespread resistance. Rapid remote detection and mapping of GDM outbreaks would enable precision pesticide applications to target high performing but resistance-prone fungicides where and when most needed, while relying on less resistance-prone protectants elsewhere. To actualize this vision, we investigated two platforms for GDM surveillance: high resolution, multispectral satellite imagery and a terrestrial robotic imaging system at the Cornell Pathology Vineyard in Geneva, New York. We evaluated several supervised and unsupervised methods to predict disease severity using satellite spectral features as input. Spectra and vegetation health indices derived from Planet Labs SkySat imagery (50cm pixel size) could differentiate between healthy and diseased vines even at low GDM severity (10% symptomatic leaf area). Automated severity ratings derived from rover-based imagery also correlated well with human scout ratings ($r > 0.75$). Our next step is to integrate the two systems by training satellite imagery on rover generated severity maps. Our results thus far indicate that both satellite and terrestrial robotic platforms are promising methods for mapping GDM incidence and severity.

P9.4-008

VALIDATION OF THE SCLEROTINIA STEM ROT FORECASTING MODEL (SKLEROPRO) UTILIZING SCLEROTIA-DEPOTS

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Text
Sclerotinia stem rot caused by Sclerotinia sclerotiorum is one of the important diseases of oilseed rape. SkleroPro is a forecasting model offers recommendations regarding the optimal time for fungicide application during flowering stage. However, validation is an integral part of the model development process if the model is to be used in decision support systems. Investigations were carried out on oilseed rape fields to assess the variables related to the germination of sclerotia and the production of apothecia. The emergence of sclerotia and the release of ascospores were monitored using sclerotia depots, and a data logger was used to record soil temperature and humidity. Further controlled experiments were performed to determine the influence of weather variables on the carpogenic germination of sclerotia. The monitoring reveals that the infestation strength varies yearly. The depots' monitoring showed that apothecia's appearance ranged from 13 May to 10 June 2021, significantly after the oilseed rape flowering stage. In the following year, 2022, the appearance of apothecia ranged from 20 April to 1st May, which was shortly before plant flowering time. Additionally, the findings indicated a significant correlation between the development of apothecia and ascospores with the soil temperature and moisture. In both years, initial germination of

sclerotia and production of the apothecia occurred after 3-5 days of mean soil temperature of 15°C and 55 to 60% soil humidity.

P9.4-009

PREDICTING STRESS CAUSED BY GRAPEVINE POWDERY MILDEW WITH NASA AIRBORNE IMAGING SPECTROSCOPY IN NAPA VALLEY, CALIFORNIA

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Text

Powdery mildews cause $42.8B in damage annually and are notoriously ubiquitous with 10,000+ host species on all crop producing continents. Grapevine (*Vitis vinifera*) Powdery Mildew (GPM; *Erysiphe necator*) is responsible for >90% of negative environmental consequences associated with vineyard management globally as effective control necessitates high frequency fungicide application. While mechanistic models to predict GPM incidence and spread exist, their accuracy is limited by uncertainty in underlying initial disease distribution. The overall goal of this work is to develop a quantitative index for GPM rooted in disease physiology that can be used to parameterize epidemiological models with NASA Airborne Visible and Infrared Imaging Spectrometer Next Generation (AVIRIS-NG) hyperspectral imagery collected over Napa Valley, CA. We compared Normalized Difference Vegetation Index (NDVI), Red-Edge NDVI (NDVIre), Plant Senescence Reflectance Index (PSRI), and others as viable early indicators of GPM-induced stress and compared their accuracy when derived from hyperspectral and multispectral (Sentinel-2) sources. We found NDVIre derived from AVIRIS-NG to be the most accurate indicator of grapevine health and vigor as relates to potential GPM-stress, especially once vines have amassed significant foliar chlorophyll. The next step for this work is to compare the distribution yielded by our quantitative index to simulations from the Gubler-Thomas.

P9.4-010

UAV-BASED MULTISPECTRAL IMAGERY FOR RAPID DETECTION OF HUANGLONGBING IN CITRUS NEPAL

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Text
Early, rapid and accurate detection of plant disease is critical in deploying effective management strategies. The application of multispectral imaging in plant disease diagnosis is getting priority due to ease of application in a large area, high confidence, and considerably low cost. Considering the significant variation in the spectral response of leaf tissues due to impact of huanglongbing (HLB) infection in citrus plants, this study is focused on determining the potential application of multispectral imaging in HLB detection. Multispectral imaging system mounted on UAV was utilized to acquire images from PCR confirmed HLB-infected and healthy plants in farmers' fields in Tanahu district of Nepal. The image acquired with UAV at flight height-30 m, are stitched, and post-processed to generate the geo-referenced reflectance map. The plant pixels were segmented from soil followed by extraction of regions of interest of healthy and infected with a polygon shapefile to prepare the training samples for machine learning methods. The dataset was divided into train and test split in 8:2 ratio. We employed a support vector machine as a classifier. The support vector machine as a classifier produced an accuracy in the range of 72–75% for HLB detection on test samples. The results indicate the potential application of multispectral imagery for rapid detection and pre-screening of HLB-infected trees in citrus orchards where PCR facilities are unavailable or prohibitively expensive.

P9.4-011

SIMPLE MODELS FOR COMPLICATED EPIDEMICS: EXPLORING THE USE OF EPIDEMIOLOGICALLY RELEVANT PARAMETERS IN PARSIMONIOUS MODELS TO INFORM EARLY DETECTION SURVEILLANCE

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Text
The Food and Agricultural Organisation of the United Nations estimated that between 20 and 40 percent of global crop production is lost to pests annually, these losses to plant disease costed the global economy approximately $220 billion annually (FAO, 2021). In the UK, the estimation of the cost of invasive species is £1.7 billion per year (Spence, 2020). To manage plant disease outbreaks, biosecurity measures against plant pathogens must consider risk-based decision making. Risk-based decision making is supported by a framework of surveillance and predictive modelling. Predictive modelling is useful for early detection, increasing the likelihood of extinction and decreasing the cost of management. For early detection to be effective, the efficient use of sparse data sets to inform models is necessary. Therefore, investigation of parsimonious models that are transferable across disparate emerging plant disease epidemics is a research priority. In this study, a model was used to estimate an epidemics discovery-prevalence (labelled the "rule of thumb" approximation) using epidemiological parameters. The results indicated that decreased surveillance efforts and increased growth rate of disease led to a reduction in the accuracy of the rule of thumb approximation. Further research is being conducted to investigate the landscape aggregation of host plants on the accuracy of this simple model and how this interacts with the dispersal capacity of the pathogen.

P9.4-012

ESTIMATING STRIPE RUST SEVERITY IN WHEAT USING RGB AND THERMAL IMAGING WITH MACHINE LEARNING MODELS

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Text

Accurate estimation of the severity of wheat stripe rust in a field is crucial for controlling the disease and reducing field losses. Field experiments were carried out during 2017-18 and 2018-19 to collect RGB and thermal images of wheat varieties with different disease resistance levels at critical growth stages. Machine learning (ML) models were developed using the combinations of indices and partial least square regression (PLSR) scores of indices with disease severity and Yeo-Johnson (YJ) transformed values of disease severity. The performance of ML models was the best with indices, while the PLSR scores of indices and YJ transformations of disease severity were unable to improve model performance. In training, the models achieved an R2 and d-index value of greater than 0.95, while in the validation, the models produced R2 and d-index values up to 0.67 and 0.87, respectively. Cubist model developed using indices was the most effective in predicting disease severity. In contrast, the Gaussian process regression model developed using PLSR scores of indices and the YJ-transformed disease severity was the least effective. The findings of this study demonstrated the capabilities of machine learning models in providing predictions of stripe rust severity in the field through the use of RGB and thermal images.

P9.4-013

HYPERBIRD: AUTOMATED, HIGH-THROUGHPUT HYPERSPECTRAL QUANTIFICATION OF FUNGICIDE RESIDUE LEVELS IN GRAPE LEAVES

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Text

Fungicides are crucial for global grape production, but overuse has financial and resistance consequences. If growers had a way to monitor fungicide activity non-destructively, they could reduce use and expense through more targeted applications and lengthened intervals. We previously found that handheld hyperspectral sensing can predict fungicide concertation with moderate-high accuracy. Here, we developed our “BlackBird” high-throughput imaging platform into an automated hyperspectral imaging platform (HyperBird) to quantify fungicide

concentration on grape leaves. The system consists of a hyperspectral camera (MSV500, Middleton Spectral Vision; 400-1000nm, 8nm resolution), a diffused dome light, X-Y sample positioning, and Z-axis focusing movement. The HyperBird acquires line images at 200 frames per second, equivalent to 20 seconds per 10-mm leaf disc sample (about 2 hours per 351-sample tray). Greenhouse-grown grape leaves are treated with known volumes of strobilurin fungicides, processed into discs, and monitored for up to 21 days. Leaf discs are sampled from varying distances from the point of deposition to examine systemic movement. Concentration is validated for a subset of samples with GC-MS/MS. The next step is evaluating machine and deep learning models to identify the most accurate method for concentration prediction. Our platform enables high throughput fungicide activity monitoring of thousands of samples daily, which can inform application timing.

P9.4-014

OILSEED RAPE WITH SCLEROTINIA STEM ROT SYMPTOMS CAN BE EFFECTIVELY PHENOTYPED USING LASER SCANNING METHOD (LIDAR)

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Text

Classical hand-made measurements of plant phenotype are precise but destructive. The aim of this work was the search for high-throughput technique that can effectively describe the shape and size of oilseed rape plants, with the main focus on its potential yield, expressed as the number of siliques. We were mainly interested in the effect of Sclerotinia stem rot on plant yielding potential. The study used Light detection and ranging technique (LiDAR) to count the number of siliques and compare the results with the traditional method.
Scans were done using FARO LS Focus 3D X 130 scanner in the ‘multi scan’ mode, mostly from three angles and 25% of full resolution capacity. We have proved that disease progression had an increasing effect on the phenotype of oilseed rape plants. Statistically significant differences between healthy and infected plants were found at BBCH 79 – BBCH 80 and this effect was progressing till plant maturity. Infected plants were shorter, with smaller number of side branches, the plants were prematurely dry and many of siliques fell off. The laser scanning gave inaccurate results for healthy plants but was reasonably effective for infected plants. The highest mean correlation between the hand-made and LiDAR-based measurements was 76.5%, but it was up to 92.1% for a single plant. In this study, the traditional method of manual measurements proved its superiority, but it was destructive, which makes it of limited use in plant breeding.

P9.4-015

DISEASE SPREAD DYNAMICS FROM MONITORING AT DIFFERENT SPATIAL AND TEMPORAL SCALES

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Text

Apple canker (AC) is a fungal disease caused by Neonectria ditissima that is primarily rainsplash dispersed and has a variable latent period from weeks to years. These features help to promote a non-random spatial pattern of disease incidence within and across orchard blocks, with hot-spots that can persist in the same location over several years. Spatial disease incidence dynamics were analysed from empirical data varying in temporal and spatial resolution. Temporal comparisons were made between yearly and monthly tree level disease incidence records. Incidence over time was also related to environmental factors, seasonal availability of wounds and expected latent periods. One set of incidence data was recorded at a precise spatial scale by recording the location of individual trees, whereas the other data set approximated location to the orchard 'bay' (± 10 m within a tree row). Disease spread patterns were also analysed both within-block (single cultivar and uniform management) and across-blocks (multiple apple cultivars; differing plant spacing and age). Spatial models were developed using empirical and mechanistic approaches incorporating the spatial and temporal differences in data resolution. Implications of spatial modelling with different resolutions of spatial and temporal scales are discussed for application to efficiently manage and predict the spread of AC in New Zealand apple orchards.

P9.4-016

EFFICIENCY OF ANTI-SHARKA CONTROL STRATEGIES

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Text

Plum pox virus is an aphid-transmitted virus responsible for sharka, a disease of trees belonging to the Prunus genus that can cause significant economic losses for growers whose trees are infected. Sharka used to be classified as a quarantine pest (QP). Without an effective curative method, for several decades the French State organized a collective control based on the surveillance and uprooting of symptomatic trees. However, sharka recently became a regulated non-quarantine pest (RNQP) according to European regulations. As a result, the French State is progressively disengaging from the management of this disease. Currently, the intensity of the regulated control is decreasing, and in the medium term it is planned that sharka control in orchards will be deregulated (target system). This transition raises questions on the efficiency (i.e. the ratio between results and resources used) of the future of sharka management: will the level of commitment of growers compensate for the State's disengagement? Will their organization enable an efficient monitoring of the disease and control of its transmission?
To address these questions, the functioning and performance of sharka surveillance systems (present, future) will be analyzed using the OASIS method. The results will be used to make recommendations to improve system efficiency. We will also use a model to simulate the spread and management of sharka in realistic landscapes, in order to compare the two systems' efficiency.

P9.4-017

UNDERSTANDING THE USE OF MULTISPECTRAL UAV DATA AND DEEP LEARNING FOR QUANTITATIVE RESISTANCE AND DISEASE CONTROL: CASE OF CERCOSPORA LEAF SPOT IN SUGAR BEET

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Text

Deep learning in computer vision is currently the principal pillar for image analysis in diverse application fields including disease monitoring in agriculture. Techniques for object detection, semantic segmentation and instance segmentation allow extracting image-based features to replace visual estimations on-site. In crop protection, one of the most time-consuming activities is quantifying disease damage. This work addresses the case of a relevant foliar disease in sugar beet cultivation, Cercospora leaf spot (CLS). We aimed automatic disease quantification using unmanned aerial vehicle (UAV), multispectral imagery, and deep instance segmentation networks. An UAV imagery pipeline is proposed and evaluated in time-series in two experimental fields. The first trial was arranged for a resistant test and considered a comparison of automatic and manual determination of disease severity (DS) in five sugar beet varieties. As second trial, a CLS spot-inoculated experiment was led with the objective to access the parameter for control, disease incidence (DI). Results of resistance test emphasized the robustness the automatic determination of DS, crucial for breeding programs; nevertheless, in the second experiment, a limited use of UAV-based DI was observed for the implementation of control activities. These results allow a better understanding of the practical use of UAV platforms and novel sensing technologies, crucial for replacing the very laborious work of visual assessments.

P9.4-018

CASE STUDY: INDIGENOUS KNOWLEDGE AND VALUES INFORMING BETTER SURVEILLANCE

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Text

Working with researchers and scientists, indigenous tribes in New Zealand have developed and adopted (the) Te Whakahononga, an approach that recognises the value of indigenous knowledge and its contribution to improve surveillance approaches and solutions. Indigenous tribes with this approach are encouraged to work with scientists,

researchers and agencies to understand the pathogens that are threatening native species and apply their own indigenous knowledge and observations for better research and surveillance outcomes. 13 indigenous tribes across New Zealand, with researchers from the Biological Heritage, National Science Challenge are working to address *phytopthera agathadicida* and *austropuccinia psidii*, plant pathogens that are a risk to New Zealands native species and significant to the retention of Maori language, culture, identity and traditions. Using traditional methods of observation and new technologies, the approach recognises and gives effect to the role of traditional knowledge in a surveillance effort by elevating indigenous and traditional knowledge holders into the science and research system and tribal authorities alongside local and central government agencies, into the biosecurity and surveillance systems. This presentation will discuss this approach and the outcomes of its application for surveillance on *phytopthera agathadicida* and *austropuccinia psidii* research.

P9.4-019

PATHOGEN ISOLATION RATE OF SESAME WILT DISEASE AND QUANTITATIVE RESISTANCE EVALUATION OF MACROPHOMINA PHASEOLINA

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Text

Sesame is one of the crops that suffer the most damage from repeated cropping, and is exposed to various diseases. In particular, sesame wilt disease appears in farms where most of the sesame is continuously cultivated. So it is necessary to find a control according to accurate pathogen identification.

Therefore, in this study, 23 farmhouses in 5 regions with wilting symptoms were collected, pathogens were separated by part, and quantitative resistance evaluation of Macrophomina phaseolina, a major pathogen by cultivar, was conducted in vitro. As a result, the pathogen isolation frequency was different depending on the separated organs, Fusarium spp., M. phaseolina, etc. were observed as isolated pathogens. The ratio of pathogen isolation from roots was 30% for M. phaseolina and 73.6% for fusarium sp. and 1% of Didymella. The pathogens isolated from the stem root were more diverse than the pathogens isolated from the roots, with M. phaseolina 27.5% and fusarium sp. 67.0%, Alternaria sp. 16.5%, Didymella 3.3% and so on. The pathogens isolated from the leaves were Alternatia sp., but most of them were saprophile, and only a few of the sesame pathogens, A. sesami, were isolated. Quantitative resistance evaluation of M. phaseolina was conducted on 36 varieties, a piece with a diameter of 5 mm was removed from the tip of strain 17-034 and inoculated together with seeds. As a result, Areum and Gangheuk sesame showed sensitivity, and Geumok sesame showed resistance.

P9.4-020

CLOUD-NATIVE, MACHINE LEARNING BASED DETECTION OF GRAPEVINE LEAFROLL VIRUS IN VITIS VINIFERA WITH NASA IMAGING SPECTROSCOPY IN CALIFORNIA, USA

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Text

Early warning systems for plant disease based on remote sensing can provide rapid and accurate information for efficient resource management, thus reducing losses, expenses, and unintended negative environmental impacts. We previously found that deploying Machine Learning (ML) on spectroscopic imagery (SI) from NASA's Airborne Visible and Infrared Imaging Spectrometer Next Generation (AVIRIS-NG) yields accurate maps of grapevine leafroll-associated virus 3 (GLRaV-3) at multiple spatial resolutions. Providing these maps to agricultural stakeholders would reduce time, expenses, and uncertainty associated with management, however, both storing SI and training/deploying ML models require significant computing and storage resources. This challenge will magnify tenfold as global SI from the forthcoming satellite Surface Biology & Geology satellite becomes available. We present a cloud-native architecture for plant disease detection to address this challenge using SI from NASA's AVIRIS-NG with GLRaV-3 as a model system. Our system processes SI into disease incidence maps using simple ML (Random Forest, optimized through SMOTE) and easily accommodates new additions and improvements, as well as shifting data modalities, without retaining potentially proprietary stakeholder information. We present an innovative system that empowers stakeholders to make data-driven plant disease management decisions informed by cutting-edge SI while preserving reproducibility and user privacy.

P9.4-021

POTENTIAL USE OF UNMANNED AERIAL VEHICLES TO MONITOR EXPERIMENTAL FIELDS FOR TESTING THE RESISTANCE OF SUGAR BEET VARIETIES TO RHIZOCTONIA SOLANI USING ARTIFICIAL INTELLIGENCE AND OPTICAL SENSORS.

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Text

Rhizoctonia crown and root rot (RCRR) caused by *Rhizoctonia solani* AG2-2IIIB can cause severe yield and quality losses in sugar beet. The development of resistant varieties by identifying the genotypes that excel in resistance attributes is one of the principal strategies for disease management. Selecting resistance requires extensive and labor-intensive field trials and breeding work. This situation opens the potential use of unmanned aerial vehicles (UAV) and imaging sensors. From 2019 to 2022, field trials with 17 varieties artificially

inoculated with RCRR were carried out in Göttingen, Germany. The field plots were scored three times during each session, and the fields were monitored biweekly by UAV with multispectral cameras. A digital scoring model was trained and implemented based on multispectral cameras and artificial intelligence. The model first classifies the number of diseased plants within plots and then assigns an overall plot score. A significant relationship was observed between the model's prediction and the one manually obtained by the experts for each plot. The presented approach allows continuous large-scale monitoring during the growing season and could support breeders in determining differences between RCRR resistant varieties.

P9.4-022

NEW TOOLS AND E-TRAPS FOR RECORDING, COUNTING AND CLASSIFYING APHIDS IN FLIGHT AND THE PROSPECT OF EMBEDDING THEM IN MOVING PLATFORMS

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Text

Aphids pose a significant threat as pests of citrus fruit crops worldwide. They feed directly on plant sap, they damage crops and reduce yields, but most importantly, they are being vectors of plant viruses. The transmission of these viruses depends on the movements of aphids between different parts of a plant, between nearby plants, and further afield. The movement of aphids influences the timing of virus epidemics.
Detection of Aphids in flight is difficult due to their size and weak-flying abilities. We present optical devices that record their wingbeat and machine learning techniques that automatically classify species based on analyzing their wing beat frequencies and harmonics during flight. The species we studied are Aphis spiraecola and Aphis gossypii (Sternorrhyncha: Aphididae) feeding on tender citrus stems.
We further show examples of how this approach can be embedded in electronic insect traps. We also discuss their prospect in being integrated in mobile platforms such as tractors and drones that scan the field and derive flying-insect densities. New optoelectronic devices illuminate up to 2m away and as the aphids fly, they modulate and backscatter light with their wingbeat that is collected and turned to an audible recording that is subsequently analyzed and classified to discern insect species.
The end goal of this effort is to develop monitoring devices that automatically quantify the risk to a crop and guide the process of taking informed preventative measures.

P9.4-023

A MODELLING APPROACH TO MAP THE RISK OF HLB IN THE IBERIAN PENINSULA

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Text

Huanglongbing (HLB), or citrus greening, is a devastating citrus disease, currently found in Asia, Africa and North and South America. At present, no cases of HLB have been found in Europe, but in the past decade one of the disease vectors, the African citrus Psyllid (AfCP), has been found in several locations in North-Western Spain and Portugal. The presence of an established vector population means there is a high risk of transmission between citrus if HLB is subsequently introduced.

We present the findings of a 1 km^2 computational model of vector and pathogen spread in the Iberian Peninsula. The density of citrus in residential areas and commercial orchards, as well as climate suitability, influence the pattern of spread. The majority of vectors disperse locally and are dependent on the availability of citrus plants, but we also account for long-distance dispersal via mechanisms such as wind or human transportation. Using the current estimated distribution of AfCP as an initial condition, results often show a pattern of slow growth of the psyllid in the North-West. However, once long distance dispersal or new introduction of psyllid into the densely populated commercial citrus regions in the South or East of Spain occurs, the population quickly increases. There is subsequently a high risk of rapid spread of HLB upon the introduction of an infected plant in this region.

P9.4-024

ARE AVOCADOS TOAST? A FRAMEWORK TO ANALYZE DECISION-MAKING FOR EMERGING EPIDEMICS, APPLIED TO LAUREL WILT

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Text

The collective action of individuals is key for protecting food systems when managing disease. We evaluate how information exchange about epidemic and economic outcomes can influence the management decisions of individuals and the resulting epidemic, in the context of the avocado laurel wilt epidemic in south Florida. In scenario analyses, we

addressed how social and epidemic networks, policy incentives, and social behaviors combine to influence growers' management decisions and regional avocado health. We built an agent-based model to simulate epidemic expansion using parameters specific to the laurel wilt epidemic in south Florida. We found that increased social connections resulted in decreased crop health since increased sharing of information reinforced the selection of less expensive and less effective management choices. Information exchange was most impactful during the lag phase of epidemic expansion, when the cost of disease management outweighed the cost of disease. Managers who were "stubborn" against adopting these cheaper and less effective management strategies contributed to greater regional health. Growers responded to policies that penalized individuals more than to policies which offered financial benefits. This agent-based model represents key aspects of decision making, demonstrating the caveats of information exchange across social networks, and can be used to inform the decisions of avocado growers in regions at risk like Mexico and California.

P9.4-025

DEVELOPMENT OF BIOAEROSOL MONITORING TECHNIQUES IN GREENHOUSES USING HIGH THROUGHPUT SEQUENCING AND QPCR

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Text

In the past, air in cities and agricultural fields has been tested for pathogenic microorganisms, revealing important information on potential risks to human and plant health. However, surprisingly, such tests are far less common in greenhouses, while the risk of pathogen dispersal forms a considerable threat to the crop. As a part of a project on air monitoring in greenhouses, our aim is to investigate if high throughput metabarcoding approaches can play a role in early detection of airborne fungal and bacterial pathogens, such as powdery mildew (*Podosphaera xanthii*) and cucumber angular leafspot (*Pseudomonas amygdali* pv. *lachrymans*) in commercial greenhouses. In order to detect potential biases in DNA extraction as well as during PCR and sequencing that might result in failure to detect certain pathogens a custom mock-community of bacteria and fungi, relevant for greenhouse crops, was created. In addition, aerosols were collected with an air sampler in a greenhouse where airborne pathogens were known to be present. These samples and the mock community were subjected to Illumina amplicon sequencing. In addition, the presence of *P. xanthii* and *P. amygdali* pv. *lachrymans* were quantified in the air samples by TaqMan qPCR. Here, we will present results on the sequencing results and comparison to the TaqMan assay and discuss their relevance early detection and monitoring of greenhouse pathogens.

P9.4-027

ESTIMATING THE SENSITIVITY AND SPECIFICITY OF CITIZEN SCIENTISTS FOR EARLY DETECTION OF PLANT PESTS AND DISEASES

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Text

Introductions of invasive plant pests and pathogens are rising sharply. For example, in Europe, ash (*Fraxinus excelsior*) has been impacted by ash dieback disease (causal agent *Hymenoscyphus fraxineus*) and is now threatened by the spread of Emerald ash borer (*Agrilus planipennis*). Early detection increases the efficacy of control measures, however the ability to survey large areas is resource limited. Citizen scientists provide an invaluable resource to increase the survey area, but to provide surveillance metrics, such as the confidence of pathogen absence or the estimated pathogen prevalence, the sensitivity (probability of correctly identifying a pathogen when present) and specificity (probability of correctly declaring a pathogen absent when absent) of surveyors must be known. In 2022, 23 volunteers surveyed up to 176 oak (*Quercus*) trees in two long-term monitoring sites in the UK that have been expert assessed for three acute oak decline symptoms. Volunteer sensitivity and specificity was calculated using the expert data as a reference. This revealed substantial variation in volunteers' ability to recognise different disease symptoms accurately. This dataset was then used to examine the utility of Bayesian models to calculate the sensitivity and specificity of citizen scientists in the absence of an expert assessor as a reference. These methods enable inference of surveillance metrics from citizen science data and optimisation of risk-based surveillance in plant health.

P9.4-029

EARLY DIAGNOSTICS AND GENETIC POLYMORPHISM OF PEACH LEAF CURL FUNGUS TAPHRINA DEFORMANS IN GREEK PEACH ORCHARDS

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Text
Leaf curl is one of the most important fungal diseases of peach, caused by the pathogen *Taphrina deformans*. The disease has been particularly pronounced in recent years in Greece, causing extensive damage characterized by hyperplasia of host leaves. Aiming to develop an approach towards early diagnosis and knowledge of the pathogenesis of the causal agents of leaf curl disease in Greek peach orchards, innovative -omics technologies were used to optimize the integrated management of the disease. Leaf samples were collected from four main peach producing areas in Greece and 44 strains were isolated using the “spore fall” method. Sequencing with species specific primers led to the molecular identification of the strains as *T. deformans*. Specific primers were also used for the successful detection of the pathogen in the extracted DNA of artificially and naturally infected leaves. Genomic DNA was extracted from isolated fungal strains and underwent HRM analysis, SSR genotyping and Illumina re-sequencing in order to assess the genetic polymorphism of the isolates. Quantification and detection threshold of the pathogen in artificially and naturally infected leaves was achieved by real time PCR using specific primers created for this purpose. This study concluded in a robust and repeatable method for detection and quantification of the pathogen *T. deformans* and indicated a higher genetic polymorphism between strains from different Greek peach producing areas than within each area.

P9.4-030

COMBINING CONVOLUTIONAL NEURAL NETWORK (CNN) WITH THE STROMATA CONTOUR DETECTION ALGORITHM (SCDA V1.0) TO DETECT AND QUANTIFY TAR SPOT OF CORN

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Text
Plant disease detection and quantification depend on accurate visual observations by human experts. However, human rater subjectivity along with labor- and time-intensive disease ratings reduce the reliability and accuracy of disease detection and the throughput needed for the surveillance of emerging diseases. Tar spot of corn, originally endemic to Mexico and Latin America, is an emerging fungal disease in the United States. To accurately detect and track this disease, the Stromata Contour Detection Algorithm (SCDA) v1.0 was developed in 2021, which detects and quantifies tar spot stromata using Red-Green-Blue (RGB) images of infected maize leaves. However, the performance of SCDA is dependent on optimal input parameters that require empirical analyses of numerous images within a specific dataset. Here, we combined the capabilities of the SCDA and Convolutional Neural Network (CNN) to eliminate the empirical search for optimal input parameters while automating the stromata detection process. A preliminary dataset of 9000 tar spot images generated by the SCDA was annotated by a human rater and then used as testing and validation datasets (8:2 rule) to train the binary CNN classifier. The trained CNN model achieved high accuracy (> 93 %) and minimal loss (> 0.15 %) for both testing and validation sets. Our approach will be critical for building an effective, high-throughput, and efficient detection and surveillance platform for

tar spot of corn.

P9.4-031

HIGH-THROUGHPUT MONITORING OF PLANT-PATHOGEN INTERACTIONS BASED ON LOW-COST IMAGING

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Text

Imaging systems enable to monitor in parallel population of plants during their development. Most Agtech commercial systems to achive this goal are very expensive. This limits the diffusion of such technologies. Cost is specially important for plant pathogen interactions since the imaging systems have to be deployed in confined environment, cannot be moved from these environments and therefore have to be replicated, i.e. be affordable. We recently proposed such low-cost network of imaging systems which operate day and night and are based on raspberry-pi nanocomputers coupled with RGB and Depth cameras (1). In (2,3) the value of these systems were illustrated on the monitoring of abiotic stress. We extend the use of these imaging systems in this communication to biotic stress.

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P9.4-032

SUCCESSFUL MODELING RELIES ON BETTER DATA: THE POTENTIAL OF DATA SHARING AND STANDARDIZATION TO BOOST CROP DISEASE MODELING, WITH AN APPLICATION TO SOYBEAN SDS IN THE US MIDWEST

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Text

Abstract text
Onset of diseases is affected by several factors, including (among others) weather, soil pathogens, crop genetics, and different management decisions. These factors impose an immense parameter space that needs to be explored and understood to allow mitigation efforts. Addressing this task necessitates a collaborative approach between researchers and other stakeholders, which can be hampered by a myriad of data sources, formats and notations used by the participating groups. Data standardization platforms can streamline collaborations, enable more efficient research progress, and help capitalize on a big-data approach. This presentation will present the role of data standardization in creating high quality data for modeling. Using the Axiom platform (by Agmatix) as an example, observational data of sudden death syndrome (SDS) in soybean crops from 7 states in the US and Canada (2472 observations), collected between 2012-2016, was used to develop a machine learning prediction model. The model, built as an ensemble of decision trees, was able to predict SDS occurrence with an accuracy of 80% across the different production environments. Sensitivity analysis found the key factors affecting SDS in our data: i) crop genetics, ii) precipitation during specific growth periods, and iii) seed treatments. This analysis demonstrates the usefulness of standardization to foster collaborative efforts, and to leverage the collective power to cover critical research gaps.

P9.4-033

EXPLORING LEAF SPECTRAL REFLECTANCE AS A TOOL FOR EARLY DETECTION OF INFECTED KIWIFRUIT PLANTS WITH PSEUDOMONAS SYRINGAE PV. ACTINIDIAE

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Text
Leaf spectral reflectance (LSR) has been studied in several pathosystems as a tool for early detection of the infection, before symptoms' appearance, which is key for an effective disease management. However, this technology has been poorly explored in kiwifruit bacterial canker (KBC), and other diseases, through a dynamic monitoring after artificial inoculation. Here, *Actinidia chinensis* var. *deliciosa* plants grown in a climate chamber were mock- (control) and *Pseudomonas syringae* pv. *actinidiae* (Psa)-inoculated and monitored with a hyperspectral spectroradiometer (350-2500 nm) for measuring LSR at 1, 2, 5, 7, 9 and 14 days post inoculation (dpi). At 2 dpi (prior to symptoms' appearance) and 5 dpi (initial symptoms) LSR of Psa-infected plants was significantly lower compared with control plants in the following regions: ultraviolet (UV) (350-364 and 350-400 nm, respectively); violet, blue and green (400-502 nm, at 5 dpi) and red (660-686 nm at 5 dpi and 671-688 nm at 2 dpi). A lower LSR in the UV region was found in infected plants throughout the experiment (except at 1 and 7 dpi), whereas lower LSR in the red region was only related to early infection (<7

dpi). Moreover, higher LSR in the infrared region (>700 nm) was related to symptoms' progression, with Psa-infected leaves showing this alteration only at 14 dpi, when all plants had heavy KBC foliar symptoms. Hence, LSR analysis seems to be a valuable tool for early detection of Psa, allowing anticipated sanitary measures

P9.4-034

HEIGHTENED CONCERN OF VERTICILLIUM STRIPE OF CANOLA IN WESTERN CANADA

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Text

Canola (Brassica napus and B. rapa) is Canada's most valuable crop, contributing $29.9 billion CAD to the Canadian economy. But recently verticillium stripe, a new disease caused by the fungus Verticillium longisporum, may threaten this success.
Verticillium stripe is a soil-borne disease that was found first in Canada in 2014, and a survey in 2015 found this pathogen present in all Canadian provinces.
Currently canola disease surveys conducted independently in each of the Prairie Provinces (Manitoba, Saskatchewan and Alberta), have reported the presence of this disease.
From 2020 to 2022, the prevalence of verticillium stripe has risen 8% in Manitoba and in 2022 the province saw an average incidence of 23%. In 2022, Saskatchewan coordinated a targeted survey on the eastern side of the province revealed a 9.7% average incidence in canola fields. Alberta found two suspected cases of verticillium stripe in 2020 and reported seven cases in 2022.
Overall, the results from the disease surveys highlight that verticillium stripe could be an increasing problem for the canola growing region of Western Canada and emphasize the importance of ongoing survey efforts to track this disease.

P9.4-035

THE FUSARIUM ROOT ROT COMPLEX OF SOYBEAN, DRY BEAN AND FIELD PEA IN MANITOBA, CANADA

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Text
Fusarium root rot of soybean, dry bean and field pea is of concern in Manitoba, Canada, as there have been increasing numbers of reports of significant losses in plant stand and yield caused by this disease. Therefore, annual field surveys of soybean, dry bean, and field pea have been conducted to determine the prevalence and severity of root rot, identify the common root pathogens and detect the presence of any new root diseases. Since 2015, approximately 40 crops each of soybean, bean and pea have been sampled per year where roots were rated for the incidence and severity of root rot disease and causal fungal species were identified. The results have shown that root rot of soybean, bean and pea in Manitoba is primarily caused by several species of *Fusarium*. The *Fusarium* species isolated in each year included *F. oxysporum*, *F. graminearum*, *F. redolens*, *F. avenaceum*, *F. acuminatum* and *F. solani*, with some *Fusarium* spp. common to all three crops. A notable finding was the identification of *F. graminearum* in dry bean and soybean. With increasing acreages of these crops in Manitoba, *F. graminearum* may be problematic in current crop rotation regimes given its cross-pathogenicity and as a source of inoculum. Collectively, the findings from this research will support the development of effective management strategies for root rot disease associated with the *Fusarium* spp. complex affecting soybean, dry bean and field pea in Manitoba and across Canada.

P9.4-036

NOW YOU SEE ME: UV LIGHT REVEALS HIDDEN SYMPTOMS OF LETTUCE DOWNY MILDEW

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Text
Compatible plant-pathogen interactions result in diverse visible plant disease symptoms. Assessing these symptoms is the basis for identifying plant disease and quantifying disease severity, such as in plant breeding programs for disease resistance. But before the appearance of visible symptoms, the interaction has already triggered fundamental changes in the plant. Optical sensors can non-invasively detect such changes, and therefore enable early disease detection and quantification as well as provide insights into processes underlying symptom development. This can be particularly useful for pathogens that induce visible symptoms only late in the disease cycle, for example obligate biotrophs like the oomycete downy mildews. We found that lettuce leaf tissue that is colonized by the downy mildew pathogen Bremia lactucae emits increased UV-A-excited blue-green fluorescence before the appearance of other visible signs and symptoms. Transcriptome and metabolome analysis suggest that this fluorescence originates mainly from the accumulation of phenolic acids, potential precursors for pathogen-induced lignin biosynthesis. We also show that UV-fluorescence imaging can be applied for early and accurate downy mildew disease severity estimation. In combination with automatic image segmentation, this can provide a tool for sensor-based phenotyping in downy mildew resistance breeding. Keywords: Fluorescence, Phenotyping

P9.4-037

DEEP LEARNING BASED IDENTIFICATION OF CUCURBIT DISEASES AT THE MICROIMAGING SCALE WITH EXPLAINABLE SALIENCY ANALYSIS

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Text

The timely diagnosis of plant diseases plays a crucial role in integrated pest management and decision-making to enhance agricultural yields. Still, rapid disease classification and thereafter management remain difficult for smallholder farmers lacking robust resources. Existing deep learning models for on-site plant disease classification from image inputs at the macroscopic scale suffer from confounding variables in their backgrounds and limited capabilities in a field setting. We present the CUCMicroNet pipeline for the visual classification of biotic and abiotic stresses in five cucurbit species. Images were collected from a smartphone camera and attachable 30x microscopic lens, focusing on disease lesions of cucurbit leaves. We compared an array of data separation and backend neural network architectures on their prediction abilities, quantified by their mean average precision and F1 Scores. The approach merging all classes of plant condition together regardless of the cucurbit species yielded the highest prediction capabilities, with an average precision of 93.2%, and recall of 82.9%. To understand and interpret the CUCMicroNet classification reasoning, GradCAM and t-SNE saliency metrics were analyzed to display regions of interest in the image. This tool is expected to aid farmers, especially in remote places in the world, to make reliable decisions regarding disease management treatments.

P9.4-038

A REMOTE PLANT HEALTH DIAGNOSTIC PLATFORM TO PROMOTE REAL TIME DIAGNOSIS AND SUSTAINABLE CROP PROTECTION APPROACHES IN EASTERN AND WESTERN AFRICA

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Text

A remote PLANT HEAlth Diagnostic (PLANTHEAD) network is being developed within The H2020 EWA-BELT project (https://www.ewabelt.eu/), based on a platform hosting photographic database and geroreferentiation. An image repository is being built-up with vouchered pictures of symptoms of the most relevant diseases and pests affecting major crops in african countries. The local farmer sends an alert by mobile phone directly to the central HUB, providing relevant information such as localization, crop management, pesticide treatments, along with pictures (macro- or microscopic) and a short description of the problem. If the HUB can solve the problem, the platform sends the diagnosis and suggested actions

directly to the farmer. If not, the platform sends the request to the first node, which takes charge of it. The request goes to a higher node if the lower one is unable to provide a solution. The node can be local, national or international. Once the solution is found, the competent node formulates a diagnosis and the farmer receives a notification of successful response. This response is stored in the database for future use and the images feed an AI-based image recognition system. The shared database will represent an extremely valuable tool for epidemiological studies, real time monitoring, modelling, and forecasting the progression of a pathogen or any pest that may raise serious food security/ safety concern.

The Potential of Seed Microbiomes

C5.6-1

SEED MICROBIOTA: DIVERSITY, ASSEMBLY AND TRANSMISSION

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Text

Seed can act as a primary inoculum source for the plant microbiota. Understanding the processes involved in the seed microbiome assembly and dynamics during seed germination and seedling emergence has the potential to be used to improve crop establishment. This presentation will summarize current knowledge on the diversity of microbial communities associated with seeds of about 50 plant species. Particular attention will be paid to the stable bacterial fraction shared across multiple plant species at a large scale. The origin, timing of arrival and succession of these core seed-associated bacterial taxa will be detailed using common bean and radish as study models. The relative importance of selection in seed microbiota assembly will be discussed as well as the bacterial genetic determinants potentially involved in seed transmission. Finally, strategies to limit seed transmission of plant pathogens through seed microbiota engineering will be addressed.

C5.6-2

FUTURE OF SEED PATHOLOGY IN THE METAGENOMICS ERA

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Text

Seeds are a cornerstone to US agriculture as the beginning steps to production of most plant species. These plant reproductive vehicles are also habitats for microorganisms, ranging from the symbiotic to pathogenic. Historically, seed has been moved without many

phytosanitary barriers. However, a growing concern in plant-based agriculture and trade is the risk of transporting pathogens with seed. Rapid advancements in metagenomics, the collective DNA in an environment, is revealing that a normal plant microbial community can contain diverse microorganisms, including saprophytes, pathogens, and beneficial microorganisms. This includes the possibility of finding microorganisms of emerging risk to human, animal, and plant health that were not previously known to occur in plant tissues. This is particularly poorly understood for seed health across most cropping systems, including elucidating the biological risk of seed transmission. Metagenomics is beginning to be used in seed health screening, so there is an urgent need to define the core seed microbiome as an overall indicator of seed health and for determining relevant metrics for plant pathogen identification and risk assessment. This presentation will discuss how research can advance agricultural and economic security by defining healthy seed microbiomes in the context of plant diseases and provide guidance on biologically-relevant risk assessment for seed transmission of plant pathogens.

C5.6-3

NEW INSIGHTS INTO TREE SEED MYCOBIOMES – A BIOSECURITY PERSPECTIVE

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Text

Tree seeds have been considered as a low-risk introduction pathway for plant pathogenic fungi, and their movement is, thus, mostly unregulated from a phytosanitary point of view. This is despite historical evidence of some harmful fungi, such as *Diplodia sapinea* or *Fusarium circinatum*, being introduced to new areas via seed import, and recent discoveries about high diversity of seed-associated fungi. Here we present the results of large-scale studies in which we use traditional plating and RNA- and DNA-based high-throughput amplicon sequencing to characterise all and viable fungi associated with traded tree seeds and seeds from botanic gardens. In these studies, we assess 1) the diversity, 2) main drivers of species composition, and 3) vertical transmission of tree seed-associated fungi. Our results show that tree seeds carry highly diverse fungi, including potential plant pathogens, and that fungal species composition is mostly determined by the host tree species, possibly due to high prevalence of vertical transmission. These results suggest high risk of introduction and establishment of seed-borne fungi in new environments and highlight the need for additional studies regarding spread and impact of tree seed-borne fungi, as well as for improved risk assessment and management.

C5.6-4

SOYBEAN AND COTTON SPERMOSPHERE SOIL MICROBIAL DYNAMICS

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Text

The spermosphere is the transient, immediate zone of soil around imbibing and germinating seeds. It represents a habitat where there is contact between seed-associated microbes and soil microbes, but is studied less compared to other plant habitats. Thus, the objectives of this study were to develop an efficient strategy to collect spermosphere soils around imbibing soybean and cotton in non-sterile soil and investigate changes in microbial communities. The method employed sufficiently collected spermosphere soil as initially defined in space by constraining the soil sampled with a cork borer and confining the soil to a 12-well microtiter plate. Spermosphere prokaryote composition changed over time and depended on the crop within six hours after seeds were sown. By 12 to 18 hours, crops had unique microbial communities in spermosphere soils. Prokaryote evenness dropped following seed imbibition with the proliferation of copiotrophic soil bacteria. Due to their long history of plant growth promotion, prokaryote OTUs in *Bacillus*, *Paenibacillus*, *Burkholderia*, *Massilia*, *Azospirillum*, and *Pseudomonas* were notable genera enriched. Fungi and prokaryotes were hub taxa in networks. Additionally, the enriched taxa were not hubs in networks. Overall, this study advances knowledge in the assembly of the plant microbiome early in a plant's life.

C5.6-5

PREVALENCE OF VIRUSES IN PASTURE AND HORTICULTURAL PLANT SEED MICROBIOMES

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Text

Agriculture Victoria Research, a state government department, has a role in research to support the pasture-based dairy industry of Victoria, as well as a role in biosecurity. Metatranscriptomic studies have been done on commercial seeds of the pasture species *Lolium perenne* and *Medicago sativa*, and the diversity of viruses seen in these data sets will be presented. Viruses present include known viruses and novel viruses. Data on the presence of some of the viruses in seedlings grown from these seed will also be discussed. Using metatranscriptome data enables other members of the microbiome present to be estimated, and this data will be examined with respect to the diversity of viruses present in the samples. The viral diversity in pasture seeds will be compared to that in a set of 9 horticulture species, including *Capsicum annuum*, *Raphanus sativus* and *Cucurbita pepo* which have been profiled for viruses. Viruses detected in select public sequence runs from the NCBI short read archive will used to compare viral seed diversity across countries.

P5.6-001

EXPLOITING THE POSITIVE IMPACT OF SEED-BORNE FUNGAL ENDOPHYTES TO ENHANCE TOMATO SEED PERFORMANCE UNDER CHALLENGING ENVIRONMENTS

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Text

Seed germination is drastically decreased by biotic and abiotic stresses. The improvement of seed germination efficiency is, hence, a strategic priority to increase food production. There exists a need for environmentally-friendly solutions to compensate for chemical treatments currently used. A way to enhance seed performance is to exploit the beneficial impact of endophytes on plant fitness. The current view is that such protection relies on chemical mediations using the large variety of molecules produced by endophytes.
Tomato is one of the most important crop worldwide undergoing important periodic losses due to abiotic and biotic stresses. Tomato seed germination and seedling establishment are particularly sensitive to salt and water stress as well as fungal pathogens. Recent studies support the potential of endophytes to improve tomato tolerance to salt stress or pathogens. The objective of BIOSTIM is to develop sustainable agronomic solutions based on metabolites produced by fungal seed-borne endophytes. We investigate the diversity of seed-borne fungal endophytes in tomato cultivars used worldwide and test the potential of their metabolites to stimulate tomato seed germination under abiotic and biotic stress conditions.
So far, 60 fungal endophytes have been isolated and taxonomically identified from 15 tomato varieties and their metabolites extracted. Their impact on seed germination under abiotic and biotic stresses are currently investigated.

P5.6-002

INHERITANCE AND VARIABILITY OF SEED MICROBIOTA IN RICE

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Text
Inheritance of microbes is crucial for the persistence of host-associated microbial communities. Although the inheritance of seed microbes has been reported from diverse plants, temporal dynamics of microbial communities from parent to progeny remain scarce. Little is known concerning intra-plant variations in progeny seed microbiota at the single-seed level. In this study, we addressed the veiled dynamics of the transmission and variation of seed bacteria and fungi in rice. We identified 29 bacteria and 34 fungi vertically transmitted across generations. Abundance-based regression models allow us to classify the colonization types of the microbes. We found that they are late colonizers dominating each community at the ripening stage. Source-sink modeling showed that parental seeds and stem endosphere are major origins of progeny seed microbiota. We further investigated the heterogeneity of progeny seed microbiota using 70 single-seed samples from a single field-grown rice. We found that high heterogeneity of seed bacteria and fungi at the single-seed level. The observed variation patterns could be clustered according to the originating panicle branch. Null modeling-based statistical analysis revealed that homogeneous selection, dispersal limitation, and ecological drift governed the heterogeneity of seed microbiota. This study gives empirical evidence for the drivers and dynamics of seed bacterial and fungal communities as an ecological continuum.

P5.6-003

ANALYSIS OF TOMATO SEED MICROBIOME AND ITS BIOLOGICAL ACTIVITIES

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Text
Microbiota associated with plants plays a critical role in promoting plant health. Especially, seed endophytes play an important role in the germination and growth of seedlings at an early stage. To analyze the bacterial endophytes inhibiting the tomato seeds we isolated metagenomic DNA from tomato seeds of resistant (Hawaii 7996) and susceptible (Moneymaker) cultivars to bacterial wilt disease after removing microbiota on the surface of them. The comparison of bacterial endophytes in both cultivars of seeds revealed the identification of several operational taxonomic units (OTUs) as core microbiota in the resistant cultivar. Furthermore, the culture collection of seed endophytes was established under various cultural conditions. Among the isolated seed endophytes, *Moraxella osloensis* YHT4-1 and *Paenibacillus peoriae* YHR2-1 showed growth-promoting and antimicrobial effects against tomato plant pathogens, respectively. To verify the effect of the isolates the synthetic community (SynCom) including YHT4-1 and YHR2-1 strains was constructed based on in vitro and in silico analysis. The capability of disease suppression of SynCom was tested on Fusarium wilt disease in a susceptible cultivar of tomato plant using seed-soaking treatment. Results showed significant disease suppression and taxonomic profiling confirmed the presence of the inoculated SynCom. In conclusion, core microbiota from the resistant cultivar demonstrated beneficial effects on tomato plant health.

P5.6-004

PARENTAL INHERITANCE OF THE SEED MICROBIOME IN EUROPEAN ASH (FRAXINUS EXCELSIOR)

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Text

The community of microorganisms associated with a plant (the microbiome) is critical to plant fitness. However, little is known on how plants recruit the microorganisms. Here, we study the extent to which maternal trees transmit their microbiome to their offspring during seed development; a process that will lead to differences among clones that are caused by microbiome differences rather than the action of ash genes themselves. We used metabarcoding of the ITS1 intergenic region and the 16S rRNA gene to compare the seed microbiome of European ash (Fraxinus excelsior). Seeds were dissected and organ-specific microbiomes of each seed were profiled. We also profiled the microbiome of mother (seed stalk) and father (pollen) trees. This data will improve our understanding of how intrinsic factors shape the development of the seed microbiome from mother and father tree to their offspring.

P5.6-005

IMPACT OF SOME SEED-BORNE PATHOGENS AND SEED HEALTH TREATMENT ON SOYBEAN AND MAIZE CULTIVATION

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Text

Seed-borne diseases are a growing concern on crops of economical and social interest around the world. To control these diseases, some strategies can be applied having in mind the necessity to consider the high diversity and specific characteristics of seed borne agents in addition to several other factors. Two common sanitary measures, seed health testing and seed health treatment may be considered key measures to successfully control important seed-borne diseases in practice. Studies on those topics have been demonstrating that the combination of those practices for crops like soybean, maize, etc is responsible to produce great impact on yields and on quality of their products. Studies on seed health treatment in soybean and maize were carried out to evaluate the efficacy of chemical seed treatment and to quantify the effects of pathogens like C. truncatum and R. solani, S. maydis and F. verticillioides on their hosts. Impacts of the seed-borne inoculum of those pathogens and the efficacy of seed health treatment on plant development and on yields varied according to the fungal species, but in average each 1% incidence of the pathogen in seed lots was able to cause yield reduction in the range of 1.0 to 2.5 %. Chemical seed treatment was able to reduce losses in the range of 80 to 100 %. Effects of the seed-borne inoculum of all pathogens could be more drastic as the environmental conditions in the present case were not well favorable for the diseases development.

Towards structure-based design of disease resistance genes

C6.6-1

HOW TO CREATE NEW RESISTANCE GENES BY THE MOLECULAR ENGINEERING OF NLR IMMUNE RECEPTORS

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Text

Nucleotide-binding and leucine-rich repeat domain proteins (NLRs) are the predominant class of disease resistance proteins. They are intracellular receptors that detect pathogen-secreted virulence effectors. In addition to singleton NLRs, plants possess NLR pairs composed of one sensor and one helper (sNLR and hNLR) that cooperate for effector detection and disease resistance.
The NLR pair RGA4/RGA5 from rice recognizes the effectors AVR-Pia and AVR1-CO39 from the fungus *Magnaporthe oryzae*, which causes the devastating blast disease on rice. The unconventional Heavy Metal-Associated (HMA) domain of RGA5 is critical for effector binding, and we identified the surfaces that mediate complex formation. Using detailed knowledge on other rice sNLRs, we created a new high-affinity binding surface for the *M. oryzae* effector AVR-PikD. RGA5 variants with this engineered binding surface perceived the new ligand, AVR-PikD, and still recognized AVR-Pia and AVR1-CO39 in the model plant *N. benthamiana*. However, these modifications were insufficient to create a novel blast resistance specificity in transgenic rice plants. This pinpoints significant knowledge gaps that limit the full deployment of this NLR-ID engineering strategy and provided hypotheses that we are testing by studying the structure and dynamics of RGA4/RGA5 complexes at the resting and the active state.

C6.6-3

RECOGNITION OF PATHOGEN EFFECTORS BY NON-CANONICAL DOMAINS IN PLANT NLR IMMUNE RECEPTORS

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Text
Nucleotide-binding leucine rich repeat (NLR) proteins activate plant defences on perception of pathogen effectors. These innate immune receptors are a critical line of defence against plant disease and are a major component of crop breeding programs for disease resistance. A molecular mechanistic and structural understanding of their function underpins efforts to engineer these proteins with enhanced/extended activities that have potential for deployment in agriculture. One route by which NLR immune receptors detect effectors is through non-canonical integrated domains that directly bind these proteins. These integrated domains likely have their evolutionary origin in virulence-associated targets of effectors, but have been genetically incorporated into NLRs to act as bait domains. In the context of the full-length receptors, binding of effectors to integrated domains likely induces conformational change and oligomerisation to generate a signalling platform. Studies of the interaction between the integrated HMA domain of the rice NLR pair Pik and the rice blast pathogen effector AVR-Pik has enabled engineering of novel disease resistance profiles in rice. Recently, the Pik NLR chassis has been used to incorporate novel domains that bind different effectors, opening new opportunities for engineering resistance to pathogen strains that infect different cereal crops. Structure-led approaches continue allow for optimisation of effector recognition potential by integrated domains.

C6.6-4

REVIVE BROKEN NLR-GENES

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Text
Besides changing environmental and climatic conditions plant pathogens are one of the major threats to food security worldwide. A major aspect of plant breeding involves introgression of natural genetic resistances that arose from co-evolution of plants with their respective pathogens. The process of identifying novel resistances and their subsequent introgression into elite cultivars is a time consuming and labor-intensive process. Typically, these dominant resistance traits, often encoded by NB-LRR-like receptor genes (NLRs), exert only temporarily resistance in the field. Fast evolving pathogens such as viruses can overcome such resistances, sometimes by a single amino-acid change in their recognized effector proteins. One example of the latter is the interaction of the non-structural movement (NSm) protein of the tomato spotted wilt virus (TSWV) with the Sw5b coiled-coil NLR protein. A single amino-acid change in NSm (C118Y or T120V) breaks Sw5b resistance. To counteract the evolutionary adaption of pathogens we propose a high-throughput screening pipeline using in vitro molecular evolution to identify gain-of-function mutations in NLR proteins to resurrect broken resistances.

C6.6-5

DESIGNER RGA5 SENSOR RECEPTORS CONFERRING ALTERED SPECIFICITY OF BLAST RESISTANCE IN RICE

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Text

RGA5/RGA4 and Pik1/Pik2 are two paired nucleotide-binding leucine-rich repeat (NLR) receptors conferring blast resistance in rice. RGA5 and Pik1 function as sensor receptors that recognize, respectively, the corresponding Magnaporthe oryzae Avrs and ToxB-like (MAX) effectors AVR-Pia/AVR1-CO39 and AVR-Pik through their integrated heavy metal-associated (HMA) domains, leading to helper NLR RGA4 and Pik2-mediated blast resistance. Previous studies have determined the crystal structures of RGA5-HMA/AVR1-CO39 complex, Pik1-HMA/AVR-PikD and Avr-Pib, and shown that RGA5-HMA and Pik1-HMA are structurally similar and yet recognize the effectors through distinct interfaces. Based on the structures, we engineered the AVR1-CO39-recognizing interface in the RGA5-HMA domain and its following C-terminal lysine-rich tail, and generated a mutant of RGA5, named RGA5HMA2, which can recognize the noncorresponding MAX effector AvrPib and confer complete resistance in transgenic rice to the M. oryzae strains expressing AvrPib. In addition, we generated another RGA5 mutant RGA5HMA5 that confers resistance to M. oryzae strains expressing AVR-PikD, in which the HMA domain harbors three resurfaced interfaces, including one corresponding to the AVR-PikD/Pik-HMA interface. Altogether, our work demonstrates that a synthetic RGA5 sensor NLR requires a concerted action of multiple interfaces within and outside the HMA domain to both recognize MAX effectors and activate helper NLR-mediated blast resistance.

Tracing the long-distance pathways of aerial dissemination of plant pathogens

C3.5-1

ESTABLISHING A CANADIAN BIOVIGILANCE PLATFORM THROUGH NATIONWIDE AERONET, HIGH-THROUGHPUT SEQUENCING, AND TRAJECTORY SIMULATIONS

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Text

A Canadian biovigilance platform is under development aiming to entail inadvertent repercussions of pest migration arising from cultural practices and climate change and ultimately mitigate fungal disease threats to agricultural productivity. Over the past 15 years and through multiple developmental phases, this platform now consists of local and nationwide spore sampling networks (AeroNET), DNA-based diagnostic toolboxes, and trajectory modeling of pathogen inocula. As a proof of concept, we investigated aeromycobiota in western Canada using weekly air-sampling over four growing seasons at three mixed-use sites in British Columbia and one season at five sites in southern Alberta. Metabarcoding via rust-enhanced ITS2 amplicons generated from air-borne fungal spores and novel bioinformatic pipelines to curated known cereal rust fungal sequences, was used for species-level identification. Wind trajectory simulations predicted the potential sources of rust urediniospores and their airborne transmission pathways. We showed that the Canadian Rocky Mountains created a geographic barrier to upper wind flow to prevent the spread of rusts and other plant pathogens. Regional climate and crop diversification conditions also explained some pronounced changes in the diversity and abundance of wheat rust pathogens. This study paves the way for pathogen monitoring and disease risk forecasting as part of the design and development of enhanced biovigilance systems in Canada.

C3.5-2

SURVEILLANCE OF DISPERSAL AND PATHOGENICITY VARIATION OF CORN RUSTS

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Text

Corn rusts, caused by *Puccinia polysora* and *P. sorghi* have posed an increasing risk for global corn production. However, knowledge of their population structure, dispersal patterns, and virulence dynamics is limited. Here, we built a pipeline using HiFi reads and Hi-C data, and successfully assembled two pathogens to haplotype-phased and chromosome-level. The genome size of *P. polysora* (GD1913) and *P. sorghi* (LN2104) is 1.71 Gbp and 320 Mbp, respectively, with a similar sized 18 chromosomes in each haplotype. Genome resequencing of 79 additional isolates revealed a clonal population structure of *P. polysora* in China with low geographic differentiation. Nevertheless, a minor population differentiated from the major population by having mutations on secreted proteins including *AvrRppC*, indicating the ongoing virulence evolution to evade recognition by *RppC*, a major resistance gene in Chinese corn cultivars. Some *P. polysora* strains showed different phylogenetic positions when maped to the phased genome and single nuclei, suggesting that somatic recombination could contribute to lineage differentiation. Combined with the mantel test and lineage distribution along typhoons, we considered that the dispersal pattern of *P. polysora* in China was not simply from south to north but affected by multiple possible origins, e.g., southeast Asia. The population genomics of *P. sorghi* and its comparison with *P. polysora* were also studied.

C3.5-3

A METACOMMUNITY MODEL FOR ENTANGLED AIRBORNE PATHOSYSTEMS: APPLICATION TO BROWN ROT SPREAD IN PEACH ORCHARDS

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Text

Plant disease dynamics are driven by concurrent host susceptibility, pathogen presence, and environmental conditions favourable to infection. While host susceptibility and environmental conditions have been widely studied, pathogen presence, in case of wind dispersal, is particularly complex to investigate. In this study we conceive a metacommunity model where local communities composed by plant hosts and the pathogen are connected through airborne pathogen dispersal, to describe the spatio-temporal dynamics of a fungal disease, attacking stone fruits, in continental France. We explicitly consider climatic drivers affecting pathogen dispersal and survival, host phenology and epidemic processes. We demonstrate the model against a real pathosystem, brown rot spread in French peach orchards, using measures of disease incidence and weather reanalysis. We eventually produce maps of risk distinguishing site vulnerability (inclination to be affected by secondary infections) and dangerousness (inclination to be generate secondary in other sites). Our results satisfactorily match past observations of brown rot incidence in France. Moreover, we found that most vulnerable regions are placed along the Rhône Valley, in the Roussillon and the Montauban plain, the first two being also the most dangerous. Our work represents a first step to use air-masses movements to inform plant protection strategies, and could be adapted to perform optimization under future climate projections.

C3.5-4

GENOMIC ANALYSIS, TRAJECTORY TRACKING, AND FIELD INVESTIGATION REVEAL ORIGINS AND LONG-DISTANCE MIGRATION ROUTES OF WHEAT STRIPE RUST IN CHINA

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Text

Understanding the origins and migration routes of phytopathogen inoculum is essential in predicting disease development and formulating control strategies. Puccinia striiformis f. sp. tritici (Pst), the causal agent of wheat stripe rust, is an airborne fungal pathogen threatening wheat production by long-distance migration. Due to large variation in geographic features, climatic conditions, and wheat production systems, inter-regional Pst dispersal routes in China remain largely unknown. In the present research, we sequenced 154 Pst isolates

sampled from all the major wheat-growing regions in China to study the Pst population structure. Western Qinling Mountains, Himalayan region, and Guizhou Plateau were found to be centers of Pst origin in China. Combined with trajectory tracking and field disease surveys, long-distance Pst migration routes from individual origins were proposed. The present findings will improve current understanding of Pst origin and migration in China and emphasize the need for managing stripe rust at the national scale.

C3.5-5

ASSESSING LONG-DISTANCE, TRANSOCEANIC AND INTERCONTINENTAL ATMOSPHERIC TRANSPORT OF SOILBORNE PLANT PATHOGENS ENTRAINED WITH AEROSOLIZED AGRICULTURAL DUST

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Text

Soilborne pathogenic fungi are a leading cause of crop disease and are primarily spread through microscopic, durable spores adapted differentially for both persistence and dispersal via soil, animals, water and atmosphere. While intracontinental aerial dispersion of soilborne fungal spores has been well established, transoceanic and intercontinental atmospheric transport of these spores entrained with aerosolized agricultural dust is understudied and may contribute to disease spread. Our NASA ROSES project seeks to address this need by integrating remote sensing, aerosol transport and comparative genomics to assess the long-distance atmospheric dispersal of the plant pathogenic, soilborne fungus *Fusarium oxysporum* (*Fo*) on global dust currents. The CAM6-MIMI climate model was modified to incorporate spore traits that influence dispersal and atmospheric survival, and was parameterized using the 2020 Godzilla dust event. We found modeling evidence of transoceanic and intercontinental atmospheric transport of viable *Fo* spores and offered a danger index for *Fo* spore deposition on susceptible agricultural zones. The main long-distance transport of viable spores and the highest danger for deposition on cropland are between the regions of Eurasia, North Africa, and Sub-Saharan Africa. This study provides key insights about Fusarium wilt epidemiology and lays the groundwork to build an operational, real-time global surveillance system of long-distance plant pathogen transport risk.

C3.5-6

USE OF SPATIO-TEMPORAL AEROBIOLOGICAL DATA OF SPORE ABUNDANCE TO UNDERSTAND THE LARGE-SCALE EPIDEMIOLOGY OF THE ASH DIEBACK DISEASE

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Text

Aerial dispersal of pathogenic fungi is a key process in disease epidemiology and an effective long-distance dispersal method. Quantification of airborne inoculum at different spatial scales can then inform disease epidemiology and management. Here, we use pollen-monitoring permanent aerobiological networks in Europe to inform large-scale epidemiology of the ash dieback disease. We focused on the ongoing epidemic in France as case study. Captured Hymenoscyphus fraxineus spores were estimated by qPCR on 696 samples from 2015 to 2018 from 31 locations with a duration of disease presence of 0 to 9 years, and were aligned with the nation-wide database of disease occurrence.
We detected H. fraxineus spores in disease-free locations, up to a distance of 318 km from the nearest recorded outbreak. Number of spores linearly increased with the distance to the closest disease record. The maximum number of spores was recorded six years after disease arrival, and then decreased, with a strong effect of the sampling year. The proportion of positive samples per year linearly increased with the time of disease presence (9-year range). While pathogen detectability increased with time of disease presence, inoculum load peaked at six years of disease presence. The use of mass air movement probability in our models will be discussed. Our results suggest an attenuation of pathogen load with time. Aerobiology proves a powerful tool to monitor pathogen colonization in airborne invasive pathogens.

P3.5-001

SINKS, SOURCES AND BOTTLENECKS OF ATMOSPHERIC CONNECTIVITY NETWORKS

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Text

Tracking and monitoring airborne plant pathogens pose serious challenges to plant epidemiologists since the dispersal medium, atmospheric air, is constantly moving in unpredictable and untraceable ways. Nonetheless, it is well known that both short- and long-distance dissemination events can occur as pathogenic propagules (fungal spores or bacteria) are released in to the air and passively transported from infected to susceptible hosts. From the epidemiological perspective, we still miss a formal description of the connectivity network that emerge from air mass movements. In this work we propose a new methodological framework to reconstruct these networks across large geographical areas. Our method relies on the analytical transformation of dynamical systems (atmospheric air circulation patterns) into network objects using a trajectory-based formulation of the node degree, an essential property of networks. The method is based on the computation of the

backward- and forward-in-time Lyapunov exponents and allows to identify critical nodes of the dynamic connectivity network that shall be prioritized for monitoring and surveillance, such as source, sink and bottleneck nodes. We compare our results to pollen count data from the French Aerobiology Surveillance Network and we test the hypothesis that bottleneck nodes, i.e., nodes with higher network betweenness, corresponds to those trapping stations where pollen diversity is higher across all seasons.

P3.5-002

PATHOTYPIC AND MOLECULAR CHARACTERIZATION OF XOC IN SENEGAL

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Text
Bacterial streak of rice (BLS), is a disease caused by Xanthomonas oryzae pv. oryzicola (Xoc), was first reported in Senegal by Trinh in 1980 and confirmed by H. Tall in 2022. BLS poses a serious threat to rice cultivation in West Africa. Characterization of the pathotypic and genetic diversity of bacterial populations is essential for the management of pathogen-resistant varieties. Pathogenicity tests show that all strains are virulent on the susceptible rice variety Kitaake. Phenotyping of Kitaake RXo1, Carolina Gold and IRBB1 showed that the Xo1 and Xa1 genes control 99% of the isolates tested, and that MLVA analysis identified 10 haplotypes grouped into 3 clonal complexes that are defined by a single locus variant (SLV) and 4 singletons differing from each other by 2 to 7 loci. In the St. Louis area where the largest number of strains were isolated, the genotypic diversity of the Podor Xoc collection is greater than the Dagana collection based on the richness estimated by the rarefaction procedure with MLG values of 4.056 and 2.517 respectively. The strains collected in 2014 are much more diverse than those collected in 2015, and progeny links are observable between haplotypes 8 and 9, and 1 and 2.

P3.5-003

SEASONAL SPORE PRODUCTION, GERMINATION AND FUNGICIDE RESISTANCE SHIFTS OF CERCOSPORA BETICOLA IN COMMERCIAL SUGAR BEET FIELDS IN THE USA

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Text
Cercospora leaf spot (CLS) caused by Cercospora beticola (Cb) is the most important foliar sugarbeet disease. Management includes cultural practices, resistant varieties and timely fungicide applications. Disease prediction models monitor conditions for disease spread and fungicide application, but do not include spore production and germination that may be important for predicting protective fungicide application prior to disease. Laboratory

experiments indicate spore germination begins in two hours at 10°C, is higher in free water and increases with time and temperature. Spores from fungicide resistant isolates tend to have a lower germination rates compared to fungicide sensitive isolates at lower temperatures. Field studies were conducted in 2021 and 2022 to study timing of Cb spore detection in commercial sugarbeet fields. Spore trap (Spornado) contents were tested for Cb DNA by PCR three times weekly from May to August. Asymptomatic leaves were tested for the presence of Cb DNA by PCR and monitored for first appearance of CLS. In both years, Cb spores were detected before sugar beet emergence and Cb DNA was detected in asymptomatic plants before CLS was observed. Continued seasonal testing showed changes in fungicide resistance to QoI and DMI fungicides. We conclude that forecasting models for CLS should include spore detection and early wetness conditions, and adjusted to recommend fungicide applications earlier in the growing season before infection by Cb.

P3.5-004

QUANTIFICATION OF AIRBORNE BASIDIOSPORES OF GANODERMA ZONATUM AT DIFFERENT ALTITUDINAL GRADIENTS AND THEIR RELATIONSHIP WITH ENVIRONMENTAL CONDITIONS IN OIL PALM

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Text

Basal Stem Rot, caused by *Ganoderma zonatum*, is one of the most limiting emerging diseases of oil palm crops located in northern Colombia. The hourly concentrations of *G. zonatum* basidiospores in the air were quantified using four 7-day Burkard volumetric samplers at different heights (1, 4, 7, and 10 m) installed on a scaffold-type structure located inside an 18-year lot of African palms (*Elaeis guineensis*) highly affected by the disease. Hourly records of air temperature, rainfall, and relative humidity at the capture site were also collected. The results obtained indicated that there are no significant differences in the concentration of basidiospores captured at different altitudinal gradients, which suggests a great dispersal capacity of *G. zonatum* when reaching higher altitudes or close to the palm canopy. The concentration of inoculum in the air varies according to the seasonality of the year. The highest concentrations of captured basidiospores occurred several hours after a rain event >5mm, being more frequent during the wet season. Regarding the seasonal patterns, a higher concentration of basidiospores was found at night (absence of solar radiation) between 5:00 p.m. and 4:00 a.m., when the air temperature was 20 to 23°C and the relative humidity was >80%. These concentrations decrease considerably during daylight hours, especially between 11:00 a.m. and 2:00 p.m., when the air temperature was 27 to 30°C and the relative humidity was <65%.

P3.5-005

DISPERSAL KERNELS ARE STEEPER THAN THE OBSERVED GRADIENTS

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Text

Measuring dispersal is important for understanding how pathogen populations change in time and space, since capacity for dispersal is a fundamental fitness component. We measured rain splash-driven dispersal of the major fungal wheat pathogen Zymoseptoria tritici (Zt) and estimated its dispersal kernel in field conditions for the first time. We inoculated field plots of wheat (Triticum aestivum) with two distinct Zt strains and measured the disease intensity as counts of fruiting bodies using automated image analysis. These measurements characterized primary disease gradients, which we used to estimate effective dispersal of the pathogen population. Genotyping confirmed the conclusions drawn from phenotypic data. While analysing the dispersal gradient data, we realized that our assumption of a point-like source was flawed. Although dispersal gradients contain information on dispersal, they are influenced by the spatial extent of the source. We solved that challenge with a theory that incorporates the spatial extent of sources to estimate dispersal kernels from dispersal gradients. We analysed the collected data using this spatially explicit mathematical model, which allows for a more accurate estimation of dispersal kernels. Additionally, we re-analysed published dispersal gradients for two other plant pathogens (stripe rust of wheat, potato late blight) and concluded that all three pathogens disperse over substantially shorter distances compared to conventional estimates.

Understanding emergence of pathogens in commercial and public forest ecosystems

C2.6-1

PATHOGEN EMERGENCE IN CHANGING ECOSYSTEMS: CASE STUDY WITH PHELLINUS NOXIUS IN EASTERN ASIA, AUSTRALIA, AND THE PACIFIC ISLANDS

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Text

In recent decades, brown root rot disease (BRRD), caused by *Phellinus noxius* of unknown origin, has emerged to cause extensive damage to diverse trees in tropical/subtropical regions. Understanding the population structure, demographic history, and potential pathways of spread is essential to determine if BRRD emergence was caused by invasion

of *P. noxius*. In this study, we characterized genetic relationships, pathways of spread, and evolutionary histories of *P. noxius* collected from 15 locations in eastern Asia, Australia, and the Pacific Islands. We analyzed patterns of genetic variation by measuring correlations in allele frequencies and genetic drift. Also, we applied the coalescent-based analyses using approximate Bayesian computation (ABC) with supervised machine learning. Population structure analyses revealed five distinct genetic groups of *P.noxius* with signatures of complex migration histories among study locations. ABC analyses indicated that the pathogen was most likely spread from a “ghost population” to Malaysia and the Pacific Islands, with subsequent spread to Taiwan and Australia. Major pathogen spread likely occurred 1,000s of generations ago, contradicting previous assumptions that *P. noxius* was recently introduced into many areas. Our results suggest that *P. noxius* has a long evolutionary history in the study region, and recent disease emergence is likely driven by anthropogenic and natural disturbances in many regions, rather than by recent introductions.

C2.6-2

CAN ALIEN FOREST PATHOGENS REPLACE NATIVE ONES? THE CASE OF HETEROBASIDION IRREGULARE AND H. ANNOSUM IN EUROPE

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Text

Heterobasidion irregulare and *H. annosum* are destructive pathogens of pines in North America and Europe, respectively. The former was introduced in central Italy during WWII and has been spreading in the range of the native *H. annosum* causing high mortality of *Pinus pinea* in natural forests, artificial plantations and urban parks. Based on its current and potential impacts, *H. irregulare* is recommended for regulation by EPPO. While *H. irregulare* is clearly fitter that *H. annosum* based on some key traits, one intriguing question is whether the alien species has the potential to replace the native congener in Europe.
We addressed this issue by using an aerobiological assay replicated ten years apart in a forest in central Italy were both *Heterobasidion* species have been coexisting. Replacement index (RI), Markov chains and geometric progressions were used to model the interspecific interaction between the two species and to assess the invasiveness of *H. irregulare*. Results showed that, in 10 years, the incidence of *H. annosum* dropped from 39.4 to 6.1%, while that of *H. irregulare* increased from 57.6 to 81.8%, with the alien pathogen replacing the native one (RI = 84.6%) and spreading at a maximum rate of 139 ha/year. This study adds new pieces of information on the invasiveness of *H. irregulare*. In addition, modelling results clearly support a potential for replacement of *H. annosum* in Europe, although the extinction of the native species appears unlikely.

C2.6-3

BOTRYOSPHAERIACEAE ASSOCIATED WITH BAOBAB (ADANSONIA DIGITATA L.) AND MARULA (SCLEROCARYA BIRREA A. RICH.) IN AGROFORESTRY SYSTEMS IN KENYA

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Text

Cultivation of Indigenous fruit trees such as baobab and marula provide key nutrients and income for smallholders and enhance diversification of agroforestry systems in the drylands of Sub-Saharan Africa. Cankers and diebacks are increasingly observed impacting baobab and marula in domestication trials and farms in Kenya, but little is known on disease occurrence and associated pathogens. Field disease incidence and severity was assessed. Fungal isolation and molecular identification were performed, and pathogenicity of isolates was evaluated on baobab, marula and additional agroforestry trees; *Vachelia xanthophloea* and *Calodendrum capense*. Nine taxa morphotypes belonging to genera *Lasiodiplodia*, *Neofusicoccum* and *Dothiorella* were identified co-occurring in both symptomatic and asymptomatic plant material. Seedlings inoculated with isolates of *L. pseudotheobromae*, *L. theobromae* and *N. parvum* showed similar symptoms with various degree of virulence ($p<0.001$). These findings suggest that species of Botryosphaeriaceae may occur as endophytes and also act as a disease complex, with the potential of infecting a wide range of trees in Eastern Kenya. Further investigation of ecology and impact of this potential threat to agroforestry systems in the African drylands, need to be performed in order to develop mitigation strategies. Further, their occurrence as endophytes may complicate disease management hence and exchange of disease free germplasm is important for stakeholders.

C2.6-4

COMPARATIVE GENOMICS TO DECIPHER ADAPTATION OF THE FUNGAL PATHOGEN AUSTROPUCCINIA PSIDII TO HOST SPECIES IN THE MYRTACEAE FAMILY

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Text

Myrtle rust, caused by *Austropuccinia psidii*, is responsible for significant economic, and environmental impacts. Brazil is the center of origin for *A. psidii*, an obligate biotroph, that has its asexual dikaryotic stage associated with epidemic outbreaks on a wide global host range. Thus far, four main genetic groups of the pathogen, with specialization to different

hosts and/or geographic regions, have been reported worldwide: two biotypes showing a strong relationship with different hosts in Brazil; a polyphagous biotype restricted to South Africa, and a polyphagous pandemic biotype, widely spread globally. In order to facilitate our understanding of the genetic relationship and evolution of the different biotypes, we sequenced the genome of three Brazilian isolates with evidence of host adaptations to *Eucalyptus* spp., *Syzygium jambos* and *Psidium guajava*. The new genomes were HiC-phased and annotated, in order to compare haplotypes with the genome of the pandemic biotype. We identified specific and shared groups of genes related to pathogenicity among the four isolates. These results are important resources to better decipher host adaptation processes to different species in the Myrtaceae family. Moreover, these results provide insights into the genetic relationships and evolutionary history of the pathogen, which are key to preventing new incursions of the pathogen around the world.

Funding: FAPESP grants 2019/13191-5; 2021/01606-6; 2022/11900-1

C2.6-5

PHYTOMONAS SP. CAUSING SUDDEN WILT DISEASE ON YELLOW BLEEDING HEART (DACTYLICAPNOS SCANDENS) (D. DON) IN INDIA

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Text

We have noticed sudden wilting of Dactylicapnos scandens and found that it is an aflagellate Phytomonas sp. The disease incidence was recorded at 72 percent and 50% yield loss. The symptom begins at the leaf margin as a water-soaked brown lesion. It spreads through the vine and branches. Dieback is also observed. Vines are dried and die within three weeks. The symptoms are also observed on the outer petal of the flower, which turns brown and flaccid, followed by drying and dropping of the flower. The cross-sectioning of one-week-old infected tubers shows dark brown symptoms, followed by blackening and hollowness within two weeks. In the third week, the tuber becomes empty and rotten. Aflagellate protozoa were found in the tubers, leaves, and inflorescences of infected plants. It measures 19.2 µm to 36 µm in length and width of 4.4µm to 9.6 µm. The pathogenicity of the protozoa was proved by inoculating 1 ml of 4 x 108 protozoa cells on the healthy tubers with a 0.45 mm syringe and then keeping them in a humid chamber at 24.2 °C and 90% relative humidity for 72 hours. It was observed that after 48 hours, the incubated tubers started showing brown discoloration upon dissection, and after 72 hours, they became dark brown and soft. After five days, it turns black and becomes mummified. The pathogen remains viable in the infected tubers for months. Phytomonas sp. can cause disease and yield losses of up to 50%, and it is transmitted through infected tubers.

C2.6-6

EMERGENCE OF CRYPTOSTROMA CORTICALE IN EUROPE

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Text

Cryptostroma corticale is an ascomycete responsible for the sooty bark disease (SBD) on maples. Native from eastern North America, the pathogen has spread over the last few decades in Europe and in the Pacific Northwest of North-America. The spores of *C. corticale* can be also pathogenic for humans causing hypersensitive pneumonitis. *C. corticale* can remain asymptomatically in host tissues, and its development has been linked to drought conditions. Here, we study the conditions of emergence and the aerobiology of the SBD in Europe. We analyzed two-year aerobiological and epidemiological data in France, and one-year aerobiological data from six European countries. Additionally, we modelled disease outbreaks and climatic data in France and Switzerland from 1990. Aerobiological detection of the pathogen was successful in countries within the host native area and with longer disease presence, such as France, Switzerland and Czech Republic, and absent in countries where the pathogen has not been reported yet. The disease was frequently reported in France and Switzerland after the severe drought and heat waves of 1990-1991, 2003 and especially 2018-2020. SBD occurrence was strongly related to the water balance in the vegetative season (April-August) of the year preceding disease report. Aerobiological surveillance can inform the spatial distribution of the SBD, and contribute to early detection in pathogen-free countries.

P2.6-001

MICROBIOME INTERACTIONS OF HETEROBASIDION FRUITING BODY AND ASSOCIATED DECAYED WOODY TISSUES

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Text
Heterobasidion annosum species complex is a major threat to the forestry industry. Fungal and bacterial communities in *Heterobasidion-infected* trees have been extensively studied for disease management, but less is known about the inter-kingdom interactions. The inter-kingdom analysis has revealed significant findings in recent studies by providing insights into the pathobiology of the disease as well as ecologically meaningful data. In this study, we aim to 1) Unravel the contribution and development of the bacteria and other fungi in the *Heterobasidion* infected wood decay process; 2) Uncover how the environmental factors and conifer pathogen drive the microbiome community structure and function changes; 3) Identify the core microbiome during the *Heterobasidion* mediated wood decay process. ITS and 16S amplicon sequencing data from Heterobasidion fruiting body and its associated woody tissue were applied in this analysis. Samples collected from managed forests and nature-reserved forests were classified into four decay classes based on the extent of decay of the wood. Our result shows that the abundance, activeness, and environmental response sensibility of bacteria and fungi are different in study materials. The *Heterobasidion* ecological correlation network is conservative. The core microbiome defined in our analysis showes huge potential in the microbiome community functioning. Forest management shows the biggest impact on the microbiome assembly.

P2.6-003

GENOMIC DIVERSITY, PATHOGENICITY AND STABILITY OF EPIPHYTIC PSEUDOMONAS POPULATION IN PRUNUS SPP. - SEARCHING FOR THE SOURCES OF NEW DISEASE OUTBREAKS

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Text
Despite advances in disease management, the bacterial phytopathogen *P. syringae* remains a threat to commercial cherry production. Aside from the known causes of canker, pathogenic potential has also been found in *P. syringae* strains from diverse environments. This study investigates regional and temporal variations in epiphytic *P. syringae* populations in cherry orchards in four regions of the UK across two years. In addition, to enrich insights into the prevalence of *P. syringae* in non-agricultural environments, we also sampled wild cherry and related plant species in woodlands nearby. A total of 12,000 bacterial strains were isolated from leaves and shoots in May and September of both 2021 and 2022. Multiplex PCR was used to identify *P. syringae* and detect the presence of genes encoding the Type 3 Secretion System (T3SS), a hallmark of pathogenicity, followed by whole genome sequencing. Intriguingly, a distinct reduction in the frequency of T3SS+ strains has been found in strains isolated from Scotland. These results indicate the reduced prevalence of potentially pathogenic strains in Scotland, and equally importantly, an interesting regional variation in *P. syringae* populations. Through further analysis of these environmental isolates,

we will improve our understanding of how evolution of epiphytic populations leads to new outbreaks of cherry canker.

P2.6-004

CERIPORIA LACERATA MAY BE A POTENTIAL TREE PATHOGEN

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Text

Ceriporia species are well-known saprotrophic fungi that cause wood decay in the forest. Although some endophytic *Ceriporia* spp. have been isolated from living plants, none of them was considered a pathogen. However, a weakly grown tree of *Delonix regia* with the white resupinate basidiocarps of *Ceriporia lacerata* (syn. *Irpex laceratus*) covered on its roots and stem base suddenly toppled over in October 2021 at the campus of National Taiwan University. *C. lacerata* was isolated from the interior white rot wood tissues and confirmed for its identity using multi-locus phylogenetic analysis based on ITS, 28S, *rpb1*, *rpb2* and *tef1* genes. To investigate the pathogenicity of *C. lacerata*, wounded and nonwounded inoculations were conducted on the stem of 1-m-high *D. regia* seedlings. Although none of the infected plants showed visible symptoms within 122 days post inoculation, *C. lacerata* caused browning of the interior wood at the inoculation site, and *C. lacerata* re-isolated at the frequency of 80% from the bark and 25% from the wood tissues. The results indicated that *C. lacerata* is able to infect living woody plants and caused wood degradation. Considering that *C. lacerata* is a potential tree pathogen, the inhibitory effects of 13 fungicides with 9 modes of action were evaluated on the mycelial growth of *C. lacerata*, and tebuconazole and propiconazole were the most effective. How *C. lacerata* affects the health of living plants and its management are worthy of further investigation.

P2.6-005

TOP DOWN AND BOTTOM UP: HOW THE INTERACTION OF TWO PATHOGENS FUELS MORTALITY OF COMMON ASH (FRAXINUS EXCELSIOR) IN EUROPE

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Text

Since the early 1990s, the invasive pathogen *Hymenoscyphus fraxineus* has been causing severe dieback of common ash (*Fraxinus excelsior*) and narrow-leaved ash (*Fraxinus angustifolia*) in Europe. Recently, several cases of windfall of ash trees with green foliage were reported in Switzerland. The uprooted trees frequently showed signs of Armillaria root disease. To shed light on the interaction of *H. fraxineus* and *Armillaria* spp. and to develop

criteria to identify potentially hazardous ash trees, we investigated frequency and severity of Armillaria root disease on common ash trees showing varying degrees of crown dieback. In addition, we conducted non-invasive static pulling experiments on a subset of the investigated ash trees to estimate their resistance to uprooting and stem breakage. We found that the probability of Armillaria root disease increases with crown dieback severity. Most *Armillaria* infections were caused by *A. gallica* and *A. cepistipes*, which primarily act as opportunistic pathogens on weakened trees. Ash trees with a reduced resistance to uprooting were more often infected by *Armillaria* spp. than trees resistant to uprooting or stem breakage. Based on these results, we are developing recommendations for practicians to assess the safety of ash trees infected by *H. fraxineus* and/or *Armillaria* spp.

P2.6-006

A CASE STUDY OF EXPANSION OF HETEROBASIDION PARVIPORUM GENOTYPES IN A NORWAY SPRUCE STAND ON PEAT SOIL

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Text

The aim of the study was to analyse the long-term expansion of root rot fungus Heterobasidion spp. genets in a 72-year-old, Norway spruce (Picea abies (L.) H. Karst.) stand on drained peat soil (Oxalidosa turf. mel. forest type. The stand was for the first time sampled for Heterobasidion root rot in 2009 and for the second time in 2019. Heterobasidion was isolated from 142 trees/stumps (28%) in 2009 and from 159 trees/stumps (30%) in 2019. In total, 29% of the analysed spruces were infected by Heterobasidion spp. In 2019, 65 Heterobasidion genets were identified. Single-tree genets represented 45% and multi-tree genets (expanded through mycelial growth via root contacts) 55% of all genets. Over a ten-year period, the total number of genets had increased by 10 and the mean number of trees infected by one genotype increased from 3.7 to 4.2. Moreover, 18 genets had expanded while 12 had disappeared. Our result indicates that rapid degradation of stumps and roots on peat soils related to shading and high moisture may limit secondary infection of Heterobasidion spp. Therefore, it is important to prevent primary spore infections, particularly reducing aboveground root damage during selective thinning.
This research was funded by JSC Latvian State Forests project No. 5-5.9.1_007q_101_21_79, “Investigation of the impact of root rot and reducing risks caused by root rot”.
Key words: Picea abies; root rot; genets; secondary infection; drained forests.

P2.6-007

ENVIRONMENTAL REGULATION OF THE HYMENOSCYPHUS FRAXINEUS LIFECYCLE

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Text

Ash dieback disease symptoms were first noticed in Europe in the 1990s, although it now transpires the pathogen, *Hymenoscyphus fraxineus*, was present at least a decade earlier. The pathogen infects ash (*Fraxinus*) foliage via ascospores ejected from apothecia formed on fallen ash leaves shed the previous year. Mortality of European ash (*F. excelsior*) is widespread across the continent due to the disease. Our study investigated environmental effects on apothecia development and ascospore ejection, and examined the relationship between ascospore density, host litter colonisation, and disease severity. Apothecia development was monitored at six sites in 2018, and seven sites in 2019, along with temperature, relative humidity, canopy closure and ground cover data. Additionally, aerial ascospore density was measured at one site in 2018 and six sites in 2019, with colonisation of *F. excelsior* litter and crown dieback severity recorded in 2020. Results revealed that temperature positively affects apothecia development, with most effect at higher relative humidity or under a sheltered litter layer. Ascospore ejection was positively affected by both temperature (with greater effect in more exposed litter layers), and relative humidity. Ascospore density was also positively related to colonisation of host litter, which in turn was related to disease severity. Our study demonstrates environmental regulation of the *H. fraxineus* lifecycle and suggests consequences for disease progression.

P2.6-008

POPLAR RUST NEVER SLEEPS: EVOLUTIONARY CHARACTERIZATION OF THE FIRST AVIRULENCE GENE IN THE POPLAR RUST FUNGUS MELAMPSORA LARICI-POPULINA

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Text

Poplar rust, caused by the pathogenic fungus *Melampsora larici-populina* (Basidiomycota, Pucciniales), is the main phytosanitary constraint for commercial poplar cultivation in Europe and other parts of the world. In the last 50 years, many rust-resistant cultivars were bred and released, but all the qualitative resistance genes released were overcome by pathogen evolution within a short period. In 1994, breakdown of the *RMlp7* resistance gene was detected in Belgium and Northern France. New virulent *M. larici-populina* individuals spread all over Western Europe in less than five years, causing very destructive epidemics, and lead to a complete replacement of the pathogen's populations. Through a genome-wide association study (GWAS), we identified a locus in the genome of *M. larici-populina*, that corresponds to the candidate avirulence gene *AvrMlp7*, whose mutation is responsible for RMlp7 resistance breakdown. To further characterize this effector, we used a population genetics approach on a set of almost 300 individuals collected throughout a 28-year period encompassing the resistance breakdown event. Genotyping at the avirulence locus highlighted two different mutations: a non-synonymous mutation and a complete deletion of this locus. The temporal survey at the candidate locus revealed that both mutations pre-

existed long before the breakdown, but at a heterozygous state. Using a reverse ecology approach, we identified the first candidate avirulence gene in this rust fungus.

Viral modification of plants and vectors

C2.4-1

MODELLING VIRAL MANIPULATION OF PLANT-VECTOR INTERACTIONS

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Text
Diseases caused by plant viruses threaten food security and ecosystem services worldwide. Almost all plant viruses are spread by insect vectors. Over the past decade or two, extensive experimental evidence has accumulated indicating plant viruses can manipulate their host to cause vectors to prefer to visit infected plants. More recent experimentation has shown how the direction and magnitude of such effects can depend on the infection status of the vector itself. There are further interactions between the infection status of the plants upon which vectors feed and vector population dynamics. Complexities also follow from distinctions between persistent and non-persistent transmission, which are expected to have significant effects on the optimal strategy for viruses to manipulate their host. Mathematical modelling offers a framework to make sense of complex interactions such as these, identifying key principles affecting spread of plant viruses when viruses can manipulate vectors and/or hosts. In this talk, I will describe some recent progress in this area.

C2.4-2

WHITEFLY-BORNE CASSAVA VIRUSES AND THE ESTIMATION OF VIRAL RETENTION PERIOD FROM ACCESS PERIOD EXPERIMENTS

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Text
Estimation of the duration that a pathogen is retained in an insect vector - i.e., viral retention period - is of particular importance for understanding plant virus epidemiology. This is typically investigated in access period experiments in which the duration of access of insects to plants is experimentally varied. In this presentation I will discuss new modelling advances for estimating viral retention period from access period experiments.

In recent decades a cassava mosaic begomovirus epidemic (CMV) spread by Bemisia tabaci whitefly has severely affected cassava yield in sub-Saharan Africa, and this was followed by

a regional cassava brown streak ipomovirus epidemic (CBSV) spread by the same vector.

I will discuss the use of the two modelling approaches for estimating viral retention period in relation to CBSV and CMV. In addition, I will discuss how low insect survival from manual transfers of insects in the laboratory may have influenced existing retention period estimates.

C2.4-3

THE ACROSTYLE, A CUTICULAR MICRO-TERRITORY WITHIN APHID MOUTHPARTS INVOLVED IN VIRUS-VECTOR AND PLANT-INSECT INTERACTIONS

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Text

Aphids are among the most frequent and economically important vectors of plant viruses in the world. The majority of aphid-vectorized viruses are transmitted in a non-circulative manner and are retained transiently on specific receptors on the surface of the stylets as the insects move from plant to plant. In order to identify receptors and explore the binding properties of aphid stylets, our team has developed various approaches, including "omics", microscopy techniques and 3D imaging, or gene function validation.
We have shown that the proteins are not uniformly distributed in the stylets conferring local functionalization of cuticular micro-territories such as the acrostyle, a discrete structure at the apex of the maxillary stylets hosting virus receptors. Interestingly, some viruses have adapted to use this structure and hijacked the acrostyle from its original function for their own benefit. Our data recently highlighted the role of the acrostyle in the retention of a saliva effector known to modulate plant responses in the early phases of aphid feeding. The acrostyle thus acts as a platform for multiple interactions capable of binding both self and nonself molecules. Furthering our knowledge of the properties of the acrostyle and the mechanisms of interaction on its surface offers avenues for disease control strategies.

C2.4-4

MULTI-INFECTION MODIFIES APHID TRANSMISSION AND PLANT TISSUE LOCALIZATION OF SUGAR BEET INFECTING VIRUSES

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Text

Aphids transmit multiple plant viruses separately or simultaneously to a new host. Sugar beet is frequently infected by several viruses transmitted by aphids. Some of the viruses infecting sugar beet (the closterovirus BYV and the poleroviruses BChV&BMYV) are restricted to the plant phloem while a potyvirus (BtMV), invades all cells of the plant. Here we investigate how BYV co-infections with the other viruses change aphid transmission and virus localization.

First, we optimized a detection method, SABER fluorescent in situ hybridization, to localize all sugar beet viruses simultaneously within co-infected tissues and cells. Using this technique, we started with BYV/BChV co-infection. Our results showed co-localization of the two viruses in the phloem cells. This correlated with a higher transmission of BYV and a lower one of BChV. Changes in viral load explain lower transmission of BChV but not higher transmission of BYV. Currently, we are testing whether changed aphid behavior on co-infected plants is responsible for higher BYV transmission. Co-infection of BYV&BtMV resulted in different localization of the two viruses in the plant tissue, compared to mono-infection, but this time it correlated with lower transmission of BYV. Other selected multi-infections are presently tested for correlation between localization, transmission, accumulation & aphid behavior, in order to understand the mechanisms behind the cellular localization and how they impact transmission.

C2.4-5

DYNAMICS OF POTATO VIRUS Y-PLANT CELL INTERACTION NETWORK

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Text

Potato virus Y (PVY) is the most economically devastating plant virus affecting the potato production worldwide. To complete its infection cycle the virus reprograms the intracellular environment of the host and a complex and dynamic interaction network is established between viral and plant proteins. This study aims at better understanding the role of viral and host proteins in such interconnected pathways. We focused in particular on two viral proteins: viral genome-linked protein (VPg) and coat protein (CP). We prepared a PVY clone with the VPg fluorescently tagged and followed the localisation of the protein during the infection cycle. Supporting the fact that VPg is a multitasking protein, the preliminary results showed that it can be observed in several plant cell organelles i.e nucleus, cytoplasm, plasma membrane and endoplasmic reticulum. We also searched for VPg interacting partners using pull-down assay. Several plant proteins have been identified and we are confirming the interaction using Yeast 2-hybrid system. Within this project we are also studying the involvement of the CP protein in the viral cell-to-cell movement. Using a PVY clone tagged with GFP we confirmed the N-terminal residues of the CP are crucial for the viral movement. We are now introducing short deletions at the N-terminus to identify the critical amino acids for the cell-to-cell movement. The knowledge acquired in this study will help developing effective agricultural management strategies.

C2.4-6

AN APHID-BORNE POLEROVIRUS SWITCHES ITS VECTOR TO THE WHITEFLY BEMISIA TABACI: AGRONOMIC IMPORTANCE, EPIDEMIOLOGY AND VIRUS-VECTOR MOLECULAR RELATIONSHIPS

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Text

Poleroviruses, like all other members of Luteoviridae are phloem-limited RNA viruses, which are exclusively transmitted by aphids. In the last decade, poleroviruses have been on the rise due to frequent recombination events between members of this group. Outbreaks with vein yellowing, leaf rolling, fruit discoloration and change in fruit taste in pepper cultivations in Israel, despite low aphid populations, led to reinvestigating the disease and its transmission by vectors. A new recombinant polerovirus was discovered, and strikingly demonstrated a shift in its insect vector from aphids to the whitefly Bemisia tabaci. Full genome of this new virus, named as Pepper whitefly borne vein yellow virus (PeWBVYV) was characterized. PeWBVYV shares homology (>95%) with Pepper vein yellows virus (PeVYV) described from Israel and Greece on its 5' end half while being homologous to African eggplant yellow virus (AeYV) on the 3' half. The recombinant PeWBVYV was not transmissible by aphids but was transmitted by B. tabaci MEAM1 species and not MED. Parameters for acquisition and transmission of the new virus by B. tabaci, competitive interactions with PeVYV inside aphids and whiteflies, proteins interacting with the new virus in aphids and whiteflies, and geographical distribution of both viruses were investigated. PeWBVYV is the first report of a whitefly-transmitted polerovirus.

F2.4-1

PLANT VIRUS INFECTION MODIFIES VOLATILE CUES INVOLVED IN MULTITROPHIC APHID-PLANT-PARASITOID INTERACTIONS

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Text

Several plant viruses manipulate their hosts to modify the attractiveness of their insect vectors, in a way that enhances their own spread. Cucumber mosaic virus (CMV) emit volatile organic compounds (VOC) that attracts its main vector, *Aphis gossypii*. However, it is not known how aphids respond to plants double infected with additional viruses such as Cucurbit aphid-borne yellows virus (CABYV). Furthermore, plants often emit volatiles after aphid feeding that attract natural enemies to limit pest damage ("call for help"). However, few studies have been conducted on how aphid parasitoids react to VOC emitted by virus-infected plants. Thus, we conducted Y-tube olfactometer assays to understand how simple and double infection (CMV+CABYV) can modify volatile cues involved in aphid-attraction and in aphid-plant-parasitoid interactions. Our results confirmed that VOC emitted by CMV-infected melon attracted *A. gossypii* but when plants became infected with CMV+CABYV aphid attraction was suppressed. We also found that in the presence of aphids, *Aphidius colemani* was attracted to mock-inoculated plants but rejected VOC emitted by CABYV-infected plants. Thus, our work reports for the first time that a plant virus can counteract the "call for help" plant defense response involved in parasitoid attraction to its aphid host. This could ultimately induce lower rates of parasitism on CABYV-infected plants reducing the effectiveness of biological control of *A. gossypii* by *A. colemani*

P2.4-002

IMPACT OF 'CANDIDATUS LIBERIBACTER ASIATICUS' ON THE EXPRESSION AND ACTIVATION OF TOLL SIGNALING PATHWAY GENES IN DIAPHORINA CITRI, THE VECTOR OF CITRUS GREENING DISEASE

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Text

Citrus greening disease, (Huanglongbing; HLB), is putatively caused by the phloem-restricted bacterium '*Candidatus* Liberibacter asiaticus'. HLB is an economically important disease of citrus worldwide. In Florida, HLB is endemic causing huge damage to the citrus industry. HLB is transmitted by the Asian citrus psyllid, *Diaphorina citri*. To date, the disease management mainly depends on insect vector control by insecticides. Toll signaling pathway proteins and receptors play crucial roles in embryonic development and immunity in insects. In the current study, we identified and performed *in silico* analyses of toll-related proteins in *D. citri*. Our findings indicate that the relative expression of Toll pathway genes was altered in the '*Ca*. L. asiaticus'-infected compared to healthy *D. citri*. Furthermore, we knockdown *pelle* and *tube*, two key genes in the Toll system using RNA interference technology. Silencing of the *pelle* and *tube* increases nymphal mortality and shortens the adult lifespan. Currently, we are investigating the effect of silencing the dorsal gene in the embryonic development of *D. citri*. In addition, we are evaluating the effect of silencing Toll genes on the susceptibility of *D. citri* to Gram-positive and Gram-negative bacteria. Ultimately, targeting Toll system by RNAi may help in mitigating the HLB disease by misfunctioning the development process, limiting the vector immunity, and interfering with the '*Ca*. L. asiaticus' transmission by *D. citri*.

P2.4-003

PLANT BACTERIAL PATHOGEN MANIPULATES THE FATTY ACIDS METABOLISM OF THE INSECT VECTOR TO FULFILL ITS NUTRITIONAL NEEDS

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Text

Diaphorina citri is the main vector for the '*Candidatus* Liberibacter asiaticus' pathogen, which is associated with citrus greening. *D. citri* transmits '*Ca*. L. asiaticus' during its feeding on the phloem sap of citrus. The mode of transmission is circulative, propagative, and persistent. '*Ca*. L. asiaticus' has a small genome (1.2 mbp). Therefore, it acquires most of its nutrients

from its hosts. We assessed the effect of '*Ca*. L. asiaticus' infection on the level of the free fatty acids in its vector. The fatty acids and triglycerides were extracted from adult *D. citri* using ethyl acetate, derivatized with boron trifluoride-methanol, and analyzed using GC-MS. Nine fatty acids were identified in the extract of *D. citri* adults. Oleic acid was the most predominant fatty acid followed by stearic acid and palmitic acid, whereas the rest of the fatty acids were present in low amounts. In general, the levels of the detected fatty acids in '*Ca*. L. asiaticus'-infected *D. citri* were lower than those found in healthy psyllids. Our findings showed that, the reduction of fatty acids in '*Ca*. L. asiaticus'-infected psyllids resulted from the higher activity of the β-oxidation to generate acetyl-CoA, which could be converted to citrate or used to convert alanine to proline in the fat body and to produce more ATP. In conclusion, our results indicated that '*Ca*. L. asiaticus' may enhance the β-oxidation of fatty acids in its vector insect to fulfill its nutrient requirements.

P2.4-004

SHORT-DISTANCE MOVEMENT OF BEMISIA TABACI MEAM1 AND TRANSMISSION OF TOMATO SEVERE RUGOSE VIRUS TO TOMATO PLANTS IN THE FIELD

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Text

The tomato golden mosaic disease, caused by the tomato severe rugose virus (ToSRV), is considered one of the most important diseases in tomato fields. The ToSRV is transmitted by *Bemisia tabaci*, from a wide range of hosts. The ToSRV arrival in commercial fields and the epidemic development are highly related to the presence of near inoculum sources. In that context, monitoring studies can be a helpful tool to understand the spread dynamic of the vector and, consequently, the disease. This study evaluates the short-range fly distance of *B. tabaci* MEAM1 through recaptures in yellow stick traps and the transmission of ToSRV by viruliferous adults through the release in an arena with tomato plants placed in knowing distances. Of the two proteins source tested (albumin and lecithin), albumin was efficiently detected by PTA-ELISA. There were no effects on mortality rates when the insects were labeled. The field tests showed that a label reduces the number of recaptured insects compared with unmarked ones. Due to its easy detection, dye labeling was considered more straightforward. Most whiteflies only achieved a 30 m distance, which was the maximum of ToSRV-infected plants detected. The results of this study reinforce the suspects that the epidemic-supporting source of ToSRV inoculum must be in the close range of commercial fields and provides new tools to understand the *B. tabaci* MEAM1 movement, based on different labeling methods.

P2.4-005

IRIS YELLOW SPOT ORTHOTOSPOVIRUS-THRIPS COMPLEX IN ONION PRODUCTION AREAS IN THE USA: PROGRESS TOWARD DEVELOPING MANAGEMENT TACTICS

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Text

Iris yellow spot orthotospovirus (IYSV) is predominantly transmitted by onion thrips (Thrips tabaci Lindeman, Thysanoptera: Thripidae) and continues to be a production constraint for seed and bulb onion crops in several onion producing states in the USA. Ongoing research over the last several years was directed toward understanding the epidemiology including the role of thrips vectors, diversity of onion thrips populations and screening of onion breeding lines for virus resistance. Onion thrips is a major pest of onion and serves as IYSV vector, however, there is limited information available on the genetic variation within and between T. tabaci populations in the USA. A total of 92 COI gene sequences of T. tabaci from A. cepa were analyzed including 83 T. tabaci specimens were collected from A. cepa from 15 locations comprising four states of the USA. 7 distinct haplotypes of T. tabaci were identified from the current collection, while 9 T. tabaci sequences retrieved from GenBank comprised 5 haplotypes. Overall, 15 haplotypes of T. tabaci infesting A. cepa were identified in the world that includes the ten haplotypes in the USA. In the phylogenetic analysis, all the populations collected during the study clustered with thelytokous lineage. Results suggested that haplotypes 1 and 7 are more frequently prevailing haplotypes in the northwestern USA, with haplotype 1 being the predominant all over the country. The eastern USA appears to have a more diverse group of haplotypes.

P2.4-006

BIASED POLLEN TRANSFER BY BUMBLEBEES FAVORS THE PATERNITY OF VIRUS-INFECTED PLANTS IN CROSS-POLLINATION

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Text

Volatile organic compounds emitted by virus-infected bean and tomato plants attract bumblebees. Bumblebees vibrate tomato flowers to extract pollen (‘buzz pollination’) and, in doing so, maximize seed production by increasing the self-pollination rate. CMV infection decreases tomato seed yield, except in the presence of bumblebees; buzz pollination activity rescues infected plant seed yield. We proposed that this may have resulted from pollinator preference for flowers of CMV-infected plants that increased their success as female parents, by increasing the probability of ovule fertilization, and that this constituted a ‘payback’ from the virus to its susceptible hosts. Here, we used a GFP marker gene for paternity analysis to determine if virus infection affected male reproductive success in bee-mediated cross-pollination. We found that bees that first visited flowers of infected plants showed a strong preference to subsequently visit flowers of non-infected plants; suggesting

the insects either find infected plant pollen distasteful, or direct contact with infected flowers repellent. The behavior of the bees to move towards non-infected plants after pollinating the infected, appears to explain the paternity data, which show a statistically significant ~10-fold bias for fertilization of non-infected plants with pollen from infected parents. Thus, in the presence of bumblebee pollinators, CMV-infected plants exhibit enhanced male, as well as female, reproductive success.

P2.4-007

THE RENEWED THREAT OF TOMATO SPOTTED WILT, A GLOBAL AGRICULTURAL PANDEMIC

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Text

With an expansive host range of over 1000 plant species across 90 families, tomato spotted wilt virus (TSWV) — since its first report in 1915 in Australia — has become a pandemic virus with an estimated economic impact over $1 billion annually. Current management strategies for TSWV heavily rely on growing single gene resistant cultivars of tomato ('*Sw-5b*' gene) and pepper ('*Tsw*' gene) deployed worldwide, combined with multiple pesticide applications to control thrips, a supervector of TSWV and a cosmopolitan pest. However, the increased virulence of TSWV through emergence of resistance breaking (RB) strains in recent years has significantly escalated what is already an unparalleled dual threat to agricultural production worldwide. Our lab reported new, more virulent TSWV RB strains, with novel RB mutations capable of swiftly disrupting single-gene resistance in all tested commercial tomato and pepper cultivars. Data on comprehensive genomic analysis, characterization, and transmission biology of three tomato-infecting RB strains from Texas, California, and Mexico and one pepper-infecting RB strain from Texas will be presented. Our progress in developing TSWV reverse genetic system (led by Dr Verchot's lab at Texas A&M University) and a potential of novel RNAi-based approach to manage the dual threat of TSWV and thrips will be discussed.

P2.4-008

VECTORS OF XYLELLA SPP. AND ITS ROLE IN GLOBAL TRANSMISSION

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Text
Vectors play an important role as sentinel in *Xylella* spp. transmission. The vectors of *Xylella* spp. belong to taxonomic groups of three different superfamilies (Cercopoidea, Cicadoidea, and Membracoidea) within the order Hemiptera worldwide. An overview of the categories of vector distribution shows contrasting results in different continents. In the Americas, sharpshooters (Cicadellidae: Cicadellinae) are major vectors while spittlebugs (Aphrophoridae) are minor; in Europe, spittlebugs and cicadas (Aphrophoridae and Clastopteridae) are main vectors. As to Asia, Africa, and Oceania, where vector information was relatively limited, sharpshooters are major vectors as well. Despite the invasion of exotic vectors, many cases of Xylella spp. transmission were taken via local insects, suggesting the inoculum source might come from imported infected plants and plant materials. For instance, both vectors in Taiwan are native insects whereas the sequence data of the pathogen is identical to America. Since there is still no effective treatment for *Xylella* spp.-caused disease, the infectious rate can be reduced by controlling vectors, detecting and removing infected host plants to prevent further spread. To prevent global transmission, preventive measures must be taken by increasing the intensity and frequency of host plant products quarantine in high risks countries to block out infected vectors and pathogens.

P2.4-009

INDUCTION OF APHID RESISTANCE IN TOBACCO BY THE CUCUMBER MOSAIC VIRUS CMVDELTA2B MUTANT IS JASMONATE-DEPENDENT

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Text
Cucumber mosaic virus (CMV) is vectored by aphids, including Myzus persicae. Tobacco (Nicotiana tabacum ‘Xanthi’) plants infected with a mutant of the Fny strain of CMV (Fny-CMVΔ2b, which cannot express the CMV 2b protein) exhibit strong resistance against M. persicae, which is manifested by decreased survival and reproduction of aphids confined on the plants. Previously, we found that the Fny-CMV 1a replication protein elicits aphid resistance in plants infected with Fny-CMVΔ2b, whereas in plants infected with wild-type Fny-CMV, this is counteracted by the CMV 2b protein; a counter-defence protein that, among other things, inhibits jasmonic acid (JA)-dependent immune signalling. We noted that in nontransformed cv. Petit Havana SR1 tobacco plants aphid resistance was not induced by Fny-CMVΔ2b, suggesting that not all tobacco varieties possess the factor(s) with which the 1a protein interacts. To determine if 1a protein-induced aphid resistance is JA-dependent in Xanthi tobacco, transgenic plants were made that expressed an RNA silencing construct to diminish expression of the JA co-receptor CORONATINE-INSENSITIVE1. Fny-CMVΔ2b did not induce resistance to M. persicae in these transgenic plants. Thus, aphid resistance induction by the 1a protein requires JA-dependent defensive signalling, which is countered by the CMV 2b protein.

P2.4-010

EFFECTS OF A VIRAL COUNTER-DEFENSE PROTEIN ON PLANT-INSECT INTERACTIONS IN ARABIDOPSIS THALIANA

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Text

Cucumber mosaic virus (CMV) infection of a host plant has been shown to induce changes in plant secondary metabolism. In *Arabidopsis thaliana* these include altered emission of volatile organic compounds (VOCs) and soluble molecules such as glucosinolates, which influence the interactions of a host plant with aphids, important viral vectors. For example, aphids confined to plants infected with Fny-CMV showed reduced growth and reproduction. The multifunctional CMV-2b protein disrupts signalling mediated by key defense and stress-related phytohormones such as jasmonic acid and has been implicated in modulating changes in VOC emission that lead to altered insect behaviour and viral spread. My research explores the mechanisms behind this phenomenon. Aphid growth rate and reproduction experiments were conducted as measures of aphid health and behaviour on *2b*-transgenic plants, which revealed that the growth and reproduction of generalist aphid *Myzus persicae* was significantly reduced when aphids were confined to plants expressing the 2b protein, while two species of specialist aphids were not significantly impacted by *2b* expression. Choice tests are also being conducted to determine the settling preferences of these aphid species on non-transgenic and *2b*-transgenic plants. This research clarifies the mechanisms behind CMV's effective manipulation of an infected host plant to accelerate its spread, with significant implications in understanding an agronomically important pathogen.

P2.4-011

MONOPARTITE AND BIPARTITE BEGOMOVIRUSES DIFFERENTIALLY INTERACT WITH TWO COMMON WHITEFLY CRYPTIC SPECIES (B AND Q)

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Text

Begomoviruses transmitted by the Sweetpotato whitefly, Bemisia tabaci Gennadius, are major constraints to vegetable production in the United States. Monopartite tomato yellow leaf curl virus (TYLCV) and bipartite cucurbit leaf crumple virus (CuLCrV) and sida golden mosaic virus (SiGMV) affect crops such as tomato, cucurbits, and snap bean. Bemisia tabaci cryptic species B (MEAM 1) is predominant outdoors, and B. tabaci Q (MED) cryptic species is limited to greenhouses. However, in recent years, B. tabaci Q has been found colonizing outdoor ornamentals and field crops in Florida and Georgia. This has raised concerns on whether the dispersal of B. tabaci Q cryptic species into the landscape could exacerbate the virus epidemics situation. Competence in the transmission of CuLCrV,

SiGMV, and TYLCV was compared between B. tabaciB and Q. Bemisia tabaci B efficiently transmitted all three viruses, whereas the Q did not transmit the two bipartite viruses. TYLCV loads in both cryptic species following acquisition was similar, but bipartite virus levels were substantially lower in B. tabaci Q than in B. Virus loads in various whitefly tissues revealed a similar trend. A series of molecular and Omics approaches were undertaken to decipher the differences in interactions between monopartite and bipartite viruses with both cryptic species. An exciting number of transmission-influencing candidates were identified, and some were functionally validated. These findings will be discussed.

P2.4-012

PRESENCE OF TOBAMOVIRUS ALTERS THE MECHANICAL TRANSMISSIBILITY OF BEGOMOVIRUS

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Text

Virus-virus interaction is a common phenomenon that causes recombination, host range change, virus transmissibility, and fluctuation of virus titer. It makes the management of virus control in the field more complicated. In this study, we tried to identify interactions between RNA viruses and DNA viruses and its implications to DNA viruses' mechanical transmissibility. Two tobamoviruses and four begomoviruses were used for analysis. TMV, ORSV, ToLCNDV-OM, and TYLCTHV are mechanically transmissible viruses, but ToLCNDV-CB and ToLCTV are not. Coinfection of one tobamovirus and one begomovirus was conducted by coagroinfiltration in Nicotiana benthamiana. Symptoms were observed at 8 dpi and systemic leaves of N. benthamiana were used for mechanical inoculation to new plants. Results showed that TMV abolished mechanical transmissibility of ToLCNDV-OM and TYLCTHV, but TMV helped ToLCNDV-CB and ToLCTV for mechanical transmission to N. benthamiana. Mechanical inoculation of TMV and ToLCNDV-OM coinfection revealed that only ToLCNDV-OM but not TMV could be detectable in oriental melon. TMV failed to inhibit the ToLCNDV-OM in non-host oriental melon. ORSV also helped ToLCNDV-CB and ToLCTV for mechanical transmission, but it didn't alter mechanical transmissibility of ToLCNDV-OM and TYLCTHV in N. benthamiana. In oriental melon, ORSV didn't help ToLCNDV-CB for mechanical transmission. Analysis of virus accumulation and the interaction mechanisms of this phenomenon is ongoing.

P2.4-013

IDENTIFICATION OF PLANT VIRUS RECEPTORS IN THE STYLETS OF THEIR INSECT VECTORS USING CRISPR-CAS9 MUTANT APHID LINES

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Text
Aphids are the only known vectors of stylet-borne non-circulative viruses comprising hundreds of species in the genera *Caulimovirus*, *Potyvirus* and *Cucumovirus*. These viruses can be acquired and inoculated from one plant to another within seconds when aphids feed. They are retained on receptors located at the surface of the cuticle of insect mouthparts. The *Cauliflower mosaic virus* (CaMV) was shown to bind the acrostyle, an organ at the tip of aphid stylets. Stylin-01, a cuticular protein from the CPR family directly accessible at the surface of the acrostyle, was shown to play a role in CaMV transmission. To characterize Stylin-01 role in viruses transmission as a receptor for CaMV and other viruses, stable Stylin-01 mutant lines were generated via CRISPR-Cas9 technology in the pea aphid *Acyrthosiphon pisum*. Our work focuses on two Stylin-01 mutant lines: one with a complete knockout of the protein and another with an alteration at the cuticle/fluid protein interface. While demonstrating a major role of Stylin-01 in CaMV transmission, our results show that at least a second cuticular protein is involved in the retention of this virus at the surface of the acrostyle. In addition, our data indicate that the transmission efficiency of *Turnip mosaic virus* (TuMV, *Potyvirus*) is poorly affected in aphid mutant lines, thus showing that Stylin-01 is not the main receptor for TuMV. All together, our data demonstrate the existence of at least two virus receptors in aphid stylets.

P2.4-014

THE INFLUENCE OF DIAPHORINA CITRI FLAVI-LIKE VIRUS ON HUANGLONGBING BACTERIA VECTORING AND PSYLLID BIOLOGY

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Text
Diaphorina citri, the insect vector of citrus Huanglongbing (HLB) disease, transmits *Candidatus* Liberibacter asiaticus (*C*Las) between plants. Insect virus provides an effective target-specific method to decrease the *C*Las transmission. The influence of insect viruses on psyllid biology and the interaction between these viruses and *C*Las remain unclear. Here, *D. citri* flavi-like virus (DcFLV), one of four associated viruses in the gut virome, was identified by high throughput sequencing and selected for further study. We show that DcFLV systemically infects *D. citri* and is vertically transmitted to the offspring. On the cellular level, dark necrotic portions were observed in DcFLV-exposed midguts that are similar to the *C*Las-exposed midguts of psyllids. We also measured significant differences on the expression level of apoptosis- and endoplasmic reticulum (ER) stress-related genes in DcFLV-infected psyllids compared to DcFLV-free psyllids. *C*Las and DcFLV co-occurred in field-collected psyllid populations and co-localized in the guts and salivary gland cells of *D. citri*. Moreover, DcFLV infection increased *C*Las acquisition in nymphs. Our study identified a *D. citri*-associated virus that localized and showed pathogenicity on *D. citri* guts. The virus modulates *D. citri* cellular functions and may affect the efficiency of *C*Las transmission.

P2.4-015

THE TRANSMISSION OF BARLEY YELLOW DWARF VIRUSES BY FOUR CEREAL APHID SPECIES

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Text

Barley yellow dwarf viruses (BYDVs) infect cereals such as wheat, barley and oats worldwide, causing significant yield and quality losses. BYDVs are frequently found in south-eastern Australia, where BYDV-associated yield losses of up to 84% in wheat and 64% in barley have been observed. They are persistently transmitted from plant to plant by aphids. The most common cereal aphids in south-eastern Australia are *Rhopalosiphum padi* (bird cherry-oat aphid), *Rhopalosiphum maidis* (corn aphid), *Metopolophium dirhodum* (rose grain aphid) and *Diuraphis noxia* (Russian wheat aphid). In this study, a glasshouse experiment was conducted to examine the efficiency with which these four cereal aphid species transmit two BYDV species (BYDV PAV and BYDV PAS-like) to wheat. Each plant was inoculated with a single viruliferous adult aphid. Plants were tested for virus presence using tissue blot immunoassay four weeks after inoculation. While there were differences in transmission efficiency between aphid species, no obvious differences were observed between BYDV species. *R. padi* was the most efficient vector of both BYDV species. Further experiments have been conducted on other hosts. This information improves our understanding of the relationship between BYDV diversity and epidemiology in an Australian context.

P2.4-016

CHARACTERISING THE INTERACTION OF THE CUCUMBER MOSAIC VIRUS 1A AND 2B PROTEINS

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Text

The cucumber mosaic virus (CMV) 2b viral suppressor of RNA silencing (VSR) is a potent counter-defense and pathogenicity factor that inhibits antiviral silencing by titration of short double-stranded RNAs. It also disrupts microRNA-mediated regulation of host gene expression by binding ARGONAUTE 1 (AGO1). But in Arabidopsis thaliana complete inhibition of AGO1 triggers another layer of antiviral silencing against CMV mediated by AGO2, de-represses strong resistance against aphids (the insect vectors of CMV), and exacerbates disease symptoms.

Recent work showed that the CMV 1a protein, a component of the viral replicase complex, regulates the 2b-AGO1 interaction (Watt et al., 2020). By binding 2b protein molecules and

sequestering them in P- bodies, the 1a protein limits the proportion of 2b protein molecules available to bind AGO1. This ameliorates 2b-induced disease symptoms, and moderates induction of resistance to CMV and to its aphid vector but does not appear to inhibit the 2b protein's VSR activity. The amino acid sequence between residues 56-60 in the CMV 2b protein has been shown to be essential for its interaction with the CMV 1a protein.

The interaction between the CMV 1a and 2b proteins represents a novel regulatory system in which specific functions of a VSR are selectively modulated by another viral protein. The finding also provides a mechanism that explains how CMV, and possibly other viruses, modulates symptom induction and manipulates host-vector interactions.

P2.4-017

TIMING OF IN-SEASON PLANT-TO-PLANT SPREAD OF POTATO VIRUS Y IN POTATO IN IDAHO, UNITED STATES

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Text

Potato virus Y (PVY) is vectored in a non-circulative, non-persistent manner by many aphid species. Knowing when PVY is moved from plant to plant may inform optimal timing of PVY mitigation strategies such as mineral oil applications. Such knowledge may also indicate whether age-related resistance (ARR), a phenomenon that is inconsistently observed in the field, can be successfully harnessed. In 2021 and 2022, a small-plot experiment to exclude alatae at various times during the growing season via a mesh barrier was established in a randomized complete block design with four replications. Six treatments included 1. no barrier, 2. barrier from before emergence (BE) to vine beating (VB), 3. barrier only from BE to presumed onset of ARR (4 weeks post-emergence), 4. barrier only from onset of ARR to VB, 5. barrier from BE to tuber bulking (12-13 weeks after planting), and 6. barrier from BE to chemical vine kill. All plots were treated with a systemic insecticide. Based on a combined analysis using a generalized linear mixed model, PVY pressure in 2021 was higher than 2022, but for both years the lowest incidence of PVY in daughter tubers was observed in treatments where alatae were excluded either all season, from BE to bulking, or from BE to vine kill. These results support recommendations for PVY mitigation efforts to extend at least to onset of tuber bulking, and that onset of ARR may not contribute substantively to PVY management in a field setting until later in the season.

P2.4-018

PARAMETERS INFLUENCING WHEAT DWARF DISEASE INCIDENCE AND LEAFHOPPER ABUNDANCE IN SWEDEN

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Text

Wheat dwarf disease is commonly occurring in many parts of Europe and Asia. The disease is caused by wheat dwarf virus transmitted by the leafhopper Psammotettix alienus. In Sweden, there have been outbreaks of the disease in winter wheat for more than 100 years with sometimes great crop losses. The primary virus infection of winter wheat occurs in autumn with adult leafhoppers followed by a secondary spread in spring by wingless nymphs. This study aimed at evaluating factors contributing to the incidence of wheat dwarf disease in Swedish fields of winter wheat and the correlation with population size of the leafhopper vector as well as weather conditions. A significant correlation between the size of the adult leafhopper population in autumn to disease incidence in the following year was found in some counties. PCR tests of pools of collected P. alienus leafhoppers revealed that they frequently were carriers of WDV, especially in autumn after a high incidence of wheat dwarf disease. The weekly average temperature as well as weekly averages of maximum and minimum temperature in autumn were found to be significantly related to the number of adult leafhoppers per field. This study shows the constant presence of WDV and wheat dwarf over the years and that autumn temperature is an important factor determining the number of leafhoppers in the field and the incidence of disease. The result provides insights that can be useful for improving management strategies of the disease.

P2.4-019

TRANSMISSION EFFICIENCY OF TOMATO CHLOROSIS VIRUS (TOCV) TO POTATO PLANTS BY BEMISIA TABACI MEAM1 AND MED AND DAMAGE CAUSED BY THIS CRINIVIRUS ON THIS VEGETABLE

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Text

Tomato chlorosis virus (ToCV), transmitted by whiteflies, causes significant damage to solanaceous crops. This study aimed to evaluate the efficiency of ToCV transmission to potato plants by B. tabaci MEAM1 and MED and the damage caused by ToCV in potato plants. Transmission efficiency was evaluated exposing B. tabaci MEAM1 and MED to ToCV-infected potato leaves for 24-h, then transferred (10 whiteflies/plant) to healthy plants (n=20) of the cultivars Agata and Asterix for a 24-h inoculation period. To measure the damage, healthy or ToCV-infected plants of the cultivars Agata and Asterix (n=15) were grown, separately, in field conditions within insect-proof cages. The infected plants were inoculated 30 days after emergence. The damage caused by ToCV was assessed by comparing the productivity and the number of tubers produced by healthy or infected plants. The transmission efficiency of ToCV to Agata plants by MEAM1 and MED was 5% and 30%, and to Asterix plants were 20% and 55%, respectively. Healthy and infected plants of the cv. Agata produced an average of 325 g and 307 g of tuber and means of 8.4 and 8.2 tubers per

plant, respectively. Healthy and infected plants of the cv. Asterix produced an average of 207 g and 160 g of tuber and means of 7 and 6.4 tubers per plant, respectively. As potatoes are vegetatively propagated using seed tubers, further studies are necessary to evaluate the secondary damages caused by ToCV on this vegetable.

P2.4-020

FIRST REPORT OF BIDENS MOSAIC VÍRUS INFECTING PATCHOULI PLANTS (POGOSTEMON CABLIN BENTH.) IN BRAZIL.

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Text

Patchouli (Pogostemon cablin) is a plant belonging to the Lamiaceae family, used for medicinal purposes and in the cosmetics industry. In 2022, 6 patchouli plants exhibiting mosaic and leaf deformation were found in a flower shop in Botucatu municipality, São Paulo state, Brazil. Total RNA was extracted from leaves of all symptomatic plants and subjected to RT-PCR using the potyvirus universal primers WCIEN/PV1. Amplicons of the expected size (800 bp) were obtained for all samples analyzed and sent for nucleotide sequencing. The nucleotide sequences obtained showed 95.83%-98.90% identity with the corresponding nucleotide sequence of an isolate (Genbank AY960151.1) of bidens mosaic virus (BiMV) identified in plants of Bidens pilosa in Brazil. Symptomatic patchouli leaves were macerated in phosphate buffer and mechanically inoculated into 9 healthy plants of B. pilosa. The inoculated plants were kept in a greenhouse for symptom observation and the viral infection was confirmed by RT-PCR using the BiMV-specific primers 8331/9046. The B. pilosa inoculated plants showed mosaic symptoms 10-15 days after inoculation, and molecular analyses confirmed the infection with BiMV in all inoculated plants. In Brazil, four viruses have been reported infecting patchouli, patchouli X virus (PatVX), tobacco necrosis virus (TNV), pepper ringspot virus (PepRSV), and an unidentified potyvirus. To the best of our knowledge, this is the first report of BiMV infecting patchouli plants in Brazil.

P2.4-021

TRANSMISSION EFFICIENCY OF BEGOMOVIRUS BY DIFFERENT POPULATIONS OF BEMISIA TABACI MED IN SÃO PAULO STATE

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Text

Begomoviruses transmitted by cryptic species of *B. tabaci* cause losses to several crops of agricultural importance. This study aimed to evaluate the transmission efficiency of the begomovirus tomato severe rugose virus (ToSRV), tomato rugose mosaic virus (ToRMV) to tomato plants, and bean golden mosaic virus (BGMV) to common bean plants by *B. tabaci* MEAM1 and MED. The experiments were conducted with one population of *B. tabaci* MEAM1 and three populations of *B. tabaci* MED collected in different municipalities in São Paulo state. Inoculum source plants were placed in separate cages with MEAM1 or MED whiteflies for a 24-h acquisition access period (AAP). At the end of the AAP, the insects were transferred, according to the virus acquired, to cages containing healthy plants of tomato and common beans for an inoculation access period (IAP) of 24-h. Total DNA was extracted 21 days after the inoculation and virus infection was confirmed by PCR using begomovirus universal primers. The transmission rates of ToSRV, ToRMV, and BGMV by MEAM1 were high and ranged from 66.5%-93.3, 46.7%-80%, and 53.3%-100%, respectively. MED populations did not transmit ToSRV and ToRMV to tomato plants. Only one MED population transmitted BGMV to a bean plant (1/15), indicating that MED is able to transmit begomovirus. The results suggest that the MED populations from São Paulo State are not good vectors of these viruses.

P2.4-022

COLLECTION AND IDENTIFICATION OF POTENTIAL XYLELLA FASTIDIOSA VECTORS - ASSESSMENT OF BACTERIA TRANSMISSION IN POLAND

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Text

A quarantine organism, the bacterium Xylella fastidiosa (Xf), is a xylem-inhabiting, vector-transmitted, Gram-negative, and very slow-growing bacterium in the Lysobacteraceae (earlier Xanthomonadaceae) family. Spread for the long distances of X. fastidiosa occurs mainly via import/export human-mediated transportation of mainly latently or symptomatically infected plant material. Short-distance distribution is usually by xylem sap-feeding insects. Until now the presence of bacterium Xf was not reported nor studied in our country. During this study over 500 individuals of insects belonging to four families: Cicadellidae, Aphrophoridae, Delphacidae and Membracidae were collected in different geographical regions of Poland. The application of real-time PCR with TaqMan probe and nested PCR for the detection of Xf, using DNA extracted directly from selected insects known as potential vectors of X. fastidiosa, allowed to predict the potential threat of bacteria transmission in our country. The results of the conducted research will be presented. This study was financed by the National Science Centre, Poland (Narodowe Centrum Nauki), grant UMO-2017/26/M/NZ9/01024.

P2.4-023

TRANSMISSION OF CUCURBIT LEAF CRUMPLE VIRUS (GEMINIVIRIDAE: BEGOMOVIRUS) BY VECTOR BEMISIA TABACI WITH DIFFERING SECONDARY ENDOSYMBIONT COMPOSITION

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Text
Cucurbit leaf crumple virus (CuLCrV), a bipartite begomovirus transmitted by the sweetpotato whitefly (*Bemisia tabaci*; Hemiptera: Aleyrodidae), is a limiting factor in the production of cucurbits in the United States. Virus transmission is in a persistent, circulative manner, and whitefly secondary endosymbionts, such as *Rickettsia*, may affect transmission efficiency. Virus transmission tests were performed from susceptible versus resistant watermelon varieties using *B. tabaci* MEAM1 harboring the maternally inherited secondary endosymbiont *Rickettsia* versus MEAM1 deficient of this endosymbiont. Results demonstrated CuLCrV transmission from susceptible watermelon to the same susceptible variety in 50% of tests using *Rickettsia*-harboring or *Rickettsia*-deficient whiteflies. CuLCrV was not detected in susceptible watermelon after transmission test whiteflies acquired virus from the resistant watermelon variety. Quantification of virus titers in leaves after whitefly acquisition access demonstrated significantly lower titers in leaves fed on by *Rickettsia*-deficient versus *Rickettsia*-harboring whiteflies. Virus titers in whiteflies post-inoculation access were not significantly different. These results highlight the importance of using resistant watermelon varieties in CuLCrV management. Studies to better understand how vector secondary endosymbiont composition affects virus transmission and viral titer in host plants are ongoing.

P2.4-024

GENOME-WIDE ASSOCIATION STUDY REVEALS LOCI ASSOCIATED WITH VECTOR COMPETENCY OF DIAPHORINA CITRI, INSECT VECTOR OF CITRUS GREENING DISEASE

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Text
Genome-wide association studies (GWAS) have revolutionized scientific insight and potential to examine complex diseases. Huanglongbing (HLB), or citrus greening disease, threatens the citrus industry at a global scale. HLB results from the growth of “Candidatus Liberibacter asiaticus” (CLas) in the phloem of Citrus species and is spread by the hemipteran insect vector, Diaphorina citri. GWAS of 600 adult D. citri collected from four citrus groves in southeast Florida, USA, was used to identify genetic loci associated with pathogen acquisition, a quantitative trait. The acquisition phenotype for each insect (absolute titer of CLas) was estimated using quantitative PCR. After shallow sequencing (1-7x coverage), haplotyping, imputation, quality filtering, and kinship analysis were performed to establish a marker panel. An association model identified hundreds of single nucleotide polymorphisms significantly associated with CLas acquisition and genomic loci. A separate reference-free K-mer based analysis was compared to the initial reference-based GWAS to cross-examine

loci deemed biologically important. Loci of interest were further assessed with Gene Ontology pathway analysis. This study provides novel insight into the genetics underlying vector biology and promotes further research to identify therapeutics which prevent insect-vector disease acquisition and transmission.

P2.4-025

HOW MIGHT MANAGEMENT INTERVENTIONS BE MATCHED TO THE STAGE OF EPIDEMICS FOR CASSAVA VIRUSES?

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Text

In recent decades a cassava mosaic begomovirus epidemic (CMV) spread by Bemisia tabaci whitefly has severely affected cassava yield in sub-Saharan Africa, and this was followed by a regional cassava brown streak ipomovirus epidemic (CBSV) spread by the same vector.

Both viruses are spread by B. tabaci - but infection also occurs in contaminated plant stock. But it is essential to understand how the relative importance of the transmission routes depends on the temporal phase of the epidemic.

In this poster we indicate how the efficacy of managing infected cuttings vs. whitefly-borne transmission changes as the temporal phases of epidemics change. We contrast findings for CMV and CBSV using recent published results on retention period for these viruses.

SATELLITE EVENTS

4th European Conference on Xylella fastidiosa

DEGREE-DAY-BASED MODEL TO PREDICT EGG HATCHING OF PHILAENUS SPUMARIUS (HEMIPTERA: APHROPHORIDAE), THE MAIN VECTOR OF XYLELLA FASTIDIOSA IN EUROPE

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Text

Philaenus spumarius L., the main vector of *Xylella fastidiosa* (Wells) in Europe, is a univoltine species that overwinters in the egg stage, and its nymphs emerge in late winter or spring. Predicting the time of egg hatching is essential for determining the precise times for deploying control strategies against insect pests. Here, we monitored *P. spumarius* eggs from oviposition to egg hatching together with the daily temperatures and relative humidities at four field locations located at different altitudes in central Spain. The collected data were used to build a growing degree day (GDD) model to forecast egg hatching in the Iberian Peninsula. Furthermore, the model was validated with field observations on the presence of newly born nymphs in different regions of Spain. The model was then used as a decision-support tool to calculate the optimum timing for applying control actions against *P. spumarius* nymphs. Our results suggest that controlling nymphs at two different dates would target the highest percentage of nymphal populations present in the field. Our model represents a first step for predicting the emergence of nymphs and adopting timely control actions against *P. spumarius*. These actions could limit disease spread in areas where *X. fastidiosa* is present.

CURRENT SITUATION OF XYLELLA FASTIDIOSA IN FRANCE

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Text
Xylella fastidiosa (*X. f.*) has been detected for the first time in France, in Corsica and PACA region during the summer 2015. Since 2020, *X. f.* has been also highlighted in Occitanie region with a significant number of new outbreaks discovered during the summer 2022. Only the subspecies *multiplex* has been reported so far in these regions, although *X. f.* subsp. *pauca* was previously reported and eradicated in one outbreak in Menton (PACA). In France, the sequence-types (ST) ST6 and ST7 were identified, but new ST88 (Var) and ST89 (Alpes-Maritimes) were recently identified in PACA on two different areas with a limited host range. In Occitanie, only ST6 has been identified but MLVA analyses suggested theses strains are genetically different from ST6 strains from PACA, Corsica and Spain. Since 2015 in France, only 5 olive trees (*Olea europaea*) have been found infected with low titer. No grapevine (*Vitis vinifera*) and no *Citrus* sp. were found infected with *X. f.*.
Official *X. f.* detection is based on the real-time PCR Harper *et al.*, 2010. The new version of the French method MA039 v6 includes an internal PCR control 18S (Ioos *et al.*, 2009) for the detection in plant and a new DNA extraction method based on the Maxwell® HT Environmental TNA kit (Promega). Compared to the traditional CTAB-Chloroform method, this new automatized protocol allows an improved sensitivity on *Olea europaea* and *Quercus* spp., safer working conditions for operators and the automatization of the process.

AUSTRALIA PREPAREDNESS: XYLELLA FASTIDIOSA AND ITS INSECT VECTORS – POTENTIAL CONTROL AND MANAGEMENT OPTIONS APPROPRIATE TO AN AUSTRALIAN CONTEXT

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Text
The spread of Xylella fastidiosa (Xf), one of the most detrimental plant pathogens currently affecting the Americas and Europe, is a reality that will likely continue due to globalisation. As such, effective preparedness of countries where the pathogen does not yet occur is paramount to shield both native flora and commercial plant-based industries from an incursion and spread.
As a first step for being prepared for a robust response should an incursion take place, a comprehensive and critical review of the existing literature regarding available control and management strategies was undertaken.
Here we will present the results of this review which was a response to the Australian National Xylella Action Plan and specifically Action 3.4 which is to Analyse literature and overseas experience to identify control and management options relevant to the Australian context.
Our results indicated that current mitigation strategies are largely deployed across those that target Xf in the host plant and those which focus on the insect vectors with the aim to minimise the impact of Xf, its vectors and the associated plant diseases caused by the bacterium. Furthermore, integrating these results, we have identified several research gaps that could limit Australia's capacity to respond to a Xf incursion. We have therefore proposed a set of 24 research recommendations that will increase Australia's preparedness to control and manage a Xf outbreak and these recommendations will be discussed.

SURVEY ON THE PRESENCE OF XYLELLA FASTIDIOSA IN OAK TREES IN FRANCE AND ASSESSMENT OF SEED-TRANSMISSION

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Text

Xylella fastidiosa (*Xf*) is a plant xylem-inhabiting bacterial pathogen that may colonize a wide range of plants and cause diseases in various crops of economic importance. According to climate model predictions, most French area could be suitable to *X. fastidiosa* subsp. *multiplex* (*Xfm*). Strains of this subspecies are likely to cause serious damages to various plant species including forest and shade tree species such as oak. A sampling campaign was therefore conducted in 2020 and 2021 in French state forests exploited for the quality of their oak trees and in woods. Quantitative PCR was used to detect *Xf*. None of the samples taken from oak trees during these two years revealed any *Xf* contamination. Whereas *Xf* introduction through contaminated plant material (horizontal transmission) most probably already occurred into Europe, *Xf* vertical transmission through seed is poorly documented. The transmission of *Xf* to the acorn was studied by inoculating stems at different vegetative stages (from flower to fruit). Transmission from acorn to seedling was also analysed by acorn inoculation before sowing. In both types of analyses, no *Xf* transmission was demonstrated, while stem colonization was observed. However, the question of seed transmission for other plant species remains an important issue for a better understanding of the mechanisms underlying the epidemiological dynamics and control of diseases caused by this European priority pathogen.

DIRECT XYLELLA FASTIDIOSA WHOLE GENOME SEQUENCING FROM PLANT USING TARGETED ENRICHMENT

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Text

Xylella fastidiosa is a gram-negative plant pathogenic bacterium with a broad host range, which causes important diseases. Endemic to the Americas, this bacterium was recently introduced to the European continent where it has been detected since 2013 in various plant species in Italy, France, Spain, and Portugal. Precise genetic characterization of *X. fastidiosa* diversity can be obtained using whole genome sequencing, but isolation of the bacterium from contaminated plant material is not always successful. In order to access the bacterial genome directly from plant infected samples, a SureSelect targeted enrichment method was developed. Enrichment was very efficient to retrieve *X. fastidiosa* genome sequence with genome coverage significantly improved for all enriched plant samples, whatever the plant species or the contamination level. Whole genome sequencing data are useful to study the epidemiology and the origin of *X. faxtidiosa* introductions, and to give new insights for surveillance and management of this quarantine bacterium.

PROJECTING CLIMATE SUITABILITY OF XYLELLA FASTIDIOSA, AN IMPORTANT GLOBAL BIOSECURITY THREAT

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Text

Xylella fastidiosa (XF) is a plant pathogen that can cause disease in a wide range of plants, making it a serious global threat to plant health. The outbreak of XF in Italy and its subsequent spread in Europe has raised serious concern about its potential impact on the production of food crops. To better understand the potential distribution of this pathogen, a Maximum Entropy (MaxEnt) model was developed using long-term climate data and data on the current global distribution of XF. The model found that annual mean temperature and precipitation of the coldest quarter were the most significant contributors to XF's potential distribution, accounting for 90.1% of the model projection. The optimal temperature for pathogen establishment was identified as 16°C. The model projected that most of New Zealand is suitable for XF establishment, and by overlaying the model output with areas where potential vectors are present, hotspots for XF establishment were identified. The model also highlighted areas where the climate is highly conducive to XF establishment, including most parts of Italy, southern France, southwestern Spain, and large areas in Portugal. These findings may be valuable for countries such as China, Australia, New Zealand, and South Africa, where the pathogen has not yet established. Overall, the MaxEnt model provides important insights into the potential global and local distribution of XF, which could aid in developing strategies to manage and control its spread.

NO NEED TO KILL XYLELLA FASTIDIOSA

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Text

Regarding the symbiotic relationship between pathogens and plants, I propose that it is not necessary to use targeted killing or isolation methods to combat Xylella fastidiosa. Instead, we can control the relationship between the pathogen and the plant in a mutually beneficial way that does not harm the host by enhancing the tree's vitality, improving its immunity, and providing drug treatments. We have already achieved success in date palms, where the relationship between the pathogen and the plant can be mutualistic, and this phenomenon can be compared to traditional Chinese medicine's approach to dealing with viruses. I believe that studying the symbiotic relationship between pathogens and plants can provide new ideas and methods for agriculture, environmental management, and biological control, and promote the development of new agricultural technologies. In the future, international cooperation needs to be strengthened to explore the mechanisms and applications of the

symbiotic relationship between pathogens and plants, providing new avenues and possibilities for achieving sustainable development.

EVALUATION OF A NEW BIOLOGICAL SOLUTION COMBINING ANTIMICROBIAL PEPTIDES AND A DELIVERY SYSTEM BASED ON AGROBACTERIUM TUMEFACIENS FOR THE CONTROL OF XYLELLA FASTIDIOSA

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Text

Xylella fastidiosa (Xf) is a quarantine bacterium that can infect the xylem of a wide range of cultivated, ornamental, and wild host plants. Xf is the causal agent of Pierce's disease in grapevine in North America, citrus variegated chlorosis in South America, and olive quick decline syndrome, notably in Southern Italy. There is currently no method that effectively prevents or cures host plants from infection. The current approaches for Xf disease management mainly rely on eradication of infected plant to reduce inoculum source, the use of insecticides to control the vector populations, and the use of healthy plant propagation material. Therefore, there is a need for new and safe biological solutions. Protecting and/or curing mature fruit trees infected by *Xf* remains a challenge but would be the best possible solution to avoid eradicating infected trees and replanting. The use of antimicrobial peptides (AMPs) represents an interesting approach due to their broad spectrum of activity against bacterial pathogens, low environmental impact, and limited evolution of resistance. EBCL will test an innovative approach combining the use of AMPs and a delivery system based on Agrobacterium tumefaciens. The main advantage of this method is that it can potentially confer resistance to mature fruit trees. Preliminary tests will thus be performed to evaluate proof of concept of this strategy for the control of Xf.

NOVEL TARGET-ORIENTED PEPTIDES WITH POTENTIAL FOR INFECTION CONTROL STRATEGIES AGAINST XYLELLA FASTIDIOSA

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Text

Peptides can be promising candidates against *X. fastidiosa* due to their broad spectrum of activity and low environmental impact. They can be targeted towards the pathogen by affecting its viability or by hampering processes such as biofilm formation or motility. Peptides can also be directed towards the host by acting as plant defense elicitors, which would induce a priming state.

In our studies, a screening platform was developed to assess the bactericidal and antibiofilm

activity of peptides using viability qPCR and crystal violet, respectively. Peptides were selected or newly designed, synthetized and tested, resulting in the identification of the peptides **1036** and **RIJK2**, which possess dual activity (high bactericidal and antibiofilm activities). In addition, a screening platform for the identification of plant defense elicitors on *Prunus dulcis*, host of *X. fastidiosa*, was set up with the peptide **flg22** using RNAseq and RT-qPCR. From the peptide library studied, we identified the peptide **FV7** as a novel plant defense elicitor. In addition to the bifunctional peptide **BP178** with bactericidal and plant defense elicitor activities, several of the above new peptides are currently being tested in the almond platform to evaluate their efficacy of infection control against *X. fastidiosa.*
Funding was provided by 101060593 EU project, and RTI2018-099410-B-C21 MCIN/AEI/FEDER, and TED2021-130110B-C43 MCIN/AEI/ Unión Europea NextGenerationEU/PRTR and MCIN FPU19/01434.

DELIMITING SURVEY OF XYLELLA FASTIDOSA SUBSP. MULTIPLEX : BRINGING TOGETHER MULTIDISCIPLINARY EXPERTISE TO SUPPORT RISK MANAGEMENT

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Text

Efficient delimitation of a newly detected area infested by a quarantine pest is a key element to enhance the effectiveness of phytosanitary measures. Xylella fastidiosa subsp. multiplex was first detected in the department Aude (Occitanie, France) in September 2020. In the following months, the reinforced surveillance implemented in accordance with Regulation (EU) 2020/1201 resulted in numerous detections close to the first outbreak, while an extra survey plan made it possible to detect several new and separate infested areas in the same department. Then, in 2021 a multidisciplinary working group in the framework of the Epidemiological Plant Health Surveillance Platform was appointed to support the risk manager with the further delimitation of the contaminated area. On-site observations, expertise and risk maps using eco-climatic data were used to define a risk-based and sequential survey plan and provide guidelines to improve the surveillance. The implementation of this survey plan resulted in findings of both contaminated and non-contaminated areas. The risk manager strongly relied on the results of this survey plan to adapt the surveillance and management strategy. This approach could be generalised to other outbreaks of Xylella fastidiosa or any other pest. It highlights the benefits of bringing together researchers (bacteriologists, entomologists, epidemiologists), inspectors, agronomists and stakeholders to devise a delimiting survey.

XYLELLA FASTIDIOSA: ACTION OF THE ENZYME LESA ON PLANT LIPIDS

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Text

Xylella fastidiosa is classified as a global threat for agriculture. After inoculation by xylem sap-feeding insects, *Xylella fastidosa* colonizes plants by different strategies including an enzymatic arsenal. Among these molecules, LesA[1] is the major enzyme among the proteins secreted by the bacteria. According to its sequence and its structure, this protein is supposed to be a member of lipase/esterase family. LesA is structurally close to LipA of *Xanthomonas oryzae* that is also involved in virulence. We produced LesA in *E. coli* and performed a number of experiments of characterization by in-vitro tests based on selected substrates to verify its activity. In a second step, we performed an untargeted lipidomic approach using total lipid extracts from *Arabidospis thaliana* leaves. The results obtained on the activity tests confirm the esterase activity of LesA but only on short carbon chains. The lipidomic results show 172 compounds significatively differents ($p<0,05$) between treated and untreated samples. Some have been identified by mass-spectrometry, suggesting that LesA could have an impact on some phospholipids. More investigations are needed to better know biological substrates of LesA and its role in plant colonization and symptoms expression in plants infected by *Xylella fastidiosa*.

[1]Nascimento, R. et al. The Type II Secreted Lipase/Esterase LesA is a Key Virulence Factor Required for *Xylella fastidiosa* Pathogenesis in Grapevines. Sci. Rep. 6, 18598 (2016)

THE FATE OF XYLELLA FASTIDIOSA IN COMMON WOODY PLANT SPECIES IN BELGIUM

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Text

In 2018, *Xylella fastidiosa* (Xfas) was detected at a Belgian arboriculture wholesaler, in olive plants imported from Spain. Despite this being a sole finding, its capacity to establish under regional environmental conditions was investigated. Colonisation of Xfas was assessed in 20 perennial woody plant species, common in the urban landscape and natural environment, under both greenhouse (10-35°C) and outdoor conditions. In three consecutive years, shoots were pin-prick inoculated with strain CFBP 8431 (ST6) from the subsp. *multiplex*. The fate of the bacterium was monitored by Harper TaqMan PCR and dPCR after 5, 12, 24 and 36 months. Overall, plants maintained a healthy appearance. Xfas detection in the inoculated part of the shoots did not indicate multiplication. Indoors, bacterial titres remained fairly constant over time, whereas they were generally lower and less stable in outdoor plants. There was

also no clear evidence of bacterial movement as detections in non-inoculated twig parts were mostly in the grey zone of quantification, except for *Castanea sativa*, *Populus nigra* and *Quercus robur*. Although a slow coloniser, xylem microbiome analyses of *Salix alba* revealed that the presence of Xfas can significantly alter resident endophytic bacterial communities at a local scale. This study shows that under the regional climatic conditions, establishment and proliferation of Xfas is unlikely, yet the pathogen may remain latent in infected tissue for an extended period of time.

FLUORESCENCE IN SITU HYBRIDIZATION COUPLED TO FLOW CYTOMETRY AND CELL IMAGING ANALYSES AS POTENTIAL TOOLS FOR XYLELLA FASTIDIOSA RESEARCH AND DIAGNOSTICS.

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Text

Fluorescence in situ hybridization (FISH) is used to directly identify microorganisms within complex samples in a few hours having a widespread application in environmental microbiology, including reports for Xylella fastidiosa detection in plant xylem vessels and insect honeydew. In Costa Rica, X. fastidiosa subsp. fastidiosa is endemic, with high bacterium prevalence and seldom disease manifestations in coffee plantations throughout the central valley, where most of the coffee is grown. We study the extent of the bacterial infection in coffee and its insect vectors. For this purpose, we implemented two FISH-based tools coupled to (i) flow cytometry (Flow-FISH), and (ii) cell imaging analyses, to study specific events of the X. fastidiosa life cycle in vitro and in vivo; as well as for the direct detection of X. fastidiosa with potential diagnostic value. For this purpose, we tested an X. fastidiosa-specific probe (KO210) and a universal probe (Eub338), using flow cytometry and microscopy. We standardized the hybridization conditions using an X. fastidiosa pure culture, we determined the detection limits of each tool, and confirmed the specificity of the KO210 probe for X. fastidiosa. Applications of the tools include detection of bacteria from plant and insect material, and in vitro cultures, with an in-depth qualitative and quantitative resolution, broadening the existing tools for research and diagnostics in X. fastidiosa.

IMPROVING THE DIAGNOSTIC CAPABILITY FOR XYLELLA FASTIDIOSA SUBSP. MULTIPLEX USING ADVANCED NANOTECHNOLOGY AND PCR-BASED ASSAYS

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Text

Xylella fastidiosa subsp. *multiplex* (Xfm) is an emerging threat to the southern U.S. pecan

and peach growing regions, causing pecan bacterial leaf scorch and phony peach disease, respectively. The current lack of reliable diagnostic tools for Xfm causes uncertainty in the specialty crop industry in the U.S. The research objective is to improve diagnostic capability for Xfm. We validated different diagnostic tools for Xfm using enzyme-linked immunosorbent assay (ELISA) and molecular-based assays, including traditional PCR and real-time quantitative PCR (qPCR). New Xfm-specific primers were generated and validated, producing expected amplicons specific to Xfm. We also developed a TaqMan qPCR assay protocol for the detection of Xfm in pecan and peach. The results of the qPCR experiments based on plant genomic DNA from petioles were equivalent to the traditional PCR amplification based on crude sap. Further improvement in detection methods is critical for the early diagnostics and subsequent timely intervention for Xfm. To address this, advanced nanotechnology was used to improve diagnostic capability. Antibody-conjugated immunomagnetic carbon nanotubes were developed to improve sensitivity and reliability of PCR-based diagnostics for Xfm. The improved PCR diagnostic methods will support production and distribution of clean plant materials during national and international movement of plant materials.

COMPARATIVE GENOMICS OF XYLELLA FASTIDIOSA SUBSP. MULTIPLEX STRAINS FROM FRANCE REVEALS PATHOGEN DYNAMICS AFTER ITS INTRODUCTION

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Text

Xylella fastidiosa is a plant pathogen responsible for numerous crop diseases worldwide. It specifically colonizes the xylem of plants and is transmitted exclusively by sap-feeding insects. X. fastidiosa has a significant adaptive capacity, as evidenced by its great genetic diversity and frequent recombination events between subspecies. Originating from the Americas, X. fastidiosa is now present in several European countries (Italy, France, Spain and Portugal) due to accidental introductions of contaminated plant material. This situation calls for a better understanding of the evolutionary dynamics of the pathogen in its new areas of distribution. Herein, we present a comparative genomics analysis of strains belonging to the subspecies multiplex that have been isolated from various host plants in France since the first detection of the pathogen in 2015. High-quality genome sequences were obtained using the PacBio sequencing technology, which allowed the identification of numerous plasmids, in contrast to American strains of this subspecies which rarely possess plasmids. We further employed a metagenomic sequencing approach combining both long- and short-read technologies to sequence additional genomes directly from infected plants in a new focus of infection. This strategy simplifies the sequencing of new strains detected in the field by eliminating the time-consuming isolation step, thereby providing access to strains that could not have been sequenced previously.

DETECTION OF XYLELLA FASTIDIOSA BY COLORIMETRIC LAMP AND DROPLET DIGITAL PCR ASSAYS

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Text

Xylella fastidiosa (Xf) is a bacterium that inhabits the xylem vessels of over 600 plants. The entry of Xf subsp. *pauca* ST53, causing the olive quick decline syndrome in Italy, represents the first outbreak in field on UE territory.
Prompt field-based and sensitive laboratory assays are urgently required for the containment strategies of the Xf spreading. A cLAMP and ddPCR assays, based on widely validated primer (Harper *et al.,* 2010), were used for Xf detection in a crude alkaline sap obtained from incubation of thin olive stem slices in NaOH-based buffer. cLAMP assay can be performed at any location in a portable isothermal block at 65°C, providing a naked-eye detectable amplification result in 40-minutes. Both assays detected the target DNA up to 10^2 CFU/mL of bacterium in crude alkaline sap without any inhibition effect. Moreover, a ddPCR assay was implemented to detect Xf in purified DNA extracts prepared from different plant and insect matrices artificially contaminated with Xf suspension..
An exstensive validation of both assays on naturally infected samples, using crude extracts and purified DNA, proved that cLAMP has remarkable potential as first screening test for a timely detection of Xf at the point of care due to its simplicity, low cost, and portability; conversely ddPCR, although requires expensive reagents/equipment and is time-consuming, is more sensitive, especially using purified DNA extracts and solves some results recorded as doubtful by the qPCR assay.

XVETORES.PT: BIOLOGY OF XYLEM-SAP FEEDING INSECT VECTORS AND POTENTIAL VECTORS OF XYLELLA FASTIDIOSA IN PORTUGAL

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Text

Xylella fastidosa was reported for the first time in Portugal in 2019; since then, this phytopathogenic bacteria was progressively spread throughout the country, and currently, 17 demarcated regions of infection have been established. This constant spread may lead to devastating economic and environmental problems, threatening Portugal's agricultural sector and plant diversity. Since there is no cure for this bacterium, vector control is perceived as the main tool to limit the spread of *X. fastidiosa*. In Portugal, some studies developed in the

Northeast region demonstrated the importance of the main and potential vectors and clarified aspects of biology, diversity and abundance; however, there is a lack of knowledge about these aspects at the national level. Therefore, the project Xvetores.pt: Biology of xylem-sap feeding insect vectors and potential vectors of *Xylella fastidiosa* in Portugal, funded by EFSA (call GP/EFSA/ALPHA/2021/07), aims to study the biology, abundance, and diversity of insect vectors and potential vectors in different agrosystems such as olive groves, cork oak forests, and urban and semi-natural areas in different regions of Portugal with different agroclimatic conditions. Furthermore, preference studies for host plants and studies on the biology of vectors under microcosm conditions will also be carried out. The Xvectors.pt project is led by IPB and integrates five other Portuguese partners, namely ISA/ULisboa, INIAV, DGAV, UP, and InPP.

MEDITERRANEAN AGRICULTURAL SPECIES, THEIR VOLATILES, AND THE POTENTIAL IMPACT ON PHILAENUS SPUMARIUS

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Text

Philaenus spumarius (Linnaeus, 1758) (Hemiptera, Aphrophoridae) is Europe's most common and widespread xylem-sap feeder insect. This insect has become a serious threat to European agriculture due to its recognized role in transmitting the phytopathogenic bacterium Xylella fastidiosa (Xanthomonadales: Xanthomonadaceae). Therefore, understanding the factors that influence the host plants' choice by this insect vector could be an important tool to manipulate the vector behaviour and implement sustainable control strategies. Therefore, in this work, the volatile profile of the leaves of three important Mediterranean crops, almond, olive, and vine were assessed. Moreover, the P. spumarius olfactory response towards two of the most abundant and common Volatile Organic Compounds (VOCs) (cis-3-hexenyl acetate and cis-3-hexen-1-ol) present in the crop leaves were evaluated. From the three plant species, in total, 83 compounds were identified. Although the plant species showed a distinct volatile profile, cis-3-hexenyl acetate and cis-3-hexen-1-ol were common and abundant. Furthermore, females of P. spumarius were significantly attracted to cis-3-hexenyl acetate and cis-3-hexen-1-ol, whereas males did not respond to the VOCs. Our results can help future implementation of approaches to manage the vector and the spread of X. fastidiosa.

XYLELLA FASTIDIOSA: A EUROPEAN THREAT UNDER CLOSE SURVEILLANCE ON THE MEDISYS PLATFORM

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Text

In December 2016 EFSA was mandated by the European Commission to carry out a horizon

scanning exercise on emerging plant pests. From that moment, and in close collaboration with the Joint Research Centre, EFSA started its monitoring and extraction of news and scientific data on plant pests based on the Medisys platform (https://publications.jrc.ec.europa.eu/repository/handle/JRC53155).
Classification is automatically and continuously carried out by the system on more than 21,000 media and scientific literature sources, from around 200 countries, using a set of more than 13,000 pre-defined keywords. The result of this activity is presented in freely available monthly newsletters (https://efsa.onlinelibrary.wiley.com/doi/toc/10.2903/(ISSN)1831-4732.Horizon-scanning-for-plant-health).
With its 54 keywords, the European situation of Xylella fastidiosa has been monitored over the last 6 years on media and scientific publications, from outbreaks to vectors, from subspecies to hosts, from detection methods to epidemiology. Since the beginning of this activity, X. fastidiosa was the pest providing the largest number of items on Medisys and the most represented pest in the horizon scanning publications (in 94 outputs over the total 108 accessible by March 2023).

ENDOPHYTIC MICROBIOMES OF WILD PLANTS SUSCEPTIBLE TO XYLELLA FASTIDIOSA INFECTION

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Text
Xylella fastidiosa (Xf) is a phytopathogenic bacterium responsible for a wide range of diseases with high economic, environmental and social impacts. The plant-associated microbiome plays a central role in maintaining fitness, but little is known about the diversity and structure of these endophytic microbial communities. It is therefore essential to study this diversity and the interactions between endophytes and plants in order to understand the biotechnological potential of these microorganisms. In this context, we aim to characterise the structural diversity of endophytic microorganisms in wild plant species susceptible to Xf infection and present in the demarcated area of Portugal. Plant samples were selected and tested by qPCR for the presence of Xf. The microbiome was analysed in plants with and without Xf using a long-read sequencing approach. The results suggest a functional importance of some core microbiome groups, namely related to the ability to degrade toxic substances, fix nitrogen and promote plant growth. They may also help to explain the lack of visible symptoms and signs of decline in these wild plants, suggesting that certain taxonomic endophyte groups may contribute to modelling the infection. Notably, this is the first study in wild plants and the identification of relevant clusters is a contribution to the understanding of the impact of Xf on microbiome dysbiosis in nature.
This work was financed by FCT (PTDC/ASP-PLA/3145/2021).

LONG-READ METABARCODING APPROACH FOR DIAGNOSIS AND EPIDEMIOLOGY IN GENETICALLY HETEROGENEOUS PRUNUS SP. ORCHARDS

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Text

Diagnostic challenges are increasing as globalisation and climate change favour the introduction of new plant pests. Xylella fastidiosa, Xanthomonas arboricola, Pseudomonas syringae and Monilinia fructicola are important pests affecting Prunus production with significant losses. Two different Prunus production systems were analysed: old orchards of P. dulcis and coexisting orchards of P. avium and P. persica, where significant areas of P. dulcis have recently been established. The high density of genetically heterogeneous Prunus sp. in which pests can coexist favours the development of highly adapted strains and/or the occurrence of coevolutionary phenomena associated with the spread and emergence of new diseases. This coexistence of phylogenetically close strains with drastically different phenotypes is a critical diagnostic challenge. In this context, molecular epidemiological studies were conducted by characterising the leaf and flower bacteriome and mycobiome of Prunus sp. using a long-read sequencing approach to assess the occurrence, distribution and impact of pests. Preliminary results showed that, in contrast to the leaf microbiome among Prunus species, significant differences were found between the flower and leaf microbiomes. Important pathogen-related groups were detected with significant impact on the microbiome structure, supporting the use of this approach for large screening phytosanitary surveys.
This work was financed by FCT (PTDC/ASP-PLA/3145/2021).

PHENOLOGY AND ECOLOGY OF PHILAENUS SPUMARIUS L. INFORM RISK ASSESSMENT FOR XYLELLA FASTIDIOSA WELLS IN SWITZERLAND

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Text

The meadow spittlebug *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) has been identified as the main vector of *Xylella fastidiosa* Wells in Europe. With a proactive approach toward a potential arrival of *X. fastidiosa* in Switzerland, we are studying the phenology and ecology of *P. spumarius* in the southern Alps so as to inform risk assessment for *X. fastidiosa* and suggest timely and effective management measures. The study of phenology allowed to identify the developmental periods, the host plants and their specificity for the nymphs in the Insubric climate. The ecology was studied across Canton Ticino according to

a stratified design that considered different combinations of environmental factors (climate, geology and topography, summarized by the Swiss Environmental Domains), habitat (vineyards, olive groves, orchards, hay meadows and pastures) and management (intensive vs. extensive). This provided information about host plants preference and specificity within different regional pools, and estimates of the density of *P. spumarius* in different habitats. Nymphs select preferentially host plants from Asteraceae, Plantaginaceae, Caryophyllaceae, Fabaceae families, in particular *Taraxacum officinale*, *Plantago lanceolata*, *Silene vulgaris*, *Trifolium pratense*. Nymphs' host preference varies according to regional pools and habitat type. Density of spittle nests is significantly higher in hay meadows compared to the other surveyed habitats and in extensively-managed habitats.

VECTORS AS SENTINELS OF PLANT DISEASES IN A CHANGING WORLD: RISING TEMPERATURES INCREASE THE RISK OF XYLELLA FASTIDIOSA OUTBREAKS

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Text

Not much is known about how a changing climate will affect the epidemiology of generalist vector-borne diseases. We developed a high-throughput screening method to test for the presence of *Xylella fastidiosa*, in its insect vectors. Based on a four-year survey in climatically distinct areas of the island of Corsica (France), we found a significant positive correlation between the frequency of vectors positive for the bacterium and temperature. Higher prevalence corresponded with milder winters. Climate projections show that the risk for *X. fastidiosa* outbreak will increase in the future. Besides calling for research efforts to limit the incidence of plant diseases in temperate zones, our work reveals that recent molecular technologies could and should be used for massive screening of pathogens in vectors in order to scale-up surveillance and management efforts. Such methods could for example target multiple plant-pathogens in vectors communities. This will contribute to better understand the drivers of plant pest spread and establishment such as the influence of climate change or ecosystem degradation or to better evaluate environmentally sound control solutions over large geographical and temporal scales.

ABILITY OF GLASSY-WINGED SHARPSHOOTER TO ACQUIRE XYLELLA FASTIDIOSA SUBSP. PAUCA FROM RIPE OLIVE VARIETIES GROWN IN CALIFORNIA, USA.

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Text

Xylella fastidiosa subsp. *pauca* (*Xfp*) is not currently present in North America but could have significant impacts on the United States olive industry if it were introduced. The state of California (CA), which has historically dealt with disease outbreaks caused by *X. fastidiosa* in

other crops, produces 70-80% of olives grown in the US. Several insect species present in CA, including the glassy-winged sharpshooter (*Homalodisca vitripennis*, GWSS) are known vectors of *X. fastidiosa* in grapevine, and could drive spread of *X. fastidiosa* in olive if *Xfp* strains were introduced. This study evaluated the susceptibility of three CA ripe olive varieties (Mission, Manzanillo, and Sevillano) to olive-pathogenic *X. fastidiosa* strain DeDonno, as well as ability of GWSS to acquire this pathogen from infected olive plants. GWSS caged on *X. fastidiosa*-infected olive seedlings for 3 days tested positive by PCR for *X. fastidiosa* at a rate of 4.8%. This shows that GWSS can acquire *Xfp* from CA ripe olive varieties and could potentially act as a vector of *X. fastidiosa* in this crop. Although overall acquisition rates were low, acquisition occurred as soon as 30 days post-inoculation of the plants in all three olive cultivars tested. Potential for GWSS to serve as a vector of *Xfp* in olive is relevant to scenarios where this subspecies is introduced to North America, or where GWSS is introduced to areas where *Xfp* is already present such as the Mediterranean.

EARLY DETECTION OF XYLELLA FASTIDIOSA INFECTION USING REFLECTANCE PROXIMAL SENSORS IN ALMOND AND OLIVE TREES

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Text

Asymptomatic infection, long incubation period and non-specific symptoms hamper early detection of *Xylella fastidiosa* (*Xf*), which is essential to successfully eradicate, contain and manage *Xf* epidemics. Our objective was to assess the response of *Xf*-infected plants by physiological traits using proximal sensors at leaf level in (i) artificially infected olive plants and (ii) naturally infected almond trees in Mallorca Island. Olive leaves were measured, sampled and tested for *Xf*-infection for 18 months. Almond *Xf* symptoms were evaluated in 150 trees in May, June and July 2022. One branch was selected per tree and leaves measured and tested each month. Our results identified, in both species, physiological traits related to anthocyanins, carotenoids, xanthophylls content as the best indicators to discriminate between plants *Xf*-infected (even in absence of visible symptoms) from those non-inoculated or testing *Xf*-negative. Hierarchical clustering and discriminant analyses showed distinct groups differentiating symptomatic from asymptomatic leaves. Moreover, machine learning algorithms revealed the predictive capability of the visible and infrared spectral information to detect *Xf* infection in asymptomatic almond leaves 1 or 2 months before symptoms appearance with overall accuracies >87%. These results allow the application of imaging techniques, which is being used in a phenotyping platform.
Funding: XF-ACTORS, ITS2017-095(CAIB), E-RTA2017-00004-C06-02 (AEI-INIA), OIAOE, BeXyl

GRAPEVINES PRIMED WITH LIPOPOLYSACCHARIDE DEMONSTRATES SYSTEMIC RESISTANCE TO PIERCE'S DISEASE AND REVEALS AN IMPORTANT PEROXIDASE MECHANISM LINKED TO THE IMMUNE MEMORY

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Text

Plants activate an enhanced defense response when challenged by a pathogen in a mechanism called priming. Molecular signatures of microorganisms known as microbe-associated molecular patterns (MAMPs) can activate priming. The bacterium, *Xylella fastidiosa* (*Xf*), is a xylem-limited pathogen containing lipopolysaccharides (LPS) considered as a MAMP. LPS isolated from *Xf* elicits a priming response from *Vitis vinifera* grapevines. Primed grapevines experienced lower pathogen titer and significantly less disease compared to naïve vines.

During the priming phase (PP) and post-pathogen challenge phase (PPCP), analysis of differential gene expression revealed considerable transcriptional reprogramming. Unlike naïve vines during the PPCP, primed vines revealed an increased number of differentially expressed genes temporally and spatially.

We identified that *VviCP1*, which is a cationic peroxidase, was upregulated during the PP and PPCP. This suggests that *VviCP1* may link the PP to the PPCP. Constitutive expression of *VviCP1* demonstrated significant disease resistance suggesting that a mechanism relying on a peroxidase mediator is important during defense priming to the immune memory. Compared to naïve vines, weighted gene co-expression analysis revealed that more genes were co-expressed in primed vines of local and systemic petioles. This signifies that in primed plants there exists an innate synchronization of the systemic response to combat *Xf*.

MOBILE DIGITAL DATA COLLECTION TOOLS: XYLELLA FASTIDIOSA OUTBREAK PREPAREDNESS & PLANT HEALTH SURVEILLANCE

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Text

Xylella fastidiosa poses a significant threat to UK plants, with the potential to cause widespread damage. As infected hosts may have long asymptomatic periods, it is crucial to have effective tools for early detection and rapid response to Xylella outbreaks. We developed a digital data collection system to coordinate Plant Health Inspectors during a rapid response to Xylella outbreaks. The system uses ESRI ArcGIS Online applications to generate survey grids around positive findings and allocate grid squares directly to inspectors. Barcodes attached to samples and scanned into the ESRI Field Maps mobile app provide full traceability and controlled data entry ensures consistency. All collected data is available on ESRI Dashboards for immediate analysis, ensuring that policy decisions and data analysis are always based on the latest data. Full deployment and training for 400+ inspectors were completed in 10 months. The system has proved to be effective in directing sample collection and providing real-time information to management teams during simulated outbreaks. These digital tools are essential for mitigating the impact of Xylella on UK agriculture and horticulture.

USE OF PLASMID PROFILES IN EPIDEMIOLOGICAL SURVEILLANCE OF XYLELLA FASTIDIOSA OUTBREAK IN THE VALENCIAN COMMUNITY, SPAIN

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Text

In the *Xf* Demarcated Area (DA) of the Valencian Community (VC), Spain, all infected plants and insect vectors harbor the same sequence type (ST) as identified by MLST analysis (*Xf* subsp. *multiplex* ST6). However, *Xf* strains harboring three plasmid profiles have been isolated to date in this DA: strains harboring one (pUCLA-ESVL) or two (pXF64-Hb_ESVL and pUCLA-ESVL) plasmids, or no plasmids. In this study, we used a PCR-based plasmid-typing approach as a tool in the epidemiological surveillance of this *Xf* outbreak. A set of hundreds of DNA samples testing positive by qPCR including infected almond trees (86% of samples), 20 plants species including crops and landscape plants identified as *Xf* hosts in this DA, as well as three species of *Xf* insect vectors (3% of samples) were typed for the presence of the two plasmids. Results revealed that four plasmid profiles were present in this DA, with a new plasmid profile being identified, that did not match any strain isolated to date. The plasmid profile most abundant was pXF64-Hb_ESVL(-)/pUCLA-ESV(-) (55%), followed by pXF64-Hb_ESVL(+)/pUCLA-ESVL(+) (24%); however, its distribution differed among municipalities being some of them more prevalent in specific regions. The geospatial associations of the different plasmid profiles and the implications in the surveillance of the *Xf* epidemic in the DA of the VC will be discussed.

Financed by Projects E-RTA2017-00004-C06-02 (AEI-INIA, Spain) and BeXyl (grant ID 101060593, EU-Horizon Europe)

DETECTION OF SYMPTOMS INDUCED BY XYLELLA FASTIDIOSA WITH HIGH-RESOLUTION MULTISPECTRAL SATELLITE DATA: ASSESSMENT WITH AIRBORNE HYPERSPECTRAL IMAGERY

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Text

Xylella fastidiosa (Xf) and Verticillium dahliae (Vd) are plant vascular pathogens that threaten global olive and almond production, restricting the flow of water and nutrients. Previous studies have demonstrated that hyperspectral and thermal imagery acquired at high spatial resolution successfully detected early symptoms of infection. Here, we assessed the feasibility of scaling-up Xf- and Vd-detection algorithms to high-resolution multispectral satellites Worldview-2 and -3 (WV 2&3) against airborne hyperspectral imagery in olive and almond orchards acquired across countries and seasons. Machine learning algorithms were trained using as inputs model-inverted plant traits, solar-induced fluorescence (SIF), and a thermal indicator (CWSI). Results obtained with multispectral satellite and airborne hyperspectral datasets reached similar accuracies for detecting advanced stages of disease development (OA=0.81/0.91; k =0.63/0.81, respectively). However, the detection of the early stages of disease development was outperformed by hyperspectral data (OA=0.74 and k=0.47 for WV 2&3) due to the inability of WV 2&3 to track critical plant traits such as NPQI, xanthophyll proxy PRIn, SIF, or anthocyanin content. Adding the thermal CWSI indicator to satellite data improved OA by 10%–15%, but the early detection of symptoms still requires specific narrow bands sensitive to Xf- and Vd-induced symptoms. Comparisons across species (almond, olive) and pathogens (Xf, Vd) will be discussed.

GENOMIC CHARACTERIZATION OF XYLELLA FASTIDIOSA SUBSP. PAUCA STRAINS ISOLATED FROM OLIVE (OLEA EUROPAEA L.) AND ALMOND (PRUNUS DULCIS MILL.) TREES IN ARGENTINA

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Text

In Argentina, *X. fastidiosa* (Xf) subsp. pauca is considered a regulated non-quarantine pest that is causing serious phytosanitary problems in traditional olive plantations over 50 years old, especially in cv. Arauco grown in La Rioja province. Using the MLST typing system, two sequence types (ST) exclusive to Argentina were identified; ST69 and ST78 being detected in olive and ST78 in almond. In this study the complete genomes of two strains of *Xf* subsp. *pauca* isolated from olive (La Rioja region) and one from almond (Catamarca region) were obtained using a hybrid assembly approach combining data from Illumina and Oxford Nanopore sequencing platforms. The assemblies were generated with Trycycler, polished with medaka + pilon and evaluated with BUSCO. All strains were assembled at the chromosomal level (~2.6 Mb) and one plasmid was found in each strain. A Maximum Likelihood tree including a worldwide collection of *Xf* genomes indicated a closer relationship of isolates from Argentina with those from Brazil. These genomes represent a valuable resource in comparative genomic studies to extend the knowledge of *Xf* outbreaks, providing

data to determine potential *Xf* origin, virulence, and evolution. These are the first *Xf* genomes obtained from olive and almond trees in Argentina.
Research funded by PICT-2020-00230, PROYECTO-INTA I090, Red CYTED, E-RTA2017-00004-C06-02 (AEI-INIA, Spain) and BeXyl (grant ID 101060593, EU-Horizon Europe)

UNDERSTANDING POTENTIAL VECTORS OF XYLELLA FASTIDIOSA IN AUSTRALIA

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Text
Xylella fastidiosa although not present in Australia is a destructive plant pathogenic bacterium, with several subspecies affecting many plant species including grapevines, almonds, peaches, apricots, and olives in many countries worldwide. It is xylem limited, and transmitted mainly by spittlebugs, sharpshooters, leafhoppers and froghoppers. Xylella and its exotic vectors have been identified by the Plant Health Committee as the number one National Priority Plant Pest for Australia, by New Zealand MPI as an 'unwanted organism' and by the European Commission as one of most dangerous plant bacteria worldwide. Our research aims to provide biosecurity agencies with tools and knowledge which can be effectively implemented to rapidly eradicate Xylella fastidiosa, or prevent and suppress its spread if there is an incursion in Australia. This project focuses on potential insect vectors of Xylella, biology, physiology and ecology in targeted horticultural crops across three states of Australia. Efforts, methodology, results, and its significance to Australian food production will be communicated and discussed.

DECIPHERING XYLELLA POTENTIAL PATHOSYSTEMS IN THE BELGIAN FLORA : A STUDY ON NORTHERN EUROPEAN TEMPERATE REGIONS WITH EMPHASIS ON RIPARIAN AREAS

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Text
The discovery of various strains belonging to three subspecies of *Xylella fastidiosa* in Europe has raised concerns about the potential spread of the bacteria to Northern regions. It is crucial to evaluate the susceptibility of European flora, which has not been exposed to this pathogen before. In a biosafety facility, we mechanically inoculated KLN59.3 GFP-labelled *X. fastidiosa* at 22°C and 28°C to assess the susceptibility of *Salicaceae*, including *Populus tremula, Populus canescens, Salix alba,* and *Salix caprea*. The movement and multiplication

of bacteria in plants were examined using PCR, real-time PCR, confocal or scanning electron microscopy. After nine months, all plants tested positive for *X. fastidiosa*, except for 57% of *P. canescens* under the 22°C growing conditions. Bacteria were detected up to 120 cm from the inoculation point for *S. alba* and were found in the roots of all species. Successful isolation was achieved for *S. alba* and *P. tremula*. The average CFU/g of plant tissue per species ranged from 1.5E+03 to 3.5E+06, with the highest figures for *P. tremula*, which also showed a high number of totally obstructed vessels observed by confocal microscopy. Additionally, more than 400 endophytic bacterial isolates obtained from *Salicaceae* xylem sap, extracted using a Scholander pressure chamber, are currently under investigation as potential antagonist agents.

XYLELLA FASTIDIOSA INFECTION ALTERS THE XYLEM MICROBIOTA IN WILD AND CULTIVATED OLIVE TREES

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Text

Nowadays, there are no tools available to cure *Xylella fastidiosa* (*Xf*) once a plant becomes infected. Research on plant-associated microorganisms is gaining increasing interest as an innate natural defense of plants to cope against infection by pathogens. In this study we have characterized the xylem-inhabiting bacterial communities in olive trees infected or not by *Xf* under natural field conditions in the Balearic Islands (Spain) by using a NGS approach. We have also evaluated the potential differences in the xylem microbiota associated to wild and cultivated (native or introduced) olive genotypes. For that, 244 samples from leaf petioles of *Olea europaea* plants were sampled and fingerprinted using an EST-SNP marker analysis to identify the olive accession. Alpha and beta-diversity measures of bacterial communities indicated that the main significant differences were associated to *Xf* infection, followed by the origin of the olive genotype (wild or cultivated) within each island (Mallorca, Menorca and Ibiza). Also, we have identified different bacterial genera significantly enriched according to *Xf* infection and olive genotype. The relationship between the genetic closeness of cultivated versus wild olive genotypes along with their differences in the xylem microbiome will be addressed.
Supported by Projects PID2020-114917RB-I00 and TED2021-130110B (AEI-Spain) and BeXyl-101060593 (EU-Horizon Europe)

EVALUATION OF OUTBREAK RESPONSE PLANS FOR XYLELLA FASTIDIOSA IN ALICANTE, SPAIN

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Text

Xylella fastidiosa is a priority quarantine pathogen in the EU. As laid down in the EU legislation, after an outbreak, a demarcated area is established delimiting the infested zone surrounded by a buffer zone, where intensive surveillance and control measures are applied. The spread of almond leaf scorch disease (ALSD) in Alicante, Spain, was simulated with a spatial individual-based epidemiological model, comparing the performance of different surveillance and control designs for outbreak management. Two survey designs were compared where the survey effort was estimated based on the population size, or in two-steps, first the sample size per hectare, and second the hectares to be inspected. Two confidence levels, buffer zone sizes and eradication radii were compared, including those set by the regulation. The effect of vector control and inoculum removal was considered in the model by reducing the transmission rate parameter in the buffer zone, greatly decreasing the number of infected trees. Without the reduced transmission rate, the number of infected trees was similar to that obtained without any intervention, even with the eradication measures in place. No major effects were observed with the different buffer zone sizes and eradication radii. Higher confidence levels resulted in better disease control but increased survey efforts. The two-step approach had the greatest survey efforts but, did not improve disease control substantially.

IS A 'HIGH QUALITY' PLANT ALWAYS HEALTHY? CHARACTERISING PERCEPTIONS OF 'QUALITY' ACROSS THE LIVE PLANT TRADE IN THE CONTEXT OF XYLELLA FASTIDIOSA.

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Text

The movement of plants through the ornamental plant trade presents a major source of risk for the introduction and spread of *Xylella fastidiosa (Xf*). Prevention is crucial and involves understanding how trading bodies assess the risks associated with procurement of host plants. We focus on how key businesses define whether a plant is 'healthy' or not. We interviewed 44 businesses across the UK such as garden centres, nurseries, growers, and wholesalers and surveyed a further 100 in one specific county identified as a potential entry point for *Xf*. 'Quality' was consistently presented as a characteristic used to judge the health of a plant. We unpacked this further to identify what quality means and how this may influence purchasing decisions. Three main themes emerged: the quality of the plant (e.g. appearance, colour), quality of the supplier (e.g. relationships, response to queries), and quality of processes (e.g. on-site husbandry, record keeping, biosecurity checks). Each of these themes presents challenges to ensuring that Xf is detected or detectable. For example, assessing plant quality is largely informed by visual inspection, which may miss vital clues that a plant is infected. Long-term relationships with suppliers may lead to (mislaid) trust that their biosecurity processes are sufficient to prevent or detect Xf. By clearly outlining factors influencing current purchasing behaviours, we will present opportunities for improving biosecurity across the supply chain.

IDENTIFICATION AND CHARACTERIZATION OF A XYLELLA FASTIDIOSA RESISTANT ALMOND GENOTYPE

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Text

Almond leaf scorch (ALS) caused by *Xylella fastidiosa* is an emerging disease in the EU and the Mediterranean that lack effective management tools. To identify *X. fastidiosa* resistance in almond, we grafted seven genotypes on infected almond trees in a commercial orchard, using the commercial cultivar 'Um ElFahem' (UEF) as a susceptible control. Each genotype was grafted on at least 10 trees with 3-4 grafts per tree. Disease incidence and severity were determined visually at 170 days post grafting. Six of the grafted genotypes including UEF, were similarly susceptible to *X. fastidiosa* with 65 to 82% of the grafts showing ALS symptoms. On the other hand, one genotype was clearly more resistant with only 12% symptomatic grafts. In the following year, none of resistant genotype grafts expressed ALS symptoms, while 87% of the UEF grafts were symptomatic. Furthermore, only 12% of the resistant genotype grafts were positive for *X. fastidiosa* in the second year, compared with 50% in the first year. We also measured *X. fastidiosa* migration and relative concentration in the grafts using quantitative-PCR and found that while *X. fastidiosa* migrated to similar distances in UEF and in the resistant genotype, its population was significantly lower in the resistant genotype. This indicates that this genotype is resistant to *X. fastidiosa* and that its resistance intensifies over time. Further characterization of the resistant genotype is underway and will be presented at the meeting.

CAN A PATHOGENIC PLANT GALL BECOME A BENEFICIAL SYMBOINT? ROLE OF AGROBACTERIUM IN DEVELOPING SOLUTIONS TO PLANT VASCULAR BACTERIAL DISEASES.

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Text

Plant vascular diseases due to infection with bacteria, like Xylella fastidiosa (Xf), are difficult to treat due to inefficient therapeutic molecule delivery. We used Agrobacterium tumefaciens (At) as a therapeutic molecule delivery tool by converting the pathogenic gall into a beneficial symbiont. This was accomplished by creating a T-DNA that included four genes from T-DNA of pathogenic At that encode cytokinin and auxin biosynthetic enzymes together with a therapeutic gene of interest (gene for production of antimicrobials) . The plant growth regulators induced cell division/gall formation. The T-DNA also encoded a

visual marker molecule (mCherry or GFP fluorescent proteins or betalain a small red colored betacyanin molecule) to observe production in symbionts. The symbiont T-DNA vector was inserted in the disarmed At EHA105 strain for plant inoculation. Our proof of concept focused on citrus greening disease, caused by the phloem-limited bacterium 'Candidatus Liberibacter asiaticus' (CLas). Upon symbiont formation, marker proteins were expressed in the symbiont, and movement into neighboring plant tissues was detected by western blot. When antimicrobial peptides were expressed, symbionts produced on CLas-infected citrus reduced foliar symptoms and CLas titer. We present that symbionts can be used as a screening tool or as a field deployable solution to deliver therapeutics for plant vascular bacterial diseases such as Xf Induced diseases.

THE SECOND MESSENGER C-DI-GMP: VIRULENCE AND BIOFIM FORMATION BY XYLELLA FASTIDIOSA

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Text

The vascular pathogen *Xylella fastidiosa* subsp. *fastidiosa* Temecula1 causes Pierce's Disease in grape plants. Bacterial proliferation blocks the transport of sap through the xylem, causing water and mineral nutrient alterations in the aerial parts of the plant. The pathogenicity mechanisms involve biofilm formation in the xylem vessels of infected plants. In the genome of Temecula1 there are few genes encoding GGDEF/EAL domain-containing proteins potentially involved in the turnover of c-di-GMP. It is known that this second messenger regulates the multicellular life style in bacteria. In *Xylella*, low levels of c-di-GMP are associated to acquisition of bacteria by the insect vector, while high levels of the second messenger are associated with the exploratory phase in plants (virulence). These phenotypes are contrary to the accepted canonical framework of c-di-GMP influencing the lifestyle of most phytopathogenic bacteria including other *Xanthomonadaceae*. We have generated mutants in GGDEF/EAL domain-containing proteins which were unavailable, investigated their role in virulence of grapevines and implemented a protocol to quantify c-di-GMP in these bacteria with the aim of certainly testing whether the c-di-GMP paradigm has an exception in *Xylella*, as it is suggested by the mutants' phenotypes. Identifying compounds which may inhibit biofilm formation by *Xylella fastidiosa* strains and unveil the mechanisms involved is also a goal.

FACTORS DRIVING INSECT VECTOR PRESENCE, ABUNDANCE AND PATHOGEN TRANSMISSION: THE CASE OF PHILAENUS SPUMARIUS AND NEOPHILAENUS CAMPESTRIS

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Text
Global trade routes have increased the risk of new disease outbreaks and enhance the invasive species spreading worldwide. *Xylella fastidiosa* is one of the vector-borne plant pathogens that provokes huge economic impact. To understand its epidemiology, it is crucial to unveil the ecological factors affecting its insect vectors. *Philaenus spumarius* and *Neophilaenus campestris* are the most important vectors in the Balearic Islands. To shed light on the ecological drivers that influence vector seasonality, a three-year macrocosm study was conducted in olive, vineyard and almond crops in Majorca. Also, transmission tests were conducted under laboratory conditions. We used Generalized Linear Models (GLMs) to assess the effect of different ecological factors (e.g., type of crop or vegetation structure) on the presence and abundance of the vectors. Moreover, we evaluate the differences in transmission of *X. fastidiosa* between both vector species using Generalized Mixed Linear Mixed Models (GLMMs) and Pearson correlation. Our results highlight that the most influential variables explaining the presence and abundance of *P. spumarius* were the vicinity of canopy and cover and for *N. campestris* cover and border vegetation compartments. On the other hand, results on transmission trials showed no correlation between the number of positive insects and the number of plants infected, and that percentage of plants infected did not depend on the species of vector.

USE OF APTAMERS AS A DIAGNOSTIC TOOL FOR XYLELLA FASTIDIOSA

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Text
Aptamers are single-stranded nucleic acids capable of adopting defined three-dimensional structures that allow them to interact with high affinity and specificity with their target molecule. To identify aptamers that specifically and differentially recognize bacterial cells of *Xylella fastidiosa* (*Xf*), 12 rounds of selection against a mixture of five *Xf* strains belonging to different subspecies were performed, and counter-selection was carried out in all rounds against bacteria belonging to 20 genera that are common inhabitants of the plant xylem. The enrichment in *Xf*-specific aptamers of the populations obtained in successive rounds of selection was analyzed by real-time PCR and rounds 11 and 12 were cloned to obtain individual aptamers and analyzed by Next Generation Sequencing (NGS). The ability of six aptamers identified to recognize *Xf* bacteria cells was studied by slot-blot and ELONA and their structural characterization was performed with m-Fold (secondary structure) and QGRS mapper (possible G-quadruplex) software. Two aptamers have been identified (X11-5F and X136699R) able to recognize with a high affinity a set of 15 *Xf* strains belonging to four subspecies being their binding capacity significantly higher than that shown against other common xylem-inhabiting bacteria. The potential use of these aptamers as a diagnostic tool for *Xf* will be discussed.
Financed by Project P18-RT-4184 from the Regional Government of Andalusia and the European Social Fund

ESTABLISHMENT OF AN IN VITRO COLLECTION OF OLIVE CULTIVARS

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Text

As part of the UN-programme for development of integrated techniques for inducing genetic diversity and improvement of vegetatively propagated and horticultural tree crops (CRP D24014) genotypes of Olea europaea were established in vitro. The research focused on developing and validate tissue culture protocols as a first step to reach induced stable mutants with tolerance to Olive Quick Decline Syndrome Disease. The cultivars ‘Coratina’, ‘Favolosa’ ‘Frantoio’, ‘Leccino’ and ‘Ogliarola‘ were kept under greenhouse conditions. Single node explants of juvenile branches from trees were used for the initiation of the cultures. The aim of this work is to study the influence of factors such as explant maturity, position within the branch on the establishment of an in vitro shoot culture of Olea europaea.The basal medium Olive medium (OM) was supplemented with different cytokinins. Significant differences in the survival percentages were found between the cultivars with SPPS statistics 27. Interestingly, the response of explants from two different donor plants of ‘Leccino’ indicates the importance of the physiological state on the establishment process. Also explants from the two branch parts showed different survival rates with apical branch part performing better. However, significant differences were found in the shoot lengths after three months. Here explants from the basal branch part produced significant longer shoots in most cultivars.

APPLICATION OF A WEB-BASED APP (CLASNIP:WWW.CLASNIP.COM) FOR THE IDENTIFICATION AND CLASSIFICATION OF FASTIDIOUS PLANT PATHOGENS

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Text

Fastidious plant pathogenic bacteria inhabit either the xylem or phloem of the vascular tissues of plants. They are usually transmitted from plant to plant by insect vectors, which also serve as hosts for reproduction and overwintering. These pathogens either resist to growth in any available media such as ‘Candidatus Liberibacter solanacearum’ (CLso), or require specific and enriched media such as Xylella fastidiosa (Xf), which makes identification and classification challenging. Here we introduce Clasnip (www.clasnip.com), a web-based platform implemented with a state-of-art classification algorithm by deriving the Maximum Likelihood estimation of the Hidden Markov Model (HMM) parameters, for rapid and reliable interspecific and intraspecific interpretation of sequences reads. To date, Clasnip has newly curated databases for the classification of potato zebra chip (CLso), grapevine pierce disease (Xf), bacterial ring rot (Clavibacter sepedonicus), soft rot and blackleg

pathogens such as Dickeya and Pectobacterium spp, and potato virus Y phylogroups. We will demonstrate the high accuracy of Clasnip in classifying CLso haplotypes and Xf subspecies from pure culture and environmental samples using Sanger, genome and metagenomics sequencing data. This intuitive platform also allows users to customize their own reference databases for expanded detection coverage. Clasnip is a decision-making tool that can facilitate disease containment and control disease outbreaks or invasions.

WHAT IS THE RISK OF XYLELLA FASTIDIOSA TO ESTABLISH IN TEMPERATE EUROPEAN REGIONS - A SCENARIO INVESTIGATED IN BELGIUM

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Text

To prepare for the potential spread of *Xylella fastidiosa* in northern Europe, where the bacterium has not yet been evidenced, addressing data gaps on both potential host plants and insect vectors is crucial. A pilot sentinel plantation was established in the infected region of Majorca to enhance knowledge for pest risk assessment. *Prunus domestica* cv. Opal, *Quercus petraea* and *Salix alba*, identified as potential keystone host polant species, along with a network of rosemary 'spy plants,' were used for symptomatic and molecular detection over four years, emphasizing the complexity of conducting sentinel plantation assays and stressing the need for long-term investigations. In addition, the potential of *Salicaceae* as an alternate host for Xylella in Belgium and Northern Europe was investigated. The flight capacity of *Philaenus spumarius* and *Aphrophora salicina* was also studied through mark-release-recapture and flight mill experiments, highlighting a possible association with riparian areas and spread over rather long distances.

Casarin, N. et al. (2022). Journal of Pest Science, doi :10.1007/s10340-022-01562-9
Casarin, N. et al. (2022). European Journal of Plant Pathology, doi:10.1007/s10658-022-02622-7
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ECOLOGICAL ASPECTS OF SPITTLEBUGS OF THE GENUS CLASTOPTERA (HEMIPTERA: CERCOPOIDEA: CLASTOPTERIDADE), POSSIBLE VECTORS OF XYLELLA FASTIDIOSA IN OLIVES IN SOUTHEASTERN BRAZIL

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Text
The species of the genus *Clastoptera* are xylem-feeder, can be potential vectors of *Xylella fastidiosa*. In olive orchards in southeastern Brazil, individuals of an as yet unidentified species of *Clastoptera* were frequently observed in association with olive plants. Then, surveys of regions and times of occurrence of nymphs and adults, natural infectivity by *X. fastidiosa*, and association with host plants of *Clastoptera* were carried out to understand their potential involvement in the dissemination of *X. fastidiosa* in olive orchards. Surveys with yellow sticky cards and visual observations in different locations in southeastern Brazil showed that *Clastoptera* occurs only in olive orchards above 1000 m altitude in the Mantiqueira Mountain Range, with population peaks of adults and nymphs between February and April. *Clastoptera* adults collected in cards were positive for *X. fastidiosa* by qPCR, indicating that individuals are naturally carrying the bacterium in olive orchards. By visual observation, adults and nymphs of *Clastoptera* were detected in shoots of olive trees and other shrub or tree plants close to olive orchards, such as *Duranta erecta*, *Salvia rosmarinus*, *Rhododendron* sp., *Tibouchina mutabilis*, and *Prunus persica*. New studies are underway to identify or describe the species of Clastoptera (apparently a new species) collected from olive trees and alternative host plants, as well as to assess their ability to transmit X. fastidiosa to olive trees.

IMPROVING PLANKTONIC GROWTH OF XYLELLA FASTIDIOSA SUBSP. PAUCA, STRAIN DE DONNO BY SUPPLEMENTING LIQUID MEDIA WITH POLYSORBATE SURFACTANTS

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Text
Xylella fastidiosa (*Xf*) is a fastidious and slow growing bacterium, difficult to grow *in vitro*. Isolates of *X. fastidiosa* subsp. *pauca* (*Xfp*) recovered from diseased olive trees in Apulia (Italy), are notoriously difficult to be cultivated in common media (PD3 and PW or PWG), and growth in liquid cultures is very limited, most likely for the abundant formation of biofilm that promotes the aggregation of the bacterial cells, forming the typical and visible ring of biofilm and cell aggregates. Such behaviour poses major constrains for testing the effects of different growth conditions or compounds on the bacterial multiplication. In this study, we tested the effect of two polysorbate surfactants, Tween-20 (TW20) and Tween-80 (TW80) at different concentrations, on the growth and biofilm formation of *Xfp* in different liquid media. TW20 and TW80 are widely used in microbiology, biochemistry, and molecular biology due to their excellent solubilization properties and low toxicity. Our results showed that TW20 positively affects the planktonic growth of *Xfp* and reduce the biofilm formation. Whereas, when TW80 was used an increase of the planktonic growth rate was recorded, but not a decrease in the biofilm formation. Overall, our results showed that by adding the two surfactants, *Xfp* planktonic growth can be significantly increased, allowing to improve *in vitro* tests aimed at evaluating the inhibition effects of compounds and antimicrobial formulations.

INTERPLAY BETWEEN BIOTIC AND ABIOTIC STRESS EXACERBATES LEAF SCORCH DEVELOPMENT IN ARABIDOPSIS INFECTED WITH XYLELLA FASTIDIOSA

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Text

Abiotic stresses, as drought, can induce xylemic vessel emboly and loss of water potential. While these responses are essential for plant survival during drought, some bacteria may evolve to explore these host responses for their own gain. Xylella fastidiosa, a vector borne xylem pathogen, specifically colonizes more than 500 different plant species. Through feeding, insects transmit the bacterium to the xylem. Xylella cells form a biofilm attached to the cell walls. Interestingly, within these plant species some develop disease symptoms, as leaf scorch, while others remain symptomless. The underlining molecular mechanisms, and the relative importance of the host response to the abiotic stress vs biotic stress as of outcome for bacterial growth and/or disease development remains unexplored. Here, we investigated the effect of drought stress on the establishment and development of symptoms in Arabidopsis infected with Xylella. Plants were exposed to drought stress for 10 days after Xylella infection. 75% of the Xylella infected leaves show rapid development of symptoms after watering, compared to either, mock infected, or normally raised Xylella infected leaves. This effect was independent of the Xylella subspecies used. Our data suggests that the host response to drought stress may increase the ability of Xylella to colonize the host. Studying the interface between drought and infection may reveal useful molecular mechanisms to create plants resistant to infection.

SCREENING AN ALMOND GENOTYPE COLLECTION FOR RESISTANCE TO XYLELLA FASTIDIOSA AND IDENTIFYING DNA MARKERS ASSOCIATED WITH THIS TRAIT

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Text

Xylella fastidiosa causes almond leaf scorch (ALS) disease in North America, the EU and the Mediterranean. Controlling the disease once trees have been infected is practically impossible, and therefore finding resistant genotypes can be an elegant solution to reduce ALS damage. We have recently identified a resistant almond genotype by conducting a small-scale screen of our almond genotype collection. In the current study we have expanded this screen to test 50 different almond genotypes. From each genotype 8 seedlings were generated by grafting genotype scions on GF677 rootstocks. Seedlings will be inoculated with *X. fastidiosa* using the needle prick method during the spring and evaluated for disease incidence, severity, and *X. fastidiosa* colonization during the summer.

The Unit of Fruit Tree Sciences in Newe Yaar has established a map of ~5000 SNP markers, evenly spread across the genome of these almond genotypes. Using this map, the Unit of Fruit Tree Sciences will lead the research to link *X. fastidiosa* resistance to specific DNA markers, which represent a region of approximately 40K bp. This will allow to identify not only markers linked to almond resistance but also candidate genes which may be relevant for the resistance phenotype. Hence, results from these experiments are expected to advance breeding efforts to generate *X. fastidiosa* resistant almond cultivars and to help understand the genetic basis of resistance to ALS.

EVALUATION OF MOLECULAR TESTS FOR THE DETERMINATION OF THE XYLELLA FASTIDIOSA SUBSPECIES: RESULTS FROM AN EUROPEAN TEST PERFORMANCE STUDY (TPS) AND THE ORGANIZATION OF A PROFICIENCY TEST (PT)

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Text
The European Commission has identified Xylella fastidiosa (Xf) as a priority pest to be addressed in the activities of the European Union Reference Laboratory for Pests of Plants on Bacteria (EURL). The detection of Xf has been harmonized among the national reference laboratories (NRLs). Currently, Xf subspecies determination requires a laborious multilocus sequence typing (MLST) test (Annex IV B of Commission Implementing Regulation (EU) 2020/1201). Over the past few years, two real-time PCR tests have been published that focus on the Xf subspecies designation. These real-time PCRs offer several advantages when compared with the implemented MLST test: firstly, they should increase the sensitivity of detection, and secondly, they should decrease the time required for subspecies designation. However, available validation data is currently rather limited. Therefore, the EURL (Netherlands Institute for Vectors, Invasive plants and Plant health-NIVIP) has scheduled a TPS on the Xf subspecies determination by these real-time PCRs. The TPS consists of 38 blind samples containing DNA from different Xf subspecies (either mixed with plant DNA or in buffer), or plant DNA and 6 controls. The results obtained from this TPS will be discussed, as well as the organisation of a PT on the subspecies designation of Xf in 2023. Results will support the addition of one or both real-time PCR tests to the Annex IV B of Commission Implementing Regulation (EU) 2020/1201.

HOW EUROPEAN VINEYARDS ESCAPED PIERCE'S DISEASE DURING THE XIX AND XX CENTURY

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Text
Pierce's disease (PD) of grapevine caused by *Xylella fastidiosa* (Xf) exhibits a limited global

distribution compared to other grapevine diseases, e.g. powdery and downy mildews. Why European vineyards were not affected by PD during the 19th and 20th centuries is a matter of scientific debate, but no clear answer has yet been provided. Here we gather phylogenetic, epidemiological, and historical evidence indicating that (i) the spread of Xf in vineyards of the southeastern USA likely occurred after the *Phylloxera* crisis in Europe (~1868-1890) and that (ii) most of American-vine species exported to Europe came from nurseries located in areas without PD risk in the USA, according to our epidemiological model. Once in France, American vines were multiplied and distributed mainly from Montpellier and Bordeaux. Using reanalysed temperature data from 1950 to 1980 we show how Montpellier would be located in an epidemic-risk zone with potentially low to very low growth rates, while temperatures between 1870 and 1900 would be below the threshold for PD epidemics (T_{av} ~ 0.5ºC lower). Our temperature-driven epidemiological model suggests moderate PD risks only in Mediterranean islands and some coastlands areas before 1980. However, global warming is accelerating since 1990 the extent and risk levels of PD in continental areas of the Mediterranean basin (e.g. Rhone valley, central Italy) and the Atlantic face of Portugal and southern France.

NANOPORE AMPLICON SEQUENCING: RAPID, SENSITIVE AND SPECIFIC DIAGNOSTIC SYSTEM TO MONITOR AND INTERCEPT XYLELLA FASTIDIOSA

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Text

Xylella fastidiosa (Xf) is a quarantine bacterium causing important diseases in various host plants. Since 2013 it has been intercepted in several European countries, starting from Italy. The recent rise in minimum winter temperatures, due to climate change, has extended the range of areas suitable for its establishment, in fact cold temperature is one of the main deterring factors for Xf establishment. The spread of this bacterium often comes from asymptomatic plant materials for which sensitive detection is needed. Moreover, in case of new outbreak or new host plant, the identification of the subspecies and the Sequence Type (ST) is required. With this purpose, an amplicon-Nanopore sequencing was developed for Xf detection and identification at the subspecie/ST level, even in asymptomatic conditions. The workflow foresees the amplification of seven housekeeping genes (MLST) and the sequencing based on MinION from Oxford Nanopore Technology, a portable, fast and easy-to-use device, paired with an ad hoc bioinformatics pipeline. Two host plants, grape and olive, have been spiked with
the Xf subspecies fastidiosa and pauca respectively, at different known concentrations (from 10^7 to 10 cfu/mL). Preliminary results indicate that this approach is a promising tool to precisely detect and identify Xf and therefore can be used to monitor the spread and the evolution of this bacterium. Finally this workflow could be applied for the correct identification of other priority pathogens.

GENETIC VARIATION WITHIN ALMOND POPULATIONS DO NOT IMPEDE LEAF SCORCH OUTBREAK IN MALLORCA

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Text

Genetic variation within host populations decreases disease risk and spread in annual crops, but this central prediction in evolutionary ecology remains largely untested in perennial crops. Around 30 years ago, two subspecies of Xylella fastidiosa (Xf) were introduced to the island of Majorca and subsequently dispersed by the insect vector, Philaenus spumarius, mainly in almond crops. During this time, almond germplasm banks distributed in various locations on the island have been naturally exposed to the pathogen. Between 2019 and 2021, the symptoms of 95 mostly local varieties of almond trees exposed to the pathogen were analyzed and monitored each year in the germplasm collections. Xf have been detected in 82 of the 95 almond varieties analyzed. In general, a good correspondence was observed between varietal response to the pathogen in the germplasm almond collection and in the field. Despite the great spatial and genetic heterogeneity found in Mallorcan almond orchards, Xf has been effectively transmitted among almond trees and orchards, causing almond leaf scorch disease in more than 1.5 million trees (83% incidence) across the island. Analysis of the data suggests that although transmission rates may be higher in host populations with genetic uniformity, vector movement is sufficient to bridge the spatial gaps between orchards containing with more resistant varieties.

SURVEYS ON XYLELLA FASTIDIOSA AND ITS VECTORS IN MONTENEGRO

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Text

Xylella fastidiosa (Xf) causes various diseases on important crops such as olives, grapevines, citrus fruits, almonds, but also forest trees, ornamental plants and plants of spontaneous flora. Since the olive is the most widespread fruit species in Montenegro, possible presence of the Xf bacterium would cause great economic losses. The paper presents the results of a three-year survey of olive trees and other host plants carried out in olive groves, nurseries, home and public gardens. With visual inspections a special attention was paid to the presence of typical and atypical symtoms. A total of 120 samples were colected and analyzed by the LAMP method (Yaseen et al., 2015), dominantly Olea europaea (39.2%), followed by Citrus spp., Prunus spp., Nerium oleander, Laurus nobilis, Magnolia grandiflora, Rosmarinum officinalis and other host plant. In the analyzed plant material phytopathogenic bacteria Xf was not detected. Bearing in mind the importance of vectors in the transmission of Xf from infected host plants to healthy ones the presence of

insect vectors in olive groves on the Montenegrin coast was monitored as well. Insects were collected on weeds, using sweep-net sampling at three locations in the area of Valdanos and two locations in the area of Radanovici. The insect population in the area of Valadanos compared to population in Radanovici was richer in number of species and more abundant in number of individuals.

A PHYSIOLOGICALLY-BASED POPULATION MODEL OF PHILAENUS SPUMARIUS

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Text

The meadow spittlebug, *Philaenus spumarius* L. (1758) (Hemiptera: Aphrophoridae), is the main vector of the plant pathogen bacterium *Xylella fastidiosa* in Europe. Effective disease containment strategies are mainly based on uprooting infected plants and control of the vectors. The vector control strategy focuses on suppressing juveniles in the herbaceous cover and preventing colonization of the olive canopy by newly-emerged adults. The design and implementation of vector control strategies can take advantage of properly calibrated population models describing and predicting the phenology and abundance of P. spumarius populations in agroecosystems.

We developed a temperature-driven physiological-based model to predict the phenology and population dynamics of P. spumarius. We parametrized model functions describing the diapause termination and age-distribution of overwintering individuals, and the temperature-dependent development and mortality rates by integrating data collected in lab experiments and literature. The model has been calibrated and validated with field data collected in Northern and Southern Italy (in the Liguria and Apulia regions).

The model can be used for a proper definition of effective Integrated Pest Management strategies to control P. spumarius populations and thus to support Xylella fastidiosacontainment.

HOST PLANT RELATIONSHIPS OF POTENTIAL INSECT VECTORS OF XYLELLA FASTIDIOSA

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Text

Xylella fastidiosa is a bacterial pathogen that causes disease symptoms in plants, such as leaf scorch and plant dieback. The outbreak of *X. fastidiosa* in Italy has affected 80% of all olive plantations and incurred a loss of €6.3 billion a year. *X. fastidiosa* has spread globally

through transport of infected plant material. Consequently, there is a high chance that *X. fastidiosa* could enter the UK from continental Europe in the future. *Philaenus spumarius* is the main insect vector of *X. fastidiosa* locally in agricultural habitats in southern Europe. The *X. fastidiosa* bacterium is transmitted from infected plants to healthy plants by *P. spumarius* feeding. My research is focussed on understanding the feeding behaviour of P. spumarius, specifically the host plant preferences of the insect in the UK.

XYLEM SAP RECOVERED FROM DIFFERENT CROP SPECIES AFFECTS IN VITRO GROWTH OF XYLELLA FASTIDIOSA SUBSP. PAUCA, STRAIN DE DONNO

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Text

Mechanisms driving *Xylella fastidiosa* lifestyle and the host susceptibility are still largely unknown. An example of successful host adaptation is given by *X. fastidiosa* subsp. *pauca* (*Xfp*) infecting olives, with a large number of cultivars (cvs) being susceptible, and resistance traits identified only in the olive cvs Leccino and FS17. In this study, the in vitro growth and gene expression of *Xfp* were evaluated in artificial liquid media enriched with xylem sap from: olive cvs Leccino (resistant) and Cellina di Nardò (susceptible), and from citrus and grapes representing *Xfp* immune species. The results show that the sap of Cellina di Nardò promoted a greater planktonic growth than Leccino. Conversely, a higher biofilm formation was detected when the sap of Leccino was used. Media supplemented with citrus sap produced the lowest values, both for biofilm formation and planktonic growth. Unexpectedly, values similar to those recorded for Cellina di Nardò were obtained using the sap from grapes. The level of expression of 15 *Xfp* genes involved in several biological processes was assessed. The multivariate analysis yielded two clusters, one including Cellina di Nardò and grapevine, and the second Leccino and citrus. These results suggest that the xylem sap composition contributes to the resistance/susceptibility of olive cvs, but as showed by the contrasting results gathered with citrus and grapes, most likely this is only one of the components of the multifactor response to *Xfp*.

CHARACTERIZATION OF THE AMYLOIDOGENICITY OF REP-WH1 DOMAINS IN PLASMIDS FROM XYLELLA FASTIDIOSA

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Text

We have been working for many years with a plasmid replication protein from *Pseudomonas savastanoi* (RepA), achieving its structural and functional characterization[1]. Such knowledge has lent to prove that its WH1 dimerization domain has amyloidogenic traits[2]. Thus, we were able to trigger the formation of amyloid aggregates, eliciting toxicity in *E. coli* by targeting the bacterial inner membrane, disrupting PMF and hampering transport through membranes, central metabolism and ATP/nucleotide synthesis and generating ROS[3,4].

Here we report the use of the homologue Rep-WH1 domains from two plasmids of *Xylella fastidiosa* (*spp. pauca* and *multiplex*) to trigger an amyloid proteinopathy. In *E. coli*, depending on the Rep-WH1 variant, it forms distinct amyloid aggregates (according to Th-S staining), drastically changing bacterial morphology, growth rates and viability. DnaK (Hsp70) chaperone overexpression, which shifts the aggregative behaviour of *P. savastanoi* RepA-WH1[5,6], also altered the aggregation pattern in one of the variants from *X. fastidosa*. Our goal is to use these Rep-WH1 variants as a control strategy against *X. fastidiosa*.

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EPPO DIAGNOSTIC PROTOCOL ON XYLELLA FASTIDIOSA: HOW NATIONAL AND TRANSNATIONAL RESEARCH FEED INTO REGIONAL STANDARDS

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Text
EPPO is one of the Regional Plant Protection Organizations recognized under the International Plant Protection Convention. One of its functions is to develop Regional Standards. EPPO has a long standing and active program for Standard setting in several areas and started a program to prepare diagnostic Standards in 1998. The objective of this program is to achieve a harmonized approach to detection and identification for regulated pests. The work is conducted by the Panel on Diagnostics and Quality Assurance in collaboration with specialized Panels (including one in Bacteriology). Panels are composed of specialists from member countries. As of March 2023, 145 Pest-specific diagnostic protocols and horizontal diagnostic Standards have been developed. The first version of the Diagnostic protocol on Xylella fastidiosa (Xf) was approved in 2003 and focused on Vitis and Citrus hosts. After the introduction of the bacterium in Italy a first revision of the protocol was prepared to include information on the different hosts of Xf (including symptoms), details on sampling and sample preparation and new tests for detection and identification. Further revisions have been initiated to incorporate outcomes of transnational research projects (e.g. Euphresco PROMODE, POnTE and XFActors). The fifth version of the diagnostic protocol was approved in 2023-02. The EPPO Secretariat is a partner in BeXyl and relevant outcomes will be considered for inclusion in the EPPO protocol.

UPDATE ON THE EUROPEAN FOOD SAFETY AUTHORITY DATABASE OF XYLELLA SPP. HOST PLANT SPECIES

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Text
Following the X. fastidiosa oubreak in Italy in 2013, EFSA was requested by the European Commission to provide scientific assistance on this plant pathogenic bacterium. EFSA conducted a pest risk assessment and published several scientific opinions on this topic, that are all included in the EFSA Journal virtual issue on X. fastidiosa (https://efsa.onlinelibrary.wiley.com/doi/toc/10.1002/(ISSN)1831-4732.XylellaVI). EFSA also released a database of host plant species of Xylella spp.. A systematic literature review allowed the compilation of lists of host plant species, along with collection of information on infection conditions, geographic locations, pathogen taxonomy and tolerant/resistant response of host plants. The database is regularly kept up-to-date with information retrieved through a comprehensive search of the latest scientific literature and EUROPHYT outbreaks notifications by Member States. The raw data are published in Zenodo platform in the EFSA Knowledge Junction community (https://doi.org/10.5281/zenodo.1339343) and interactive reports are available in the freely accessible Microstrategy platform (https://www.efsa.europa.eu/en/microstrategy/xylella). The EFSA database of Xylella spp. host plant species represents a key tool for research, risk assessment and risk management. The main findings will be presented, particularly focusing on plant hosts of Xylella fastiosa recently reported.

COMMUNITY STRUCTURE AND SEASONAL ABUNDANCE OF POTENTIAL XYLELLA FASTIDIOSA VECTORS AND RELATED TAXA IN XYLELLA-SUSCEPTIBLE CROPS IN SOUTHERN NEW SOUTH WALES, AUSTRALIA

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Text
Xylella fastidiosa (*Xf*) and its vectors (xylem-feeding leafhoppers and spittlebugs) are considered the single greatest threat to Australian plant biosecurity and Australian horticultural industries. Despite this, potential native vectors of *XF* in vulnerable crops in Australia are unknown. Thus, the aim of our study is to examine the diversity and seasonal abundance of leafhoppers and spittlebugs present in *XF* vulnerable crops and to discover if any have the potential to be vectors of *XF*. We did this by trapping and sweep netting, every two to three weeks, in *XF* vulnerable crops (wine grapes, citrus, olives and cherries) and surrounding native vegetation in one of the main horticultural areas of southern NSW. The results of this trapping effort and possible implications for the transmission of *XF* and other plant pathogens in Australia are discussed here.

THE POTENTIAL DIRECT ECONOMIC IMPACT AND PRIVATE MANAGEMENT COSTS OF AN INVASIVE ALIEN SPECIES: XYLELLA FASTIDIOSA ON LEBANESE WINE GRAPES

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Text

Xylella fastidiosa subsp. *fastidiosa* (Xf) has not been reported in Lebanese vineyards. The average gross revenue generated by Lebanese wine growers is estimated as close to US$22 million/year for 2015-2019. The potential quantitative economic impacts of an Xf outbreak and particularly, the private control costs have not been assessed yet for this country as well as for others which Xf may invade. Using Partial Budget approach at the farm gate, we estimated that a hypothetical full spread of Xf on Lebanese wine grapes would lead to maximum potential gross revenue losses of almost US$ 11 million for an average recovery period of 4 years, to around US$ 82.44 million for an average grapevine life span period of 30 years in which infected plants are not replaced at all. The first yearly estimated additional management cost is US$853 per potentially infected hectare. For a recovery period of 4 years, the aggregate estimated additional cost would reach US$2374/ha, while the aggregate net change in profit would be US$-4046/ha. The observed costs in this study support the concerned policy makers and stakeholders to implement a set of reduction management options against Xf at both national and wine growers' levels. This re-emerging alien biota should not be neglected in this country. This understanding of the potential direct economic impact of Xf and the private management costs can also benefit further larger-scale studies covering other potential infection areas and plant hosts.

REPRODUCTIVE BIOLOGY AND EGG PARASITOIDS OF PHILAENUS SPUMARIUS

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Text

The control of *Philaenus spumarius* (Hemiptera: Aphrophoridae), the main vector of *Xylella fastidiosa*, currently relies on measures against nymphs and adults. We studied the reproductive behavior of this insect and its egg parasitoids with the aim of providing new targets and approaches for control strategies against the vector. Main investigations intended to describe: i) a molecular marker of the reproductive phase in females, ii) the duration of ovarian parapause and timing of egg maturation in different climatic areas, iii) the variations in association with the symbiotic bacterium *Wolbachia*, iv) egg parasitoid communities. The results showed that: i) the expression level of the vitellogenin gene is a good marker of egg development phase, ii) the maturation of eggs and the duration of the ovarian parapause is highly dependent on the environmental conditions, iii) the prevalence of *Wolbachia* is highly variable among populations and seems to follow an increasing trend toward colder areas, iv) egg parasitoids [*Ooctonus* spp. (Hymenoptera: Mymaridae)] are present in both North and South Italy. These results start shedding light on some poorly known biological aspects of *P. spumarius*, thus providing useful information for designing new and more effective control strategies that take into consideration the reproductive biology of the main vector of *X. fastidiosa*.

PHILAENUS SPUMARIUS VIROSPHERE: THE BEXYL PROJECT

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Text

The use of virus-based biocontrol technologies as sustainable alternatives to pesticides in pest management has been extensively discussed for decades. The application of next-generation sequencing (NGS) to viral metagenomic analyses of insect populations has significantly increased the discovery rate of viruses, leading to the identification of new virus-based tools for pest management. In this perspective, the Horizon Europe-funded BeXyl project includes a task, within the work package 5, which aims at identifying potential biocontrol agents from the virosphere of the *Xylella fastidiosa* vector *Philaenus spumarius* (Hemiptera: Aphrophoridae). Eight European *P. spumarius* populations were sampled from fields characterized by distinct ecological traits, and their transcriptomes were analyzed using NGS. In this work, we present the preliminary composition of the European virome assigned to *P. spumarius*. Beside the evaluation of potential pathogenic viruses to be exploited as bio control agent, the most recurrent viruses will be further characterized alone or in combination with other stress factors to ascertain if they produce fitness cost for the vector and can be regarded as potential tools in an integrated pest management perspective. In conclusion, the identification and characterization of virus-based biocontrol agents can contribute to the development of more efficient and environmentally friendly pest management strategies.

CHARACTERIZATION OF THE METABOLIC DIVERSITY OF XYLELLA FASTIDIOSA STRAINS BY HIGH-THROUGHPUT PHENOTYPING

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Text

Xylella fastidiosa (*Xf*) is a fastidious-growing bacterium with a wide range of host plants, being able to live with a commensal or pathogenic life style in hundreds of plant species depending on the association among specific *Xf* strains and host genotype. *Xf* phenomic characterization is key to establish potential alternative or adaptative metabolic pathways changes on certain host plants. A metabolic network using the sequences of 22 different *Xf*-strains has been proposed, and a high-throughput phenotyping approach was used to characterize the metabolic fingerprinting of seven *Xf* strains belonging to the three main subspecies isolated from several hosts. The number of carbon sources used ranged from 35 to 75, while compounds used as nitrogen sources varied from 12 to 37; with only between 14 and 22 % of the compounds being used by all the strains studied. The model developed with the *Xf* core gene set identified several key functions and the results obtained with the phenotyping microplates supported the proposed metabolic network. Different new defined minimal media have been designed based on the metabolic network and results from metabolic fingerprinting indicating that growth and biofilm formation differed according to the

type of carbon and nitrogen source used, although in general all strains had lower growth compared to that obtained in PD3 medium.
Financed by Projects E-RTA2017-00004-C06-02 (AEI-INIA, Spain) and BeXyl (grant ID 101060593, EU-Horizon Europe)

THE EPPO INSPECTION STANDARDS FOR XYLELLA FASTIDIOSA: HOW NATIONAL AND TRANSNATIONAL RESEARCH FEED INTO REGIONAL STANDARDS

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Text
EPPO is one of the Regional Plant Protection Organizations recognized under the International Plant Protection Convention. One of its functions is to develop Regional Standards. EPPO has a long standing and active program for Standard setting in several areas including inspection. Inspection Standards are developed to provide information to phytosanitary inspectors on when and how to inspect consignments or places of production of plants for planting and include sampling guidance (how lots are identified, and what is the unit of inspection). The EPPO Inspection Standards PM 3/81(3) Inspection of consignments for *Xylella fastidiosa* and PM 3/82(3) Inspection of places of production for *X. fastidiosa* were developed by the EPPO Panel on Phytosanitary Inspections following the introduction of *X. fastidiosa* in the EPPO region. Both Standards, were first approved in 2016 and have been revised twice, with the most recent update in 2022 incorporating additional sampling guidance (e.g. pooling of samples) from the EU H2020 project XF-ACTORS. Both Standards include common sections, e.g. information on vectors of *X. fastidiosa*, host plants, symptom description and general elements for phytosanitary inspections. The EPPO Secretariat is a partner in BeXyl and relevant outcomes will be considered for inclusion in the EPPO Standards.

DYNAMIC MODEL OF THE XYLELLA FASTIDIOSA PATHOSYSTEM: AN EXAMPLE ON ALMOND TREES FROM SOUTH-EAST SPAIN

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Text
The almond tree is one of the most widely grown species around the Mediterranean basin, in

southern Spain alone it generates an estimated net economic impact of €9 billion/y. However, since 2016, the xylem-feeder bacteria *Xylella fastidiosa* has spread and infected crops from the Balearic Islands to peninsular Spain. The main transmission vectors of *X. fastidiosa* in the Spanish Mediterranean are the spittlebugs *Philaenus spumarius* and *Neophilaenus campestris*. Despite all the management measures taken, the situation has become critical in certain areas, such as the northern part of the province of Alicante, Spain. As an alternative to the institutionally-sanctioned use of pesticides and crop eradication campaigns, other strategies which are currently in preliminary experimental stages are being sought out that directly target the pathogen stages. One such approach currently being investigated in our laboratory, involves the use of antibacterial endolysins, which are peptidoglycan-lysing enzymes derived from bacterial viruses.
To support these efforts, we present a dynamic model of the entire *X. fastidiosa* pathosystem. Our model is based on field data and literature. This model allows us to develop simulations of diverse scenarios, which help to identify where different measures should be implemented. By incorporating laboratory data, our results show a first insight into the required effectiveness of lysins to control the spread of the bacteria.

XYLELLA FASTIDIOSA AFFECTS THE ALMOND TREES IN THE PROVINCE OF ALICANTE (SPAIN). SINCE WHEN?

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Text
In 2017, Xylella fastidiosa (Xf) reaches the almond trees of the province of Alicante (Spain). Our project (AICO 2021/331) funded by valencian government consists of developing mathematical-computational models that incorporate environmental variables. For validation, our model representing the spread of the plant pest must be fitted to the actual data available. Here we ran into a difficulty, because we are not sure the date of the analysis of the samples coincides with the date on which the tree was infected. The origin (time and coordinates) of the infection is unknown. So, the question is, when and where did the contagion of the almond trees really begin in the province of Alicante?
Our hypothesis is Xf has been around long before 2017. It is based on the observation and analysis of the production of almonds both in the province of Alicante and in Spain. Since almost twenty years ago, we observe a decline in almond production and cultivated area due to abandonment by farmers that suffer low crop yields, while production has increased in other areas in Spain. Despite new plantations have a higher yield, the permanent decrease in the harvest confirms the unstoppable destruction, if not remedied, of the traditional Valencian almond tree.
Although there are several causes such as inclement weather, scant help from agricultural insurance and disproportionate increase in production costs, the downward trend being almost 50% in some varieties could confirm our hypothesis.

POTENTIAL ALTERNATIVE WOODY PLANTS FOR THE RENEWAL OF “POST-XYLELLA” AGRICULTURE IN SALENTO (ITALY)

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Text

Since its first discovery in 2013, the epidemic of Xylella fastidiosa subs.pauca ST53 in few years devastated the olive orchards of 3 provinces in Salento (Puglia, Italy) causing huge economic, landscape and environmental problems as well raising the need to rebuild the main and almost unique arboreous coverage, increasing biodiversity and resilience of the agro-ecosystem. The identification/evaluation of alternative woody agricultural and agro-forestry crops/species, both native and potentially adaptable to the soil/climatic conditions of Salento, is the starting point to regenerate and reconvert the future agriculture living together the bacterium. In the framework of a wider national project, the research is aimed to study the main aspects of alternative plants in term of agronomic requirements, characteristics, cultivation, protection, uses, investments and market. The information will be available as a “reasoned catalogue” that draws data/info from bibliographical research, specialist consulting, small farming experiences and pilot projects intercepted in the infected area. Furthermore, for the species not present in Salento, pathogenicity tests will be carried out, by controlled artificial/vector inoculation and periodic qPCR analyses, to verify their immunity or the level of resistance of the selected species. The results are addressed to producers/technicians to guide investments or pilot field trials as well to policy makers to plan strategies and funding measures.

KNOWLEDGE ON VECTOR’S FAUNA FOR XYLELLA FASTIDIOSA INVASION PREPAREDNESS: XYLEM FEEDERS IN ALBANIAN OLIVE ORCHARDS

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Text

Since its first occurrence in Southern Italy, *Xylella fastidiosa* (hereafter Xf) has gained importance, especially in matters of its management and limiting its transmission. In regions non-affected by the bacterium, such as Albania, it is necessary to assess the risks of spread, notably by understanding the ecology of its vectors. Surveys took place over 12 months from August 2015 and July 2016 in two Albanian regions (Vlora and Tirana), where olive production is important covering 14 olive orchards. The aim was to investigate the presence of Auchenorrhyncha species with particular attention to xylem feeders. Identified Auchenorrhyncha species belonging to families Aphrophoridae, Cercopidae, Cicadellidae, Issidae, Flatidae were found. In Vlora, 4 out of 11 species captured were xylem feeders, whereas, in Tirana, 3 species were captured. The most abundant xylem feeder species was *Philaenus spumarius* in both regions. Vlora showcased a higher presence of *P. spumarius* in olive trees than in Tirana. Adults of *P. spumarius* and *Neophilaenus campestris* were the most frequent in August, September, and October 2015. The agroecological analysis noted that the Vlora area shows a higher richness of Auchenorrhyncha in terms of the number of

species. Further, the analysis of real-time Lamp assay confirmed the absence of *Xf* in the tested individuals. This study has public implications and pointed out that this country is still a free territory from *Xf*, at least in the surveyed areas.

TRANSLOCATION OF QUORUM SENSING MOLECULES FROM TRANSGENIC ROOTSTOCKS TO NON-TRANSGENIC SWEET ORANGE SCIONS INCREASES RESISTANCE AGAINST BACTERIAL DISEASES

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Text

One of the greatest challenges for sustainable food production is the decrease of productivity due to pathogens, which makes urgent studies on plant-pathogen interactions regarding disease management. Previously, we developed a genetically modified citrus variety, carrying rpfF gene from *Xylella fastidiosa*, which was able to produce diffusible signaling factor (DSF), a quorum sensing molecule, and cause “pathogen confusion” to *X. fastidiosa* and *Xanthomonas citri*, thus attenuating CVC and citrus canker (CC) symptoms. Currently, we are investigating whether RpfF-expressing GM rootstocks could translocate DSF and improve the disease tolerance to non-transgenic scions. Hence, we verified that RpfF was produced at high levels in “Carrizo” rootstocks and triggered tolerance to CC in non-transgenic scions. Based on this, we also transformed “Swingle" rootstock, resulting in 3 GM lines (GM-SR1- 3), onto which non-GM “Valencia” sweet orange (NGM-SO) was grafted. To prove DSF translocation, *X. citri* was used as a biosensor due to its rapid infection process. Thus, leaves from each NGM-SO/GM-SR and control (NGM-SO/NGM rootstock) were challenged with *X. citri*-GFP. Genes regulated by DSF were monitored through RT-qPCR. The lines showed significantly lower symptoms and bacterial titer compared to the control. Furthermore, significant modulation of DSF-responsive genes demonstrated the potential use of GM-rootstocks to translocate DSF and confer disease tolerance to non-transgenic scions.

AGRUSAVER PUGLIA XYLELLA FIELD TRIAL

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Text

Agruresina(AgruSaver) Organic
Fertilizer

For year 2021, the percentage of desiccated shoots for the year 2021 varied from as low as 3% for the treated trials compared to the control trees that exhibited on average 50% desiccated shoots.

? The comparison of molecular analysis results in terms of CFU/ml, considering the end of the treatments period for both years of experimentation (November 2021 and 2022), shows a percentage reduction for the treated trials up to 98% and for the control up to 43% for the low experimental test.

? For the low experimental Xylella field there was a production of olives, vegetative season 2022.. The treated olive trees produced significantly more olives compared to the control trees. Even the average weight of the olives seems to reach the highest values for some of the treated trials (e.g., OS-SW-F) as compared to the control trees . The Agru-S-F treatments produced 18 times more fruit compared to control trees in 2021 and in 2022 the OliveSaver-SW treatments produced 45 times more fruit compared to the control trees.

Hence, these natural biostimulant/organic fertilizer products seem to have the potential to stimulate the plants immune system and make the plants "cohabit/disease tolerant" in an environment where the Xylella fastidiosa disease is endemic.

Big Ideas in Agricultural Microbiome Science: A Community-based Interactive Workshop

COMPARATIVE FUNGAL DIVERSITY AND DYNAMICS IN PLANT COMPARTMENTS AT DIFFERENT DEVELOPMENTAL STAGES UNDER ROOT-ZONE RESTRICTED GRAPEVINES

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Text

The root-zone restriction cultivation (RR) technique is used to achieve superior fruit quality at the cost of limited vegetative and enhanced reproductive development of grapevines. Fungal interactions and diversity in grapevines are well established, but fungal diversity under RR is unexplored. DNA from rhizosphere, plant compartments, including white roots ,leaves, flowers, and berries of rr and control plants (c) was extracted at full bloom, veraison, and maturity stage. QIIME2 performed analysis based on the ITS1 region. RR primarily affected the fungal communities of the soil and plant compartments at different growth stages. Fusarium, Ilyonectria, Cladosporium and Aspergillus spp observed in the rhizosphere overlapped with the phyllosphere at all phenological stages, having distinctive abundance in grapevine habitats. Peak richness and diversity were observed in the rhizosphere at the full bloom stage of C, white roots at the veraison stage of RR, leaves at the maturity stage of RR, flowers at the full bloom stage and berries at the veraison stage of C. Except for white roots, the diversity of soil and plant compartments of treated plants tended to increase until maturity. At the maturity stage of the RR & C, the abundance of Aspergillus spp. was 25.99 and 29.48%, respectively.

Moreover, the total soluble sugar content of berries was 19.03 obrix and 16 obrix in RR and C plants, respectively, at the maturity stage.

DO SPERMOSPHERE IS ESSENTIAL TO CROP PROTECTION?

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Text
Crops are carriers of microbiome communities, these have different biological functions and are still dynamic in time and space. Among these spermosphere communities, the microbial community present in the seeds at the time of germination has been studied as an essential form of production of healthy and resistant plants. Therefore, the objective of this research was to describe information about the biology and handling of the spermosphere and in relation to cultivated plants. For this, an analysis of Web of Science was conducted for resuming the principal's pieces of information about it. However, some fresh thirty articles were selected. In this paper, the microbial diversity in seeds is also dependent on the place of origin and the cultivated variety and is regulated by environmental factors. Therefore, the modulation of this ecological niche can be useful in relation to several work processes, including the treatment of seeds with the use of microorganisms, which can promote resistance to abiotic and abiotic factors and plant growth. understanding at the molecular level of the community and, therefore, the production sites with microbiomes that can favor the resilience of commercial crops. Research can be carried out to understand biology and ecology, as well as the correct management of the microbial community.

Biological induced resistance in plants against pathogens using beneficial microbes and natural substances

POTENTIAL OF DUCKWEED EXTRACTS TO CONTROL THE ANTHRACNOSE AND STIMULATE GROWTH OF BEAN PLANTS

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Text
Duckweed species are small free-floating aquatic plants belonging to the Lemnoideae subfamily which are found in a wide variety of climates worldwide. They have been mainly exploited for bioremediation, but several properties (fast growing, easy harvest, etc.) make them highly attractive for other applications. In this work, the duckweeds *Landoltia* sp. (La), *Lemna* sp. (Le) and *Spirodela* sp. (Sp) were cultivated under nutritionally controlled conditions and yields measured during 5 weeks. Ethanolic extracts at 25 and 50 $mg.mL^{-1}$

were tested in vitro against *Colletotrichum lindemuthianum*. Bean plants (*Phaseolus vulgaris*) grown in greenhouse were sprayed 3 days before challenging with the fungus and disease severity assessed at 7 and 12 days after inoculation (dai). The effect of extracts on length and dry weight of shoot and root was determined at 9 days after seed treatment. Landoltia, a recently segregated genus from Spirodela, exhibited the highest fresh yield, *i.e.* 239 g/ m^2/ week. All extracts reduced the mycelial growth of *Colletotrichum* by c. 32%. Although all of them did not significantly affect conidial germination, Sp-extract reduced the formation and markedly the melanization of appressoria after 48 h of incubation. All extracts reduced anthracnose severity locally and systemically at 7 dai. Systemic effect at 12 dai lasted only on La-treated plants. No extract affected elongation and dry weight of roots, but La- and Sp-treatments enhanced these variables on shoots.

DEHYDROASCORBATE INDUCES RESISTANCE AGAINST ROOT-KNOT NEMATODES IN THE SAME AND A SUBSEQUENT RICE GENERATION

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Text

Ascorbic acid is a plant antioxidant that regulates various physiological processes. Exogenous treatment with its oxidized form, dehydroascorbic acid (DHA) induces systemic resistance in rice against root-knot nematode Meloidogyne graminicola. Transcriptome analysis on roots of DHA-treated plants revealed induction of genes related to plant immunity, antioxidant activity, salicylic acid (SA) and diterpenoid production. Biochemical measurements, combined with use of chemical or genetic inhibitors confirmed that H_2O_2, SA and diterpenoid accumulation contribute to DHA-induced resistance. DHA was observed to protect plants from nematode infection for up to 14 days after treatment. Based on this knowledge an experiment in a naturally nematode-infected rice field in Bangladesh was executed using biweekly applications, and significant control efficacy was observed.
In an intergenerational experiment, the offspring of lifelong DHA-treated plants was also found to be less susceptible to M. graminicola than offspring of mock-treated control plants. The transcriptional changes in the offspring showed remarkable similarities to those of their parents. This intergenerational acquired resistance was found to be dependent on ARGONAUTE 4, an important player in the RNA-directed DNA methylation pathway. This resistance phenotype was however not inherited further.
Taken together, our results reveal interesting potential for DHA to be used as novel nematode control agent in rice.

COMPLEMENTARY PEPTIDES REPRESENT A CREDIBLE ALTERNATIVE TO AGROCHEMICALS BY ACTIVATING TRANSLATION OF TARGETED PROTEINS

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Text

The current agriculture main challenge is to maintain food production while facing multiple threats such as increasing world population, temperature increase, lack of agrochemicals due to health issues and uprising of weeds resistant to herbicides. Developing novel, alternative, and safe methods is hence of paramount importance. Here we show that complementary peptides (cPEPs) from any gene can be designed to target specifically plant coding genes. External application of synthetic peptides increases the abundance of the targeted protein, leading to related phenotypes. Moreover, we provide evidence that cPEPs can be powerful tools in agronomy to improve plant traits, such as growth, resistance to pathogen or heat stress, without the needs of genetic approaches. Finally, by combining their activity they can also be used to reduce weed growth.

ACTIVATION OF SEED DEFENSES THROUGH DEFENSE PRIMING

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Text

Yield losses due to seedborne pathogens have a major economic impact on the global food market. We attempted to develop sustainable strategies to stimulate pathogen resistance from the seed that have a negligeable impact on seed quality parameters, such as germination rate and homogeneity. Our approach consisted in applying defense priming treatments using plant resistance inducers (PRI) such as methyl jasmonate (MeJA) and β-aminobutyric acid (BABA) on developing or mature seeds. After a first stage of technique optimization, results from tomato and bean treated seeds showed extensive transcriptome reprogramming, with a strong effect on defense pathways. To correlate these data with pathogen resistance, we analysed developing and germinating seeds treated with different PRIs and their effects on the growth of different pathogens including bacteria and fungi. First results obtained on tomato showed a marked growth inhibition on A. brassicicola from specific PRI-treated seeds, while the effect on C. michiganensis was minor. Our next step is, now, the identification of the molecules responsible of the fungistatic effect present in treated seeds.

DESIGN AND APPLICATION OF A PLANT-IMMUNE BIOSENSOR FOR SCREENING AND DISCOVERY OF BIOCONTROL AGENTS AGAINST NECROTROPHIC FUNGAL PATHOGENS

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Text
To date, the screening of most microbial biocontrol agents has been conducted *in vitro* thereby restricting our ability to predict biocontrol behaviour in planta. Here we developed a novel approach, non-destructively capturing plant immune-biosensor output (via gene-based reporter system) in real-time for screening of beneficial microbes with plant immunity inducing properties. We focused on biocontrol agents capable of suppressing disease caused by destructive necrotrophic fungal pathogens such as *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, both for which limited host resistance exists. Searching transcriptomic databases for genes of the model plant *Arabidopsis thaliana* responsive to necrotrophic fungi and to beneficial microbes, we identified the *GLUTATHIONE S-TRANSFERASE PHI7* (*GSTF7*) gene. We designed a *GSTF7:luciferase* reporter system in stably transformed Arabidopsis for non-destructive observation of *GSTF7* expression in planta, and used this system to screen in high-throughput a collection of candidate microbes. We identified a *Streptomyces* isolate which protected plants against *S. sclerotiorum* and *R. solani*, but not against a bacterial pathogen. Treatment of plants with either the *Streptomyces* culture or its cell-free fermentation extract induced a range of stress and defense related genes and hormone signaling pathways. Our study demonstrates that *GSTF7* is a suitable marker for the rapid and preliminary screening of beneficial microbes for crop protection.

EVIDENCE OF CROSS-PROTECTION BETWEEN GEMINIVIRUSES IN TOMATO AND THE ESCAPE PHENOTYPE OF THE INVASIVE RECOMBINANT TOMATO YELLOW LEAF CURL VIRUS -IS76

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Text
Whereas cross protection was extensively studied and applied in plant protection strategies (PPS) with RNA viruses, this phenomenon was rarely reported with ssDNA viruses of the family Geminiviridae. Using tomato yellow curl virus (TYLCV), a worldwide economically important tomato geminivirus belonging to the genus Begomovirus, we formally demonstrated the existence of cross protection in geminiviruses. When a TYLCV clone is inoculated in susceptible tomato plants already infected with a mutated version of the clone (8 CG deletion in the intergenic region), its accumulation is at least 100 times lower than its accumulation in non-pre-infected plants. The protection effect persists at least two months after superinfection and was also observed in isogenic plants carrying the resistant gene Ty-1, irrespective of the super-inoculation mode, mediated by agrobacterium or the whitefly vector Bemisia tabaci. As TYLCV is not mechanically transmitted, cross protection is not easily implementable in PPS. However, as of now, it sheds new light on the unusual fitness of TYLCV-IS76, a TYLCV recombinant that easily superinfect TYLCV infected plants in spite of its high nucleotide identity with TYLCV (98 %). Indeed, considering that another TYLCV recombinant, exhibiting the same recombination pattern and the same genetic distance with TYLCV, is unable to establish an infection in tomato plants already infected with TYLCV, TYLCV-IS76 seems to exhibit a cross-protection escape phenotype.

SYMBIOTIC COMPATIBILITY BETWEEN ORYZA SATIVA AND ARBUSCULAR MYCORRHIZAL FUNGI GENOTYPES IMPACTS RICE'S

GROWTH AND MYCORRHIZA-INDUCED RESISTANCE AGAINST XANTHOMONAS ORYZAE PV ORYZAE

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Text

Arbuscular mycorrhizal fungi (AMF) are beneficial to their plant hosts in many ways. By increasing nutrient availability, AMF can improve the growth and tolerance of their host to abiotic stresses. AMF symbiosis can also reduce symptoms and pathogen load on infected plants, both locally and systemically, through a phenomenon called Mycorrhiza-Induced Resistance (MIR). However, there are few reports on rice mycorrhization, despite the high potential of this symbiosis in a context of rational water use.
We analysed the symbiotic compatibility (mycorrhization & arbuscules rate) between six rice genotypes (Nipponbare, IR64, Kitaake, Zhongua 11, Phka rumduol, Azucena) and three AMF species (*Funneliformis mosseae, Rhizophagus irregularis* and *R. intraradices*). Using both phenotypic and rice gene expression studies, we analysed the impact of AMF on rice growth and defence responses to *Xanthomonas oryzae pv oryzae* (Xoo) infection. Our results show differences in symbiotic compatibility depending on the combination studied. AMF induced either beneficial, neutral or negative effects on rice growth, but also on the extent of Xoo symptoms. Overall, *R. irregularis* induced the most beneficial effects, both by stimulating rice growth and by reducing symptoms caused by Xoo. To further investigate MIR at the molecular level in rice leaves, we analysed by qPCR a set of plant marker genes (development, hormones, nutrient transport, defence) of two rice genotypes with contrasting responses to AMF.

MULTIOMICS IDENTIFIES KEY ANTIFUNGAL COMPOUNDS AND BIOSYNTHETIC GENE CLUSTERS DURING LIQUID-STATE FERMENTATION OF A BIOCONTROL STREPTOMYCES

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Text

New, safe and sustainable fungicides are required to fill a growing gap in the crop protection market left by a decline in public acceptance or increasing regulation of synthetic chemical controls. An opportunity exists to exploit bacteria such as *Streptomyces*, well known for their ability to produce antimicrobials but where up to 90% of their metabolic potential is yet to be discovered and characterised. From a unique terrestrial and plant-associated collection of Actinobacteria we identified through traditional in vitro bioactivity screens a *Streptomyces* strain linked to potent antifungal activity against a broad range of necrotrophic fungal pathogens in crops. Whole genome sequencing revealed the strain encodes a predicted repertoire of over 50 biosynthetic gene clusters and has the capacity to produce up to 35 novel compounds. To facilitate identification of causal gene clusters and develop fermentation strategies for improved industrial scale antifungal compound production, we undertook a simultaneous targeted metabolomics and untargeted transcriptomics (RNAseq) approach to subsample *Streptomyces* cells, cell-free extracts and

volatiles during a fermentation time-course and link differential gene expression and metabolic profile to antifungal bioactivity. The process enabled rapid identification of candidate biosynthetic gene clusters underpinning activity, including PKS and volatiles.

THE ROLE OF DEFENSE PHYTOHORMONES IN INTERACTIONS WITH ENVIRONMENTS

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Text

In nature, plants are constantly surrounded by diverse microbes called plant microbiota that promote plant health and are attacked by microbial pathogens that threaten plant health. At the same time, plants are exposed to fluctuations in physical factors, including abiotic stresses. Phytohormones are small molecules produced and perceived in plants that govern coordinated plant responses to the environment. Signaling pathways mediated by phytohormones intimately interact antagonistically or synergistically to form phytohormone signaling networks, which enable plants to activate appropriate and effective defense responses as well as to balance defense and growth. However, the intricated hormone signaling network is also exploited by pathogens by interfering with hormone signaling or producing hormones (mimic) to benefit pathogen fitness. I will discuss the role of phytohormone signaling networks in interactions with environments, including biotic and abiotic factors.

FACILITATING THE LAB-TO-FIELD TRANSACTION OF AGRICULTURAL BIOLOGICALS

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Text

Crops are confronted with numerous abiotic and biotic stresses that affect productivity and quality. In response, crops can activate defence mechanisms with varying specificity. Stresses can also be alleviated by biologicals, which are compounds derived from dead or living organisms that can mimic cues to promote defence and growth. Even if biologicals have been shown to reduce rates of disease by activating host defence or by direct toxicity, safeguard yield and improve product quality, there are, nevertheless, hurdles in converting these findings into field practice.

In this talk, I will discuss new techniques, which can be used to screen and assess biologicals, such as automated phenotyping and spectral analysis. This could help in accelerating a sustainable adaptation of biologicals. I will present data on crop fitness, yield and product quality in potato and tomato and discuss the incorporation of biologicals in future breeding strategies and their role in the agro-ecological system.

Farmer and societal acceptance is another important step to transfer findings regarding biologicals from lab to practice. We have therefore assessed the knowledge, attitude and practice (KAP) of biologicals among small-holder farmers, agro-dealers and policymakers in Ethiopia, Kenya and South Africa.

In general, a better understanding - both regarding molecular mechanisms and stakeholders' perceptions - is necessary for biologicals to be efficiently integrated in future agricultural practices.

IDENTIFICATION OF A STREPTOMYCES NECROTIC ELICITOR INVOLVED IN ANTIFUNGAL ACTIVITY, PLANT DEFENSE STIMULATION AND BACTERIA FITNESS IN THE RHIZOSPHERE

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Text

Rhizospheric microbiota harbors bacterial strains participating in plant immunity and resistance to root diseases. Recently, we identified a *Streptomyces* strain AgN23 isolated from grapevine rhizosphere, which produce a broad spectrum of antifungal metabolites and activates hypersensitive responses (HR) in *A. thaliana*. A metabolomic approach lead to the identification of a candidate compound produced by AgN23 which may impair sphingolipid metabolism in plants. Sphingolipid metabolism of plants is involved in HR, thus we characterized the role of this metabolite through a reverse genetic approach, based on the construction of AgN23 knock-outs strains. These mutants showed a reduced antifungal activity and are unable to inhibit Inositol Phosphorylceramide Synthase activity, a crucial enzyme in plant sphingolipid pathway. The induction by AgN23 of markers associated with HR or immune responses was compromised in AgN23 knock-out strains: nuclear calcium influxes, necrotic lesions, defense gene expression, and production of camalexin. Finally, we explored the role of the candidate metabolite in the soil and found that it is involved in the rhizosphere colonization by AgN23. Thus, we identified a specialized metabolite produced by a *Streptomyces* strain which is involved in antifungal activity, plant defense stimulation and strain fitness in the plant environment. Further work will aim to investigate how this strain and its cognate metabolite structure the rhizospheric microbiota.

BIOSURFACTANT-PRODUCING BACTERIA AS RESISTANCE-INDUCERS IN PLANTS

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Text

Biosurfactants, which are secreted by a range of bacterial genera including *Pseudomonas* and *Bacillus*, play an important role in bacterial motility and attachment to surfaces, biofilm formation, and antimicrobial activity against a broad range of microorganisms. The most extensively studied biosurfactants are rhamnolipids, which are glycolipids mainly produced by *Pseudomonas aeruginosa* strains, and cyclic lipopeptides (CLPs), which are produced by selected taxonomic groups within the *Pseudomonas* and *Bacillus* genus. CLPs consist of a

hydrophobic fatty acid tail linked to an amphipathic oligopeptide that is partly or completely organized in a cyclic structure. Plant-associated *Bacillus* strains produce three main families of CLPs: surfactins, iturins, and fengycins. *Pseudomonas* CLPs are much more diverse and are currently classified into at least 17 different groups.
Biosurfactants also play a vital role in the interaction of their producers with plants, either as resistance inducers or as phytotoxins contributing to plant pathogenesis. They achieve these effects by interacting with the lipids in the membranes of microorganisms and plants. In particular, the role of rhamnolipids and selected *Pseudomonas* and *Bacillus* CLPs in resistance induction in rice will be further discussed. Evidence will be presented that some biosurfactants may induce resistance in plants by releasing elicitors from pathogens and other microorganisms rather than through direct interactions with the plant.

CROSS-PROTECTION: MILD STRAINS AS A PROMISING BIOCONTROL TOOL OF PLANT DISEASES

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Text
Until recently, plant virus management strategies have largely been limited to controlling the viral insect vectors, using resistant or tolerant varieties, and the implementation of hygiene protocols. However, these approaches are often insufficient. Especially newly emerging mechanically transmitted viruses are difficult to control as they do not rely on insect vectors for their transmission. In this lecture, we focus on cross-protection (also known as superinfection exclusion) as a biocontrol strategy to combat viral diseases in crops. Cross-protection is the underlying mechanism of plant vaccination against viral infection, in which plants are ‘vaccinated’ at an early stage of plant development using a mild or attenuated viral isolates to protect the plants against a subsequent infection with a more aggressive isolate belonging to the same viral species or genotype. Efficiency of protection depends largely on genome sequence homology between the protective and challenging isolates. Although the phenomenon of cross-protection was already described in late 1920s, its practical use in biocontrol is much more recent. In 2015, the first mild plant virus for plant vaccination (PepMV CH2 isolate 1906) was approved as active substance for plant protection in Europe. Since then, vaccination has been widely used for protecting greenhouse tomato crops against pepino mosaic virus (PepMV). Research is ongoing to translate this methodology to other emerging viruses in important crops.

CROSS-PROTECTION IN PLANT VIRUSES: HOW CLOSELY RELATED DO PROTECTING AND CHALLENGING VIRUSES NEED TO BE?

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Text

Cross-protection is a biocontrol method to protect plants from damage caused by pathogenic viruses. This strategy of cross-protecting a plant with a mild strain to prevent subsequent infection by related severe strains was first described in 1926. Although cross-protection was first evidenced nearly a century ago, the underlying mechanism(s) remain(s) poorly understood. In particular, although the genetic relatedness between cross-protective and challenge strains has been emphasized in a number of papers, the percentage of identity required for cross-protection remains unknown. This lack of data has led to a long and sometimes unsuccessful empirical search for cross-protective strains against some viral pathogenic ones. Recent papers have shown that cross-protection occurs only among variants of the same strain and not between variants of different strains for the citrus tristeza virus (Closterovirus) and the pepino mosaic virus (Potexvirus). In order to determine the percentage of identity required between the cross-protective and the challenge strains for cross-protection to be effective against the grapevine fanleaf virus (GFLV, Secoviridae), different cross-protective variants with decreasing sequence homology with respect to the challenge variant were selected and inoculated on Nicotiana benthamiana. Our results suggest that in addition to the genetic relatdeness, the entry point of the cross-protective and challenge strains impacts the cross-protection success.

ESSENTIAL OILS AS POTENTIAL ALTERNATIVE BIOCONTROL PRODUCTS AGAINST FUNGAL PLANT PATHOGENS AND WEEDS

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Text

Since sustainability of farming systems, and the environment preservation are crucial issues, it appears mandatory to offer farmers feasible alternatives to synthetic pesticides. Owing to their natural richness in active principles, essential oils (EO), biosourced products with a high added-value, display a wide range of biological properties, which could find applications in crop protection. Within the framework of PhytEO & DEPHYTOP phytomanagement projects (funded by ADEME) and carried out on trace element-polluted soils, the *in situ* cultivation of 3 aromatic plant species, namely *Coriandrum sativum* L., *Salvia sclarea* L. and *Angelica archangelica* L., led to the obtention of different EO. Thus, the main goals of this study were, on one side, to evaluate the in vitro direct effect of these molecules, against several phytopathogenic fungi, among which *Fusarium culmorum* and *Zymoseptoria tritici* are of major concern, due to the resulting foliar diseases, that may cause yield losses up to 70%. On the other side, the study focused on *in vitro* herbicidal effects of the different EO, against *Lactuca sativa* L. and *Lolium perenne* L., two plant species commonly used for phytotoxicity assays. Our first results demonstrate the antifungal effect of all the tested EO against the target fungal phytopathogens, as well as their potential to inhibit seed germination, and root elongation of the two tested plant species. EO from coriander and angelica, in particular, displayed higher efficiency.

CONSERVED DOWNY MILDEW-ASSOCIATED MICROBIOMES REDUCE PLANT DISEASE AND FUNCTION AS TRANSFERABLE RESISTOBIOMES

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Text

Plant microbiomes can protect plants against microbial attackers as is evidenced by the existence of disease suppressive soils. These are thought to be created by the plant in response to pathogen attack. Plants have been shown selectively promote a plant-protective microbiome in response to infection by e.g. the downy mildew of *Arabidopsis thaliana*, *Hyaloperonospora arabidopsidis* (Hpa). However, microbiomes are complex, microbiome studies have infamously poor reproducibility and evidence for protective microbiomes is often anecdotal. In this study, we show that distinct cultures of the obligate biotroph Hpa have accumulated the same bacteria that together reduce disease and can be vertically transmitted to a next population of plants growing on the same soil. We found that during routine maintenance of laboratory cultures Hpa typically is co-inoculated with an Hpa-associated bacterial microbiome (HAM) that is formed by a small number of bacterial species and subsequently dominates the phyllosphere of infected plants. Remarkably, bacterial isolates of the same abundantly-present species were found to be isogenic even when obtained from distinct and segregated cultures across Europe. We show that the HAM members benefit from Hpa infection, but that reversely HAM reduces Hpa sporulation when co-inoculated with Hpa spores. Our results thus suggest that infections with Hpa can over time result in the formation of 'resistobiomes' that contribute to plant disease resistance.

INDUCTION OF RESISTANCE IN SUGARCANE AGAINST RED ROT THROUGH PLANT DEFENSE ACTIVATORS.

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Text

Red rot caused by Colletotrichum falcatum globally challenging disease of sugarcane growing countries. Colletotrichum falcatum could decrease weight of cane by up to 29% and sugar loss by 31%. The emergence of new races of pathogen ultimately breakdown the resistance. In this study five plant defense activators salicylic acid (SA), citric acid (CA), K_2HPO_4, benzoic acid (BA), and KH_2PO_4 were evaluated at three levels of concentrations under randomized complete block design (RCBD). Biochemical analysis of catalase (CAT), superoxide dismutase (SOD), Hydrogen peroxide (H2O2 peroxidases (POD),) and total phenolic contents (TPC) of treated and un-treated leaves were performed. Amount of CAT, SOD, H2O2, POD was estimated in moderate susceptible variety. Maximum impact of management was recorded by salicylic acid with 16.15% disease incidence, followed by benzoic acid (21.32%), K_2HPO_4 (27.85%), citric acid (32.37%), KH2PO4 (38.40%) and control (78.83%) under field condition. The range of CAT activity was estimated as (3.20-3.86 µg/g), SOD (1.61-1.97 µg/g), H_2O_2 (0.84-1.05 µg/g) and POD was (1.81-2.16 µg/g) while, TPC concentration was higher in treated (15688 µg/g) as compared to untreated (12823 µg/g). In treated leaves, all of these altered biochemical contents were observed more or less to be restored which helped the plants in disease recovery.

Key words: biochemical, CAT, challenging disease, salicylic acid, H2O2

INDUCTION OF RESISTANCE BY PANTOEA ANANATIS AND PERCEPTION OF GLYCOLIPIDS IN TOMATO

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Text

In a context of sustainable agriculture aiming to reduce the use of synthetic pesticides, studying PGPR (Plant Growth Promoting Rhizobacteria) bacterial strains or molecules derived from these microorganisms (used in the biocontrol of fungal diseases) represents a major research challenge. A large number of studies have shown that microorganisms produce biomolecules that can be perceived by the plant and induce an innate immune response. For example, natural amphiphilic glycolipids from microorganisms, such as rhamnolipids, can induce resistance against pathogens in several plants. Recently, new glycolipids have been identified in *Pantoea ananatis*. The objective of the project is to test this new strain and its glycolipids for their ability to induce resistance against phytopathogenic fungi in tomato.
We demonstrated that this strain had direct in vitro antifungal activity against *Botrytis cinerea*. Root bacterization of tomato plants with *P. ananatis* allowed us to observe foliar protection against *B. cinerea*, demonstrating the establishment of an ISR (Induced Systemic Resistance). In addition, a stimulation of the growth of the aerial part of tomato plants was highlighted, showing the potential of *P. ananatis* as a PGPR strain. Using different markers of perception and signaling, such as extracellular ROS production and MAPK phosphorylation, we observed that glycolipids were perceived by plant cells and could therefore participate in defense responses in tomato.

THE USE OF NATURAL BIOSTIMULANTS APPLIED BY SEED COATING ON WHEAT TO IMPROVE TOLERANCE AGAINT FUNGAL PATHOGENS

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Text

Coating seeds with beneficial microorganisms appear to be as a promising approach to maintain the productivity of plants under stressed conditions. Their effect in inducing defense response of the plant to foliar or soil borne fungal diseases was evaluated. In this study, we tested the bacteria Burkholderia, the fungus Trichoderma and the yeast Meyerozyma, for their ability to control fungal disease both under controlled conditions. These biostimulants were tested on seedlings driven in a hydroponic medium in an oxygenated nutrient solution.

Daily sampling of the leaves made it possible to carry out biochemical analysis (peroxidase, phenolic compounds and H2O2 levels). On the other hand, infections by pathogenic fungi (Septoria tritici) can reveal the induction of defense reactions according to the evolution of the necrotic spots on leaves. Later on, inoculations are made by a soil borne pathogen through the injection of a suspension of Fusarium spores into the nutrient solution on wheat roots. Biochemical analysis performed in an infectious context revealed an increase of markers linked to defense response. This was also confirmed through molecular analysis performed at the leaf level which revealed the presence of acquired systemic resistance through the overexpression of genes involved in defense response. Alltogather these results showed the presence of both an inducing and priming effect of the microorganisms tested leading to the protection of the target plant.

NEIGHBOR MEDIATED SUSCEPTIBILITY, TOWARDS AN ECOLOGICAL VIEW OF PLANT IMMUNITY

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Text

Among the major scientific questions that remain to be solved in plant pathology is how plant-pathogen interactions hold up in an ecological context? Indeed, for most interactions, we have very little idea of how interactions between plants and microbes are affected by the ecological environment in which they live. At the same time, the concept of eco-immunity has been described. Broadly speaking, this concept aims to understand and explain variation in immune response, in other words, to determine why and how biotic and abiotic factors contribute to variation in the immunity of a living organism. An ecological context that seems interesting to study for its positive impact on the control of epidemics is varietal mixtures. However, this general benefit is not systematic and can be suppressed by specific allele combinations. This highlights the need to know the genetic basis of neighbor-regulated immunity in order to make better use of mixtures. According to our first results, the plant immunity regulated by the neighborhood relies on inter-root communication events. One of our hypotheses is that this inter-root communication would be ensured by specialized metabolites exuded into the rhizosphere that has been described in the context of allelopathic mechanisms. Other phenomena that could be involved include nutrient competition in the root compartment, but the molecular mechanisms by which immunity is regulated under mixed conditions have yet to be described.

NEWLY SYNTHESIZED BENZIMIDAZOLE–2–CARBAMATE MOLECULES SHOW SUPPRESSIVE ACTIVITIES AGAINST PLANT PATHOGENIC FUNGI AND OOMYCETES

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Text
Most bioactive compounds are designed and synthesized by heterocyclic chemistry. Benzimidazole and its derivatives are important heterocycles that are critical subunits for pharmaceutical and biological molecules. This study was carried out to investigate the effect of 44 newly synthesized benzimidazole compounds on Alternaria, Rhizoctonia, Cochliobolus, Fusarium, Lasiodiplodia and Pythium, which are pathogens of major crops worldwide. Well diffusion method was done using 100 mL of two different concentrations of benzimidazole compounds (1000 and 5000 ppm). The molecules designated as EBI.bB4, EBI.eB1, EBI.fB2, EBI.gB1, EBI.gB2, EBI.aB4.S, EBI.aB5.S, EBI.eB1.S and EBI.gB1.S showed suppressive effects on fungal and oomycete growth at 1000 and 5000 ppm. When these fungicides were tested against Trichoderma harzianum, a beneficial biocontrol fungus, the fungus was not affected. This suggests that these newly synthesized fungicides may work synergistically with T. harzianum in suppressing growth of the pathogenic fungi. Scanning electron microscopy showed that the fungicides resulted in malformation, bursting, swelling and hyphal tip collapse of the mycelia. The study shows that nine new fungicides show promising results in reducing growth and resulting in deformations of the mycelia of A. alternata, R. solani, C. hawaiiensis, F. solani, L. pseudotheobromae and P. aphanidermatum pathogens. The benzimidazole compounds had no effect against the biocontrol agent T. harzianum

APPLICATION OF RNA-BASED BIOPROTECTANTS AGAINST VIRUSES FOR SUSTAINABLE CROP PRODUCTION IN A CHANGING CLIMATE

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Text
The European ERA-NET SusCrop Project BioProtect aims to contribute to reducing the application of chemical pesticides through the creation of a platform for the development and cost-effective production of nature-friendly dsRNA-based bioprotectants. dsRNA is a natural and environmentally safe molecule originating from RNA interference (RNAi) pathways involved in gene regulation and pathogen defense. Its exogenous application to plants leads to the activation of the RNAi pathway which can be programmed against specific pathogens according to the designed dsRNA nucleotide sequence. In addition, dsRNA has the potential to trigger pattern-triggered immunity (PTI) thereby reinforcing crop protection through a second host defense pathway. In this project, high-quality (hq) dsRNA with homology to TMV and TuMV were formulated to increase leaf uptake and stability. The antiviral protection effect triggered by spraying the formulated hq-dsRNAs is tested in crops infected with GFP-tagged target viruses. Moreover, non-specific hq-dsRNAs are used to determine the contribution of PTI, which may influence target specificity. In the light of climate change, the effect of increased temperature on virus replication and movement and on the efficiency of hq-dsRNAs is determined. Our results show that the treatment with hq-dsRNA results in plant protection by programming the ability of plants to defend themselves against target pathogens with their own native defense mechanisms.

EFFICIENCY OF THAI MEDICINAL PLANT EXTRACT, MICRO-ORGANISM AND SALICYLIC ACID FOR CONTROL OF ANTHRACNOSE DISEASE OF CASSAVA

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Text

This study was to evaluate the efficiency of Thai medicinal plant extract, micro-organisms, and salicylic acid for controlling the anthracnose disease of cassava caused by *Colletotrichum gloeosporioides*. Three Thai medicinal plant extracts including garlic (*Allium sativum*), Wildbetal leaf bush (*Piper sarmentosum*), and curry paste at concentrations of 5000, 10000 and 15000 ppm compared with 3 isolates of *Bacillus subtilis* at concentrations 10^6 cfu.ml^{-1} and salicylic acid at concentration 500 ppm by using the poisoned food technique method compare with Mancozeb fungicide and water control. The results showed that Mancozeb 80% WP has the highest antifungal activity at 100%, which non-significance difference with *B. subtilis* CaSUT 008–2 with 98.24%. The curry paste extract at 15000 ppm has an inhibition of 73.05%, followed by Wildbetal leaf bush extract and Garlic extract at 15000 ppm can inhibit 64.73 and 42.32% respectively, and salicylic acid at 500 ppm can inhibit 42.07%. After that, all 7 treatments were tested in the greenhouse condition. The results show that Mancozeb 80% WP has high inhibitory activity against anthracnose disease at 7 and 14 days at 13.50 and 14.33% respectively, curry paste at 15000 ppm can inhibit at 11.67 and 11.33%, and salicylic acid at 500 ppm can inhibit at 11.83 and 10.33%, respectively. In conclusion, curry paste and salicylic acid have the ability as an alternative solution for managing anthracnose disease in cassava.

LESSONS FROM VIRUSES: HOW TO INDUCE RESISTANCE TO INSECT PESTS

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Text

Many plant viruses have evolved the ability to susceptibility or to trigger mild resistance their insect vectors. Certain viral mutants trigger antibiosis. For several geminiviruses and several RNA viruses, specific viral gene products have been identified that modify plant biochemistry and physiology in ways that influence their plant hosts' interactions with, respectively, whiteflies and aphids. Experiments with cucumber mosaic virus (CMV) and several potyviruses indicate that the induction of resistance to aphid settling can enhance the dispersal of viruliferous aphids and promote dissemination of viruses that utilise the 'non-persistent' mode of transmission. Modification of the host phenotype occurs through a combination of virus-induced changes in emission of volatile organic compound and in accumulation of repellent substances in plant tissue. Work with CMV in arabidopsis and tobacco has shown that this re-tuning of plant defense depends on interactions of several viral proteins with each other and with host factors influencing the pattern-triggered immunity pathway and signalling that is largely dependent upon jasmonic acid. Understanding how viruses and their gene products modify plant-insect relations will lead to more effective means of inducing natural resistance mechanisms that protect crop plants against insects

and, in the case of insects that vector pathogens, provide indirect but potentially highly effective protection against infectious disease.

EFFECT OF BROCCOLI RESIDUES INCROPORATION INTO FIELD UNDER DIFFERENT TIMES ON THE OCCURRENCE OF COTTON VERTICILLIUM WILT, STRUCTURE AND FUNCTIONAL PATHWAYS OF RHIZOSPHERIC MICROBIAL COMMUNITY

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Text

Cotton verticillium wilt (CVW) is widespread and is responsible for serious economic losses. Crop residues incorporated into soil is an important measure for controlling soil-borne diseases in agricultural management practices. The objective of this study was to evaluate the influence of broccoli residues incorporated into soil under different years (BRF, BRS and BRT) on the incidence of CVW, the population of *V. dahliae* in soil, and the soil microbial community structure and functional genes using metagenomics analysis. Results demonstrated that the disease indices dramatically decreased by 34.67%, 66.67%, and 62.67%, respectively, compared with that under the CK treatment. The *V. dahliae* populations also decreased, by 40.89%, 61.64% and 36.74%, respectively. Principal component analysis (PCA) showed that significant changes were found on the bacterial communities composition among treatments. Redundancy analysis (RDA) revealed that the structures of microbial communities were driven mainly by NH_3^--N and NH_4^+-N. Spearman's correlation analysis showed that function pathways of bacterial community was negatively correlation with OM, NH_3^--N and NH_4^+-N, and positively correlation with AP in fungal community. In addition, nitrite reductase (*NarGHI*, *NirBD* genes) were decreased in the nitrogen cycle, sulfate adenylyltransferase (*PAPSS*, *CysNCD*, *Sat* genes) and serine O-acetyltransferase (*CysE* gene) were decreased in the sulfur cycle compared with CK.

STRUCTURE-ACTIVITY OF THE LIPOPEPTIDE SURFACTIN AS ELICITOR OF PLANT IMMUNITY

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Text

Cyclic lipopeptides (CLPs) are amphiphilic secondary metabolites produced by plant beneficial rhizobacteria such as *Pseudomonas* and *Bacillus* that are important for the biocontrol activity of the producing strains. This is due to their strong antimicrobial activity but also to their plant immunity eliciting potential leading to a systemically-expressed higher resistance to subsequent infection (ISR). We have recently shown that CLP perception by plant cells relies on a unique process based on docking into specific lipid domains of the plasma membrane rather than being recognized via high-affinity protein receptors. In this

work, we show that immunity activation by a particular CLP is fine-tuned by precise structural traits in the molecule. We compared natural variants of the *Bacillus* CLP surfactin for their potential to stimulate early plant immune-related events such as ROS and RNS, for activation of specific transcription factors and for ISR triggering in *Arabidopsis*. Altogether our data revealed that slight structural changes in the fatty acid length and single amino acid substitutions at key positions in the peptide cycle strongly impact the activity and are thus of unsuspected importance in triggering both immune responses and ISR. We discuss these results in the context of co-evolution assuming that *Bacillus* species have adapted the structure of their CLPs (e.g. surfactin) according to their endophytic or epiphytic life style.

IDENTIFICATION OF RESISTANCE LOCI FOR FOLIAR DISEASES IN THE COMMON WHEAT LINE 'KIJIL'

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Text
Wheat is one of the world's most important cereal crops. With climate change in recent years, fungal diseases caused by wheat stripe rust, leaf rust and powdery mildew have had a significant impact on the safe production of wheat. The cimmyt strain ‘KIJIL’ has good resistance to wheat rust and powdery mildew, but the genetic mechanism of resistance is not known. In this study, we used the F5 RILs to identify diseases at multiple environments over a number of years, and combined with Genotyping-by-sequencing (GBS) genotyping to locate disease resistance QTL. The new seedling powdery mildew resistance locus *QPm.hzau-7BL* and the adult stage concurrent resistance locus *QYr/Lr. hzau-5DL* were identified in 'KIJIL'.The adult disease resistance genes *Lr46*, *Yr30* and *Yr27* were also localized. In response to the identification of new disease resistance loci, a variety of molecular markers were developed for breeders to use.

CLONING AND FUNCTIONAL ANALYSIS OF AN ADULT PLANT RESISTANCE GENE LR68 FOR WHEAT LEAF RUST

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Text
Wheat is the second largest food crop in the world. Leaf rust disease caused by Puccinia triticina (Pt) is one of the important disease factors affecting the global wheat yield, which can cause the loss of wheat yield up to 50% in epidemic years. Breeding resistant wheat varieties is the most economical, environmentally friendly and effective way to control wheat leaf rust. Therefore, it is particularly important to continuously explore and utilize excellent resistance loci to breed durable resistant varieties. According to previous studies, the adult resistance gene Lr68 was initially located on chromosome 7BL, which showed good resistance to wheat leaf rust at adult stage and was widely used in CIMMYT and even global wheat breeding. In this study, F5 RIL population containing 674 and 696 Lr68 single gene lines and 27 γ-ray mutants of 10 families were constructed respectively. The markers designed by RNA-seq

were used for fine mapping of Lr68. At the same time, the 7B chromosome of Arula was separated, deeply sequenced and spliced without reference. The candidate interval of Lr68 was determined by combining the fine mapping markers sequence, and the functional verification and related mechanism analysis of the candidate genes were carried out. This study will provide new markers, new genes and new germplasm for breeding wheat varieties resistant to leaf rust in the future, and providing theoretical basis for revealing the inheritance of adult plant resistance to wheat rust.

INDUCTION OF PLANT DEFENCE AGAINST MYCOSPHAERELLA IN CUCUMBER

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Text

In cucumber cultivation systems, *Mycosphaerella* has been a common problem for years. It can infect the plants through lesions in the leaf or stem and often penetrates through a fresh flower into the fruit. This results in loss of production, quality reduction, and poorly marketable products. Controlling *Mycosphaerella* is difficult as the disease has often spread in the greenhouse before being visible. We have set up a method to investigate induction of plant defence against this fungus. Infecting flowers in vitro with *Mycosphaerella* and examining the degree of fungal infestation in the developing fruit can be linked to the plant's defence. Plant treatment of young plants with methyl jasmonate lead to an increase in plant defence metabolites in the flowers with subsequent lower disease severity or even absence in the fruits. Besides, we detected a *Bacillus* strain inhibiting fungal growth in vitro. Applying foliar spray with *Bacillus* may be used additionally as a treatment against *Mycosphaerella*. Essential is the early and preventive application in young plants.

A NEW APPROACH FOR THE DESIGN OF CROSS-PROTECTION TRIAL IN THE FIELD

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Text

Cross-protection is a biocontrol method whereby the primary-infection of a plant with a mild (cross-protective) virus strain prevents it from the damage caused by the subsequent infection by another (challenge) strain of the same virus. Among the factors influencing the effectiveness of cross-protection in the field, the steady mild symptoms induced by the cross-protective strains and the genetic relatedness between the cross-protective and challenge strains seem to be crucial. To our knowledge, when developed in the field, the complex genetic diversity of

the cross-protective and challenge virus strains is rarely considered at a local scale before implementation. Hence, our strategy is to implement cross-protection against grapevine fanleaf virus (GFLV, *Nepovirus*) using a combined phenotypic and genetic approach in a long-term vineyard experiment. Our first campaigns in a highly GFLV infected plot in Burgundy led to the selection of candidates, i.e. GFLV-infected vines displaying mild symptoms while producing similar fruit yields compared to GFLV-free vines. Using high throughput sequencing, consensus sequences of GFLV RNAs found in these mildly symptomatic vines were compared to those of the challenge isolates found in a specific area of this plot where the trial will be designed. This experiment should eventually lead to the identification of key features responsible for the success of cross-protection and guide its implementation in the field.

GRAPEVINE DECLINE DUE TO THE 161-49 C ROOTSTOCK SEEN AS A DYSFUNCTION OF THE ROOT HOLOBIONT

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Text

The grapevine rootstock 161-49 C (Vitis berlandieri x Vitis riparia) is a "qualitative" rootstock which has been widely used in France. However, for the past ten years, serious and unexplained problems of 161-49 C decline have been reported. In the plot, the affected vines show a reduction in vigor, with in particular a clear reduction in the height of vegetation and in the diameter of the stems. In Burgundy, it is not uncommon to find plots affected by more than 50%, generating considerable yield losses, impacting both the production and the sustainability of the vineyard. In this context, we wanted to test the hypothesis that soil nutrient biodisponibility can impact the level of this decline (prevalence), and the root system is analyzed as a holobiont.
Our results reveal major modifications of the vine holobiont during the decline of this rootstock. The mineral composition of the rootlets as well as their anatomical structure are strongly modified. This also has repercussions at the scale of microbial communities. Moreover, modulations in the accumulation of certain metabolites are also observed.
Obtaining bioindicators of grapevine holobiont dysfunction in a situation of root decline will allow us (i) to better understand these risks, particularly in a context of climate change which generates abiotic stresses such as water stress and (ii) to better characterize root immunity as a component of the grapevine holobiont functioning.
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GRAPEVINE BIOSTIMULATION: A LEVER TO PRIME LEAF ELICITOR-INDUCED RESISTANCE TO DOWNY MILDEW

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Text

The induction of crop resistance to cryptogamic diseases by Plant Defense Elicitors (PDE) is an attractive strategy to reduce chemical pesticide use. However, the variability of its effectiveness in the field limits its development. One of the reasons is that, unlike fungicides which act directly on pathogens, PDE solicit plant immunity, the performance of which may depend on the plant physiological status. In addition to PDE, other products used in agriculture, biostimulants (BS), are described to improve certain functions of the plant. In this context, the objective of this work was to determine whether a BS, via effects on grapevine physiology, could increase effectiveness of a PDE.
We have developed an experimental methodology (on vitroplants) to characterize the effects of a BS (plant extract). Added to the culture medium, it (i) accelerates the start of the plant growth, (ii) has an impact on the plant aerial and root development and (iii) has an effect on the metabolome and the phytohormone content of leaves, stems and roots. Moreover, in response to a PDE (another plant extract, applied on leaves), the plant defense responses are induced and a protection against Plasmopara viticola (the downy mildew agent) is obtained only for biostimulated vines. Therefore, the biostimulant may act by priming the plant defense elicitor action. Thus, this study demonstrates the relevance of the use of BS to optimize the effectiveness of a PDE.
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CHITOSAN REDUCES STOMATAL PENETRATION AND PATHOGEN CELL-WALL DEGRADING ENZYMES PRODUCTION, AND INDUCES DEFENSE MECHANISMS IN BOTH BREAD AND DURUM WHEATS INFECTED BY ZYMOSEPTORIA TRITICI

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Text
Bread (BW) and durum (DW) wheats are both challenged by Zymoseptoria tritici, the hemibiotrophic fungus responsible for septoria triciti blotch (STB) disease. Developing more eco-friendly control strategies is needed in order to move towards a more sustainable plant protection approach. This study aims at investigating the ability of chitosan to induce

resistance in the two wheat species against Z. tritici. Leaf spraying of this compound provided a strong reduction of necrosis area, by 63 and 66% on BW and DW, respectively. No direct antifungal activity was observed in vitro against Z. tritici. In planta monitoring of the infection process revealed that chitosan did not prevent fungal epiphytic growth nor leaf colonization, but strongly inhibited stomatal penetration. Moreover, significant reduction of fungal cell-wall degrading enzymes and protease activities were observed in both wheat species. During the early stages of infection, chitosan induced defense responses on both wheat species, such as stimulation of endo-β-1,3-glucanase, peroxydase, phenylalanine ammonia lyase and lipoxygenase activities. Moreover, H202 and phenolic compound accumulation at the site of fungal penetration attempts were observed. Although few differences were recorded, the patterns of the defense responses induced were overall similar in the two species. These results clearly suggest that chitosan could be an interesting resistance inducer that could be used to control STB on both BW and DW.

THE ROLE OF PLANT VOLATILES IN ACTIVATING NATURAL DEFENSES AGAINST HLB AND ITS VECTORS

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Text

Huanglongbing (HLB), also known as citrus greening, is a bacterial disease that severely restricts citrus production in citrus-growing regions across the globe. Recent research has demonstrated that activating the immune system of citrus plants can help to limit HLB. This activation is primarily achieved through the salicylic acid (SA) signaling pathway by triggering plant defenses. Our research team has developed a new method for activating the immune system of citrus plants using organic volatiles that act as an alert signal for imminent attacks, prompting the plants to induce their defenses. By exposing eight citrus species to six different volatiles using a polymer diffuser, we found that almost all combinations of volatiles and citrus species could activate the SA pathway. The most potent volatile was (Z-)3-hexenyl propanoate [(Z)-3-HP], which was further investigated for its impact on the biology of both HLB vectors in defensively activated plants. We observed that exposing plants to (Z)-3-HP reduced the oviposition of the two psyllid vectors, *Trioza erytreae* and *Diaphorina citri* by more than 50%. We also studied the behavior of their parasitoids, *Tamarixia dryi* and *Tamarixia radiata*, in response to this defensive activation and found that both parasitoids were attracted to plants activated by (Z)-3-HP exposure. Finally, we present preliminary results on the effect of defensive activation through exposure to (Z)-3-HP on the acquisition and multiplication of HLB.

ANTAGONISTIC POTENTIAL OF DIFFERENT RHIZOSPHERIC BACTERIA FOR THE MANAGEMENT OF PECTINOLYTIC PATHOGENS CAUSING BLACKLEG OF POTATO

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Text
Blackleg, a bacterial disease caused by a group of pathogens belonging to Pectobacterium and Dickeya species inflicts incredible losses to potato crops in major potato growing areas of Punjab. The aim of this study was to isolate, identify and evaluate the quorum quenching (NAHL degrading) bacterial strains from potato rhizosphere against Pectinolytic bacteria causing infections in potato. A total of 78 morphologically different isolates were retrieved from 50 potato rhizosphere samples. Therefore, after initial screening of 78 isolates, only 24 isolates showed NAHL degradation activity in a 96 well plate biosensor based assay. Furthermore, remaining 24 strains were passed through different NAHL degradation assays like plate streak and TLC plate assays but providentially all showed NAHL degradation less or more. Finally, all these NAHL degrading test strains were evaluated under QQ tuber assay to agonize Pectobacterium based QS-regulatory processes. Thus, on the basis of QQ tuber assay, 2 test strains did not reduce maceration, 6 partially reduce maceration while 16 test strains exhibited complete degradation or inhibition of the NAHLs with no maceration in tubers as compared to positive and negative control. Out of 16 QQ strains, 6 were identified as Bacillus spp., 3 as Pseudomonas spp., 2 as Variovorax spp., 2 as Arthrobacter spp. and 1 each as Rhodococcus erythropolis, Commomonas and Delftia species.

DESIGN OF SUSTAINABLE BIOCONTROL STRATEGIES AGAINST SEPTORIA TRITICI BLOTCH OF WHEAT

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Text
Limiting the use of synthetic pesticides is critical to increase sustainability in agriculture and food security. Wheat is the main staple food in Europe and one of the top-3 in the world. Wheat production is challenged by diseases, being septoria tritici blotch (STB) the most damaging one in Europe. The control of this foliar disease is extremely challenging since it is caused by the fast-evolving pathogen Zymoseptoria tritici, and the methods traditionally used to control it, mainly genetic resistance and fungicides, are losing their efficacy. We propose that the strategic use of microorganisms as biological control agents (BCAs) inhibiting fungal growth and interfering with the infection of pathogens is a sustainable and effective method to control diseases, including STB. The main goals of our work are to identify BCAs adapted to wheat in Spain and the Mediterranean basin, and to determine the evolutionary potential of Z. tritici to erode BCA-based control. To achieve the first objective, we are currently generating a collection of endophytic microbes of wheat leaves from the field, and we are screening these endophytes for their antagonistic activity to Z. tritici. This work will bring applied solutions for the biocontrol of STB of wheat in the Spanish fields, as well as providing important knowledge about wheat endophyte communities and their mechanisms as BCAs.

ULVANS INDUCE RESISTANCE IN WHEAT AGAINST FUNGAL LEAF DISEASES

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Text

Ulvans are water-soluble sulfated polysaccharides extracted from green seaweeds that have previously been reported to induce resistance in plants against various pathogens. This study investigated their ability to induce resistance against two major foliar fungal diseases in wheat, Septoria leaf blotch (SLB) and powdery mildew (PM). Wheat plants were sprayed with an ulvan solution and subsequently inoculated with either *Zymoseptoria tritici* or *Blumeria graminis* f.sp. *tritici*, which are responsible for SLB or PM, respectively. Results showed that ulvan-treated plants exhibited both reduced disease severity, by 45% for STB and 42% for PM, with a clearly impact on the fungal colonization compared to control plants. Moreover, the expression levels of defense-related genes in wheat plants were analyzed using qRT-PCR. Ulvan treatment upregulated the expression of several defense-related genes involved in the phenylpropanoid pathway and pathogenesis-related protein synthesis. In the case of the *Z. tritici*-wheat interaction, ulvans reduced disease severity and the density of asexual fruiting pycnidia, while positively regulating several defense-related genes. Interestingly, the treatment did not substantially alter the wheat leaf metabolome, suggesting a low metabolic cost associated with induced resistance. These findings suggest that ulvans have potential as a natural and environmentally friendly tool for plant disease management.

ENHANCING SOIL PHOSPHORUS AVAILABILITY AND WHEAT IMMUNITY AGAINST ZYMOSEPTORIA TRITICI USING THE PSEUDOMONAS SIDEROPHORE ISOPYOVERDINE

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Text

To promote agroecology and sustainable agriculture, looking for eco-friendly alternatives to chemical fertilizers and pesticides is encouraged. The aim of the present study was to evaluate the potential of isopyoverdine, a siderophore from *Pseudomonas putida* strain BTP1, to enhance the bioavailability of iron and phosphorus in the soil and to induce resistance in wheat against the hemibiotrophic fungal pathogen *Zymoseptoria tritici*. *In vitro* assays revealed that isopyoverdine co-solubilizes both iron and phosphorus from goethite (iron oxides), thus revealing the interest of this siderophore to increase the available amount of these nutrients. *In planta* experiments were also performed to validate these findings and to assess the ability of isopyoverdine to stimulate the wheat immune system through the roots. The bioassays were

carried out in a hydroponic system containing modified Hoagland medium with the addition of goethite sorbed with phosphate ions, supplemented or not with isopyoverdine, and inoculated or not with *Z. tritici*. Disease severity, fungal sporulation, as well as plant growth traits were assessed. More specifically, plant defence mechanisms potentially induced by isopyoverdine in both priming and eliciting conditions were explored using cytological, molecular and biochemical markers. The expected results will provide new knowledge about the valorisation of bacterial siderophores for crop health and nutrition.

THE BACILLUS SUBTILIS LIPOPEPTIDE MYCOSUBTILIN PRIMES DEFENSE MECHANISMS IN WHEAT TOWARDS ZYMOSEPTORIA TRITICI

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Text

Plant induced immunity is a valuable eco-friendly tool that fits with sustainable agriculture and healthy food. Here, we reveal that mycosubtilin, a lipopeptide from the bacterium *Bacillus subtilis*, protects wheat against the hemibiotrophic fungal pathogen *Zymoseptoria tritici* through dual (direct and indirect) mode of action and that the indirect one relies mainly on priming rather than the elicitation of plant defense mechanisms. Foliar application of the biomolecule primed, during the early stages of infection, the expression of 80 genes associated with sixteen functional groups, including responses to pathogens, abiotic and oxidative stresses, secondary metabolism, cell-wall structure and function, and primary metabolic pathways (carbohydrate, amino acid, protein, lipid, and energy metabolisms). Interestingly, mycosubtilin also primed the accumulation of several flavonoids during the period preceding the fungal switch to the necrotrophic phase. This priming-based bioactivity of mycosubtilin against a biotic stress could result from its interaction with leaf cell plasma membranes that may mimic an abiotic stress-like stimulus in wheat plants, as supported by cytological bioassays, as well as the regulation of genes associated with abiotic stress-responses and early signal transduction in the treated plants. This study provides new insights into the use of mycosubtilin as a biocontrol compound with two distinct modes of action against *Z. tritici*.

RESISTANCE IN WHEAT AGAINST ZYMOSEPTORIA TRITICI INDUCED BY ULVAN REQUIRES THE FLAVONOID TRICIN

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Text

Ulvan, a heteropolysacharide from green seaweed, can induce resistance against a wide range of fungal plant pathogens. However, the defense responses and signaling mechanisms are not yet fully understood. Therefore, our work aimed at investigating the role of wheat metabolites in ulvan-induced defense responses against *Zymoseptoria tritici* by using an inhibitory approach. For that, the third leaf of three-week-old wheat plants (cv. Alixan) was infiltrated with water (control), 1-aminobenzotriazole (ABT; 0,1mM), 2-(difluoromethyl)arginine (DFMA; 2mM) or trinexapac-ethyl (TXPE; 0,1mM) using a needless syringe. ABT, DFMA and TXPE inhibit enzymes involved in the biosynthesis of HDMBOA-Glc (benzoxazinoid), putrescin (polyamine) and tricin (flavonoid), respectively. Three hours after infiltration, plants were sprayed with water (control) or ulvan (10mg·mL^{-1}) and, two days later, inoculated with *Z. tritici*. Then, wheat plants were incubated under high humidity condition (>90%) for 48h and kept at 20±4°C and 16h of light for further 21 days when the percentage of necrotic leaf area was visually estimated on the third leaf. The percentage of necrotic third leaf area recorded for control plants (infiltrated and treated with water) was 27%. Ulvan spraying reduced this variable by 37% in leaves infiltrated with water, ABT and DFMA but failed to do so in those infiltrated with TXPE, suggesting that tricin may play an important role in ulvan-induced defense responses against *Z. tritici*.

NEW BIO-SOURCED ELICITORS FROM A SUGAR BEET BYPRODUCT INDUCE RESISTANCE IN WHEAT AGAINST ZYMOSEPTORIA TRITICI

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Text

The current context of agroecology requires the discovery of new ecofriendly plant protection compounds. Plant resistance inducers are considered as one of the most promising alternatives satisfying such requirements. Here, we assessed the efficacy and the modes of action on wheat against *Zymoseptoria tritici* of a set of 31 molecules, including γ-aminobutyric acid (GABA), pyroglutamic acid (PGA, bio-sourced from sugar beet byproducts), M1 (derived from PGA), and 28 other molecules functionalized from M1 using green chemistry. Foliar application of the molecules provided significant protection rates (up to 63 % disease severity reduction) for GABA, M1 and 15 molecules functionalized from M1. A structure-activity relationship study highlighted the importance of all chemical groups of the M1 pharmacophore in the activity of the molecules. In vitro bioassays revealed that they did not exhibit any direct activity towards the pathogen, indicating that the protection conferred by the molecules is due to host defense activation. Further characterization of the most efficient molecules (M1, M2 and GABA) confirmed this finding and revealed that these three molecules up-regulate the expression of genes encoding for lipoxygenase, phenylalanine ammonia-lyase, peroxidase) and pathogenesis-related protein 1. This study reports a new

family of bio-sourced elicitors inducing resistance in wheat against *Z. tritici*, hence providing new insights into the sustainable control of this pathogen.

MORINGA FROM SUSTAINABLE CULTIVATION IN SOCIAL COMMUNITY

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(1) MoringaVerde, Augsburg, GERMANY

Text
Moringa from sustainable cultivation exclusively from Cuba & Latin America, for the commercialization of medicinal plants and with knowledge transfer for healthy nutrition. Professional and ecologically sustainable cultivation and distribution of Moringa and other Cuban plant-based foods.
Creating jobs and helping small farms to distribute their products in sense of social entrepreneurship.

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MARVIC‘s farmers and partners in Cuba and Latin America produce bionatural products in the heart of Cuba & Latin America. Initially out of self-interest, as a source of income for their families, we support them on site, with currently two of our employees, to establish social partnerships with the producers / farmers in the sense of fair trade. The preservation of the natural resources as well as the yield areas in the region is a matter of concern for us, which we actively accompany with training courses on agriculture. #CSR, #SocialEntrepreneurship www.marvic-europaverde.com www.moringaverde.de
Through collaboration and training of regional partners, supporting social entrepreneurship, sustainable economic growth and ecosystem is promoted in Cuba and Latin America. .
The cultivation areas of our partner are known for the use of nature-friendly techniques, while preserving biodiversity. This is supported by direct sourcing/partnering with farmers and farms dealing with primary food processing (“Test-beds”) with the use of effective microorganisms (EM). (E.M.)

IMMUNITY PRIMING UNCOUPLES THE GROWTH-DEFENSE TRADEOFF IN TOMATO

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Text
Plants have developed an array of mechanisms to protect themselves against pathogen invasion. The deployment of defense mechanisms is imperative for plant survival, but can come at the expense of plant growth, leading to the “growth-defense trade-off” phenomenon. Following pathogen exposure, plants can develop resistance to further attack. This is known as induced resistance, or priming. Here, we investigated the growth-defense trade-off,

examining how defense priming via Systemic Acquired Resistance (SAR), or Induced Systemic Resistance (ISR), using microbial and chemical priming agents, affects tomato development and growth. We found that defense priming can promote, rather than inhibit, plant development, and that defense priming and growth tradeoffs can be uncoupled. Cytokinin response was activated during induced resistance, and found to be required for the observed growth and disease resistance resulting from ISR activation. ISR was found to have a stronger effect on plant development than SAR. Taken together, our results suggest that growth promotion and induced resistance can be co-dependent, and that defense priming can, within a certain developmental window, not necessitate a trade-off on growth, but rather, encourage and drive developmental processes and promote plant yield.

MENOAGE WITH MORINGA

BRUNNER Robert Ingo. (1)

(1) MoringaVerde & partners, Augsburg, GERMANY

Text
„MenoAge":

Anti-aging is the subject of our century. Proteins are the key to solve it.
Glycosylation is one of the most important posttranslational modifications found in organisms from bacteria to humans.
Moringa seeds contain the antibiotic pterigospermine and fatty acids such as linoleic acid, linolenic acid and behenic acid, as well as secondary plant substances such as tannins, saponins, phenolic compounds, phytates, flavonoids and lectins. In addition, there are fats, fiber, proteins, minerals, vitamins A, B and C, and amino acids.
Determining exact glycosylation patterns is not only important in research and development but also in clinical settings where they can serve prognostic as well as diagnostic purposes.

Glycosylation is one of the most important posttranslational modifications found in organisms from bacteria to humans.
The addition of glycans, branched oligosaccharides, determines the structure and function of proteins and other macromolecules.
However, unlike the other three types of macromolecules (nucleic acids, lipids, and proteins), glycans are still on the margins of contemporary science.

MARVIC's partner GENOS is specialized in high-throughput glycan analysis using methods developed over 15 years of experience in academia and partnerships with clients in the clinical, pharmaceutical, and biotech industries.

Why glycans?
Glycosylation is one of the most important posttranslational modifications found in organisms from bacteria to humans.

TRANSCRIPTOMIC AND FUNCTIONAL ANALYSES REVEAL THE DIFFERENT ROLES OF VITAMINS C, E AND K IN REGULATING VIRAL INFECTIONS IN MAIZE

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Text

Maize lethal necrosis (MLN), is caused by maize chlorotic mottle virus combined with a potyvirid, such as sugarcane mosaic virus. However, the host response as well as the resistance mechanism of maize to MLN remains largely unknown. Here we obtained four complete gene expression profiles from PBS, SCMV, MCMV and S+M inoculated maize plants via comparative analysis of SMRT and Illumina based RNA sequencing. Both single infection and co-infection of SCMV and MCMV had significant effects on photosynthesis, reactive oxygen species scavenging, and some pathways related to disease resistance in maize. Through CMV-VIGS assays, we demonstrated that silencing *ZmGalDH* or *ZmAPX1*, two vitamin C biosynthesis related genes, promoted viral infections. However, MCMV and S+M infections were inhibited when *ZmTAT* or *ZmNQO1*, the genes involved in vitamin E or K biosynthesis, were silenced. Furthermore, we tested 54 maize inbred lines for MCMV and SCMV resistance, and two MLN-resistant inbred lines, S766 and Shen137, were identified. Moreover, we demonstrated that exogenous application of vitamin C solutions could effectively suppress viral infections and relieve the symptoms of MLN, while spraying vitamin E and K had the opposite effects. The antiviral roles of these three vitamins probably depended on their regulatory effects on the expressions of salicylic acid responsive pathogenesis-related genes. These findings the roles of vitamins C, E and K in conditioning viral infections in maize.

IDENTIFICATION OF POTENTIAL TARGETS OF A NEW ANTI-TMV COMPOUND GLY-15 CONTAINING PYRIMIDINE HETEROCYCLE AND MOROXYDINE SKELETON

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Text

We have previously reported that the new compound GLY-15 containing pyrimidine heterocycle and moroxydine skeleton structure has good anti-TMV activity (Yu et al., 2022. Pest Management Science). The combination of chemical molecular probes and mass spectrometry is used to identify the potential target proteins of the compounds. Here, we constructed a biotin modified active probe by covalent bonding biotin with GLY-15 using an active small molecule probe, and thereafter confirmed its e anti-TMV activity in Nicotiana glutinosa. Total proteins extracted from TMV infected N. tabacum plants were incubated with biotin probes P-GLY-15, and then, using the interaction between biotin and avidin, we enriched and purified the GLY-15 binding protein using an avidin coated solid phase carrier. Finally, after separation by SDS-PAGE gel, we selected specific bands for LC-MS/MS mass spectrometry analysis. The results showed that major host proteins pulled down by the XX probes include Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Fructose-bisphosphate dehydrogenase (FBA), Malate dehydrogenase (MDH), Mannose-binding lectin (MBL1), Annexin, Form dehydrogenase, mitochondrial (FDH), and Germin-like protein (GLP). Nevertheless, more precise verification of this molecular interaction between GLY-15 and

viral proteins should be performed in a subsequent study using methods such as molecular dynamics simulation or Tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS).

PSEUDOZYMA APHIDIS SUPPRESSES MAMP-TRIGGERED IMMUNITY AND ACTIVATES LOCAL AND SYSTEMIC INDUCED RESISTANCE

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Text

Beneficial microorganisms need to overcome the plant defense system to establish on or within plant tissues. Like pathogens, beneficial microbes can manipulate a plant's immunity pathways, first by suppressing and hiding to establish on the host and then by inducing resistance to protect the plant. In the current study, we demonstrated that although *Pseudozyma aphidis* can activate microbe-associated molecular pattern (MAMP)-associated genes, it does not activate MAMP-triggered callose deposition and can, moreover, suppress such deposition triggered by Flg22 or chitin. While MAMPs associated gene activation by *P. aphidis* was not dependent on salicylic acid, jasmonic acid, or ethylene signaling, suppression of MAMP-triggered callose deposition required the salicylic acid and jasmonic acid signaling factors JAR1-1 and COI1 yet did not rely on EIN2, NPR1, or the transcription factor JIN1/MYC2. Later on, *P. aphidis* or its secreted metabolites prime plants' defense machinery via local and systemic induction of PR genes in a manner independent of SA, JA, and NPR1. Finally, *P. aphidis* fully or partially reconstituted PR1 and PDF1.2 expression in SA related mutants, but not in a JA related mutant, after *B. cinerea* infection, suggesting that *P. aphidis* can bypass the SA/NPR1, but not JA, pathway to activate PR genes. Thus, either partial gene activation is sufficient to induce resistance, or the resistance can be directed through other pathogen-resistance genes or pathways as well.

METABOLOME ANALYSIS OF ARECANUT PALM RESPONSE TO YELLOW LEAF DISEASE

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Text

The arecanut palm, Areca catechu L., family Palmae is an important herbal medicine which has potential for the treatment of parasitic diseases, digestive function disorders and depression. Yellow leaf disease (YLD) caused by phytoplasmas is a destructive disease of arecanut palm. Leaves of 6 diseased palms and 6 healthy ones were collected in Baoting, Hainan, China(109.67°E, 18.41°N) and sent to BGI (https://www.bgi.com/) for untargeted metabolome analysis. Compared to healthy palms, 71differential metabolites (DMs)were detected in diseased palms and 46 ones were up-regulated. By KEGG pathway rich analysis, 6 DMs were involved metabolic pathways; 4 DMs were involved biosynthesis of secondary

metabolites; 3 DMs were involved phenylpropanoid biosynthesis. Besides, there are 8 pathways which enriched 2 DMs including cyanoamino acid metabolism, aminoacyl-tRNA biosynthesis, phenylalanine metabolism, glucosinolate biosynthesis, purine metabolism, biosynthesis of amino acids, 2-Oxocarboxylic acid metabolism, phenylalanine-tyrosine and tryptophan biosynthesis. The result providing important information for analyzing the mechanism of pathogen-plant interaction.

CHITOSAN: A COMPLEX SUBSTANCE WITH GREAT POTENTIAL FOR GLOBAL INTEGRATED PEST MANAGEMENT AND FOR REDUCING FARMERS' DEPENDENCE ON SYNTHETIC FUNGICIDES

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Text

Due to its numerous biological properties, its absence of toxicity and its biodegradability, chitosan is well studied and already used in various fields of application such as nutraceuticals, water treatment and plant protection. The interest of chitosan in agriculture, and more particularly in crop protection, has been known for decades and has allowed it to obtain the status of basic substance in Europe. Despite its strong potential as tool for reducing the use of pesticides, its development has been hindered by its complex nature and a lack of knowledge on the parameters influencing its biological efficacy. The knowledge obtained in recent years on the structure-activity relationship of chitosan as well as the technological advances that have taken place at the same time have allowed the industrial development of new well-characterized chitosans called of "second generation" and consequently the emergence of new chitosan-based products specifically designed for their application in agriculture. Thanks to an optimized composition and a controlled inter-batch reproducibility, these natural products have demonstrated interesting plant resistance inducer and fungistatic activities, and therefore represent promising solutions in the objective of reducing the use of pesticides, in a strategy of integrated pest management (IPM).

SELECTION BY PLANT OF PLANT-PROTECTING PSEUDOMONAS POPULATIONS EVIDENCED VIA AN EXPERIMENTAL EVOLUTION APPROACH

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Text

Plant interacts with multiple microbial players, together constituting the plant holobiont. At the level of their roots, a specific rhizospheric microbiota is shaped by their exudates. Within this microbiota, certain bacteria are capable of stimulating the growth of the plant and protecting it against plant-pathogens. Today, most molecular studies on plant-microbe cooperation are performed on one plant inoculated by one bacterium, and despite the multitude of strategies developed, plant-beneficial bacteria generally have very low efficacy in protecting plants grown in the field. This might be largely related to the fact that we do not know how plant-

beneficial bacteria may interplay with other members of the rhizomicrobiota and with the plant. To understand the adaptive mechanisms involved in plant-bacteria cooperation, our group has developed an experimental evolution approach, carried out on a synthetic community, made up of 10 cooperative bacteria belonging to distinct species from the Pseudomonas group, evolving in presence and absence of plants, for 400 generations. A metabarcoding analysis allowed us to investigate the evolutionary dynamic of the synthetic community. We observed rapid changes of the community composition and a clear effect of the plant in controlling the direction of changes in Pseudomonas populations' assembly. We are now exploring the genomic and phenotypic modifications of evolved populations, especially concerning their plant protection traits.

IDENTIFICATION OF COMMON FUNGAL EXTRACELLULAR MEMBRANE (CFEM) PROTEINS IN FUSARIUM SACCHARI THAT INHIBIT PLANT IMMUNITY AND CONTRIBUTE TO VIRULENCE

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Text

Fusarium sacchari is one of the primary pathogens causing pokkah boeng disease (PBD) in sugarcane in China. Common Fungal Extracellular Membrane (CFEM) domain-containing proteins only exist in fungi and play a significant role in the interaction of pathogens and plants. However, the CFEM proteins in F.sacchar were not identified and functionally resolved.

Of the 20 FsCFEM proteins identified from the genome of F. sacchari, 16 FsCFEM genes were cloned while 4 FsCFEM proteins (Fs06761, Fs08184, Fs10706, and Fs13617) were identified as effector proteins that could suppress the programmed cell death (PCD) triggered by BCL2-associated X protein (BAX) in Nicotiana benthamiana. Subcellular localization analysis revealed that these four effectors could enter plant cells and mainly located in the cell membrane and nucleus. By the yeast YTK12 secretion system, the signal peptides of four CFEM proteins were functionally verified. The expression levels of these four effector proteins were significantly increased in the Fs-infected sugarcane plants. Through fungal gene knockout, three genes (Fs06761, Fs08184, and Fs13617) were found to be important pathogenic factors for pathogens.

Our results support that the FsCFEM effector proteins were virulence effectors and might be involved in regulating plant immunity.

ENDOPHYTIC STENOTROPHOMONAS MALTOPHILIA D1B AND TRICHODERMA ASPERELLUM AAUTLF PRODUCES ANTIFUNGAL COMPOUNDS AND EXHIBITS ADDITIVE PLANT GROWTH-PROMOTING EFFECTS

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Text
Present study was carried out to isolate 177 bacterial endophytes and 57 fungal endophytes, out of which; 143, 51 and 40 isolates were obtained from roots, stems and leaves, respectively, of the tomato crop in decreasing order. A maximum of 112 endophytes were isolated during monsoon followed by 64 and 58 isolated during pre-monsoon and post-monsoon periods, respectively, indicating the rich diversity in bacterial and fungal endophytes of tomato crops from different locations of Assam, India. Bacterial endophytic isolate D1B and fungal isolate AAUTLF showed the highest antifungal activity against the pathogen *Fusarium oxysporum* f. sp. *lycopersici in vitro* and *in vivo*. Based on 16S rRNA gene of bacteria and 5.8r DNA of fungal endophytes the most effective bacterial and fungal isolates against FOL were identified as *Stenotrophomonas maltophilia* D1B and *Trichoderma asperellum* AAUTLF, respectively. The bacteria produced antifungal compounds including Benzotiazole, Oleic acid, Phenyllactic acid and 3-(Hydroxy-phenyl-methyl)-2,3-dimethyl-octan-4-one. However, *Trichoderma asperellum* AAUTLF produced an antifungal compound, 6-pentyl-2H-pyran-2-one, which also possesses growth-promoting characteristics. This would be highly important for the source of antagonistic strains and biocontrol of tomato Fusarium wilt, as well as other plant fungal diseases.

Fusarium wilt disease of banana: how to tackle a pandemic?

ORNAMENTALS AS ALTERNATIVE HOSTS OF FUNGAL PATHOGENS CAUSING FUSARIUM WILT IN BANANA

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Text
Fusarium wilt, caused by a suite of *Fusarium* spp. formerly known as *F. oxysporum* f. sp. *cubense*, is a major threat to the cultivation of bananas globally. In addition to persistent chlamydospores, the fungus likely survives in the field between crop cycles in alternative hosts like grasses and weeds. However, it is unknown if other Zingiberales, grown as ornamentals in the same geographical areas as bananas, are hosts of *Fusarium* spp.. We conducted greenhouse assays to test whether a Race 1 (R1, aka *F. phialophorum*) and Tropical Race 4 (TR4, aka *F. odoratissimum*) strains, can infect ornamentals. *Ensete ventricosum*, *Stretlizia reginae* and *Musella lasiocarpa* showed no external symptoms with either strain. In contrast, *Heliconia latispatha* and *Musa coccinea* displayed external symptoms after inoculation with TR4. Inoculation of *H. latispatha*, *H. psittacorum*, *M. coccinea* and *M. velutina* with R1 also resulted in wilting leaves. We recovered isolates from distinct organs of all studied plant species and confirmed their identity by PCR. Re-isolated

strains caused disease symptoms on Gros Michel or Grand Naine banana plants. The susceptibility of some ornamental species and the presence of *Fusarium* strains as asymptomatic endophytes in others, with remaining pathogenicity, calls for a revision of current containment protocols for Fusarium wilt. The relevance of these species as reservoirs and potential vehicles for dissemination, should be further examined under natural conditions.

EVALUATION OF RESISTANCE OF MUSA CULTIVARS TO FUSARIUM OXYSPORUM F. SP. CUBENSE TROPICAL RACE 4 IN COLOMBIA

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Text

Fusarium oxysporum f. sp. cubense Tropical race 4 (TR4) has severely curtailed banana production in the tropical regions of the world. In 2019, TR4 was reported in Colombia, and more recently in Peru and Venezuela. TR4 is virulent to all Cavendish type banana cultivars, including Cavendish clones, which are the source of 99% of banana exports. However, the production of traditional cooking bananas plays an important role in food security in many developing countries. The response of traditional plantain cultivars from Colombia against TR4 is still largely unknown. In this study, we have assessed the TR4 resistance response of 10 Musa cultivars with plants grown under semi-controlled conditions. The incidence and severity of the disease were calculated. The banana accessions evaluated showed different degrees of susceptibility to TR4 with incidence values from 25 to 100% for Manzano and Pelipita, respectively; and severity values between 6 and 35%. Additionally, the quantity of pathogen DNA was compared between inoculated cultivars using ddPCR technology, showing a correlation between the quantity of pathogen DNA and the Fusarium wilt disease severity rating. The traditional cooking bananas of Colombia are susceptible to TR4 under semi-controlled conditions, this result, raise alarms up for all cooking banana farmers, the establishment of strict containment and biosecurity measures are fundamental.

EARLY DETECTION OF FUSARIUM WILT IN BANANAS USING RAMAN SPECTROSCOPY: A PROMISING NON-DESTRUCTIVE DIAGNOSTIC TOOL

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Text

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) is a serious threat to banana production worldwide, particularly with the spread of the tropical race 4 (TR4) strain. With no cure for the disease and popular banana varieties such as Cavendish having high

susceptibility, early detection and timely control measures are crucial. Recent research has focused on developing advanced diagnostic techniques such as polymerase chain reaction (PCR) and next generation sequencing (NGS) technology. One promising method is Raman spectroscopy (RS), which can detect FOC infection in banana plants with minimal or no damage. Our research found that Raman signals from banana leaves can reliably indicate FOC infection, particularly early at the asymptomatic stage. The key vibrational bands of RS from carotenoids allowed for the early detection of FOC, up to 40 days after inoculation and 35 days before characteristic wilting symptoms appeared. As the disease progressed, the accuracy of FOC detection improved to over 90% by 61 days after infection. The speed of processing time, ease of use, and non-destructive nature of data collection make RS an ideal early detection tool, improving the effectiveness of mitigation practices to eliminate, contain, and quarantine FOC.

EVALUATION OF THE BIOCONTROL CAPACITY OF NATIVE MICROORGANISMS AGAINST FUSARIUM OXYSPORUM F. SP. CUBENSE

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Text
Fusarium Wilt is an economically important disease of bananas caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). It causes severe losses in the yield and quality of bananas and is extremely difficult to control conventionally using chemical fungicide. Biological control offers an eco-friendly alternative for sustainable plant disease management. In this context, the objective of this research is the determination of the biocontrol capacity of native microorganisms against *Fusarium oxysporum* f. sp. *cubense*. For the isolation of native rhizospheric microorganisms, samplings were carried out in the region of Perené and Satipo in the central jungle of Peru. Thirty rhizobacterial isolates were screened for antagonistic activity in dual culture, and isolate 27 showed the highest antagonistic activity (81,52% mycelial growth inhibition) against Foc. The metabolites of isolate 27 inhibited mycelial growth of Foc by 80%. Based on the morphological, physiological and phylogeny analysis with 16S rRNA sequence the isolate 27 was identified as *Burkholderia sp*. This is a preliminary study of the SATREPS project (Japan - Peru): "The Project on establishment of an alert system for *Fusarium oxysporum* f. sp. *cubense* the banana and plantain wilt pathogen, and biological mitigation strategy of the pathogen"

GENETIC RESISTANCE FOR LASTING CONTROL OF FUSARIUM WILT

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Text
The first Fusarium wilt pandemic of banana ended with genetic resistance; Cavendish was found to be resistant to Race 1. In the same way we predict that TR4 will be halted by genetic resistance but, this time more persistently if resistance genes are pyramided. Resistance to TR4 exists in wild diploid lines. Analysing these resistance sources allows

development of molecular markers for use in breeding programs. If these resistance sources prove to be independent of each other, the markers will permit selection of combined resistant traits in future cultivars.
Molecular markers are used routinely in crop breeding, enabling early selection of germplasm and, for resistance breeding, reducing the need for pathogenicity testing at each generation. In banana, the sterility of cultivated lines and requirements for parthenocarpy increases the complexity of breeding. We have identified molecular markers for resistance from a Malaccensis source and these markers are being used for selection in international breeding programs. Additional segregating populations have been generated using the resistant diploid Calcutta 4 and a Pisang Jari Buya-derived line, to determine if these traits map to the same or different chromosome locations from that of the Malaccensis resistance locus. The ultimate molecular marker is an actual resistance gene itself and once identified, such a gene can be used as a perfect marker in breeding programs or alternatively be deployed by genetic manipulation

SPECIFIC DETECTION OF FUSARIUM OXYSPORUM F. SP. CUBENSE AND ITS RACES BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

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Text
Loop-mediated isothermal amplification (LAMP) can be used for rapid, simple and easy detection of plant pathogens. In this study we established LAMP for specific detection of the banana wilt pathogen *F. oxysporum* f. sp. *cubense* (*Foc*) and its races, based on the genomic information. *Foc*-specific detection primer set was designed on a candidate effector gene *ce15*. The gene was present in all *Foc* isolates tested, but absent in other formae speciales of *F. oxysporum* and non-pathogenic *Fusarium* spp. A *Foc* race 1 isolate 160527 possessed *ce15* on the contig 2, and its contig 2-partly-deficient mutant lost pathogenicity to banana (cv. Shima-banana; Matsui 2022). Races of *Foc* can be distinguished by the retention pattern of the putative effector genes. Race SR4 (subtropical race 4) possesses *SIX7* and *SIX8*, TR4 (tropical race 4) possesses only *SIX8*, and race 1 has neither. We designed primer sets for the specific detection of *SIX7* and *SIX8*, respectively. We could propose the rapid detection of *Foc* and its races by combination of LAMP with these primer sets. Extraction of genomic DNA, used as a template, from plant tissues and fungal mycelial cake took about 10 min using Template Prepper kit, LAMP reaction was completed in about 30 min and the determination can be made immediately after the reaction. The detection limit of the template DNA was 0.5 x 10^{-2} ng/µl reaction mixture for all the primer sets.

IMPROVING RESISTANCE TO FWB IN EAST AFRICAN HIGHLAND BANANAS

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Text

Breeding for fusarium resistance in East Africa has been accomplished through a collaboration between the International Institute of Tropical Agriculture (IITA) and national partners in Tanzania and Uganda. These breeding efforts have produced a number of promising hybrids with resistance to both race 1 and TR4. Four of these hybrids have been advanced to multilocational trials and release is expected within the next two years. Integrating fusarium resistance, however, is only a single consideration as new hybrids must also meet consumers demands and perform at least as well as commonly grown check varieties in both consumer preference trials and in terms of agronomic performance. The described hybrids display up to a 60% yield increase and have consumer preference scores comparable to commonly grown varieties. A new methodology for incorporating recombinant breeding into banana improvement has been developed and deployed. This should decrease the time required to produce new varieties combining multiple desired traits.

FUSARIUM OXYSPORUM F. SP. CUBENSE TR4 INTERACTIONS WITH BANANA AND ALTERNATE HOSTS, AND ORIGIN OF THE PATHOGEN IN THE MIDDLE EAST

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Text

Fusarium oxysporum f.sp. *cubense* tropical race 4 (TR4), the causal agent of wilting and plant mortality of Cavendish banana varieties, is considered one of the most devastating soilborne fungal pathogens of this crop worldwide. The pathogen was first detected in South East Asia in the 1990s, and has since spread worldwide, being discovered in Jordan and Lebanon in 2014, and Israel in 2016. Disease symptoms include leaf chlorosis and wilting, with internal vascular discolorations of rhizomes and pseudostems and eventual plant mortality. TR4 isolates from Israel were tested for pathogenicity, and identification from symptomatic plants was reconfirmed by PCR. Resistance/susceptibility screening of banana germplasm, included 6-7 resistant, 6-8 tolerant, and 15-16 susceptible genotypes. In inoculated resistant plants, TR4 was not detected within the vasculature while in susceptible wilted plants conidia and mycelia were observed within these tissues. TR4 colonization of roots and shoots of certain weed species growing in infested soils of banana plantations indicated systemic infection of these hosts without disease symptoms. Artificial inoculation of citrus, mango, avocado and grapevine seedlings indicated symptomless seedling root colonization by TR4. Genome sequencing of TR4 isolates (two from Israel, one from Jordan, Philippines, and Indonesia each) and comparison to 11 additional worldwide isolates by SNP analyses indicated that TR4 in Israel originates from Jordan.

Harnessing Culture Collections for Improved Plant Health

THE NATIONAL COLLECTION OF PLANT PATHOGENIC BACTERIA (NCPPB)

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Text

The NCPPB is one of the longest-established collections of its type; its aims are to maintain representatives of all known culturable bacterial plant pathogens and exemplify the standards for housing and supply of reference and type strains.
In 1947 bacterial plant pathogens from the National Collection of Type Cultures, some dating back to the early 1900s, were combined with a collection maintained by W J Dowson at the Botany School, Cambridge, for the Agricultural Research Council.
On his retirement in 1956 the collection of 200 isolates moved to the Plant Pathology Laboratory of the Ministry of Agriculture, Fisheries & Food and was recognised as a National Collection.
The NCPPB, funded by the Dept. for Environment, Food & Rural Affairs since 2002, continues its primary role of providing scientific support to the UK Plant Health Service. It houses circa 4000 lyophilised strains. Each batch is checked for purity, authenticity and viability immediately after lyophilisation. Enough ampoules are made to ensure that supply demands are met before the end of the batch's shelf life – measured in decades – and for some accessions examples of each previous batch continue to be housed, so comparisons can be made between the earliest lyophils and those made today.
The NCPPB is a founder collection of the United Kingdom Biological Resource Network (UKBRCN), is a member of the European Culture Collections' Organisation (EPPO) and is an affiliate member of the WFCC.

High-throughput sequencing in plant virology: from discovery to diagnostics

USE OF HIGH THROUGHPUT SEQUENCING FOR PLANT MATERIAL CERTIFICATION AND RELEASE OF QUARANTINED PROPAGATIVE PLANT MATERIAL AT FOUNDATION PLANT SERVICES IN DAVIS, CALIFORNIA, USA.

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Text
High throughput sequencing (HTS) is an important component of routine testing procedures at Foundation Plant Services (FPS) at the University of California, Davis for plant material certification and quarantine import programs. The US Department of Agriculture-Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ) and California Department of Food and Agriculture (CDFA) have approved the use of diagnostic testing protocol that replaces biological indexing with a combination of HTS and polymerase chain reaction (PCR) testing for release of plant material. Research conducted at FPS indicates that this protocol results in more accurate test results compared to biological indexing using woody and herbaceous indicators for virus detection. FPS scientists conducted side-by-side studies comparing the efficacy of indexing to PCR and HTS testing in grapevine, Prunus, and rose. The results of these studies indicate that biological indicators give false negative results a significant percentage of the time and do not provide sufficient sensitivity in detecting target viruses or unknown viral pathogens. Similar results were obtained in studies conducted on Prunus and pome fruits by other clean plant centers. The streamlined testing methods yield the most accurate information about the phytosanitary status of material, expedite release times, and reduce potential risks from the transmission of vector-mediated viruses in the field.

HIGH-THROUGHPUT SEQUENCING IDENTIFIES CO-INFECTION OF TURNIP YELLOWS VIRUS AND ASSOCIATED RNAS IN SWEDISH OILSEED RAPE

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Text
In autumn 2018, the presence of green peach aphids (Myzus persicae) in southern Sweden presented a risk for infection of winter oilseed rape (OSR; Brassica napus) with turnip yellows virus (TuYV; genus Polerovirus, family Solemoviridae). Therefore, a survey was carried out in spring 2019 with random leaf samples from 46 OSR fields in southern and central Sweden using DAS-ELISA. TuYV was detected in all fields except one, and in the counties of Skåne, Kalmar and Östergötland, the average incidence of TuYV-infected plants was 75% and reached 100% for nine fields. Analyses of the CP gene of nine Swedish TuYV isolates revealed highest identity to TuYV isolates from the UK. One OSR sample was selected for high-throughput sequencing using rRNA-depleted total RNA and Illumina technology. The assembled TuYV sequence of 5661 nt covered the complete genome except for the terminal ends. A phylogenetic analysis showed a close relationship between the Swedish TuYV isolate from OSR and European isolates from pea. In addition, near full-length sequences were obtained for TuYV-associated RNA (TuYVaRNA; 2841 nt) and TuYVaRNA2 (2795 nt), which both shared highest nt identity with German pea isolates of TuYVaRNAs. Similar to other polerovirus-associated RNAs, the two TuYVaRNAs had two ORFs encoding proteins for replication. Potentially, co-infection with TuYVaRNAs could increase the severity of disease and more studies are required to study their incidence and effect on crop plants.

METATRANSCRIPTOMICS APPROACH TO STUDY THE VIROME OF ECONOMICALLY IMPORTANT CROPS

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Text

The use of high throughput sequencing (HTS) has expanded our perspective on the distribution and diversity of plant viruses. Furthermore, due to the increasing number of versatile and improved HTS technologies and the decrease in the cost per sample, implementing HTS has facilitated the diagnosis and discovery of novel viruses. This study aimed to examine the putative virome of economically important crops. Leaf samples were collected from vegetables, ornamentals, and row crops. Information on different nucleic acid extraction methods for HTS, including double-stranded RNA and total RNA, will be presented. Library preparation was performed from pooled samples before sequencing in an Illumina platform. The sequenced libraries were mapped to the host's reference genome, and the resulting sequences were de novo assembled. Both nucleic acid extraction methods successfully yielded sequences of good quality. A metatranscriptomics analysis revealed complete genome contigs of a variety of known and unidentified putative RNA and DNA plant viruses co-infecting the same host. The information obtained in this investigation will help develop a broader perspective on other viruses present in the tested plant species to determine whether co-infections with other viruses are a factor that might influence (negatively or positively) plant physiology, product quality, and yield.

THE ADDED VALUE OF PREPUBLICATION HTS DATA SHARING FOR THE DISCOVERY AND CHARACTERIZATION OF A NEW POTATO TORRADOVIRUS

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Text

High throughput sequencing (HTS) is a powerful technology for the detection of plant viruses. In 2014, the U.S. Customs and Border Protection intercepted potato tubers originating from South America. In 2016 the Netherlands Food and Consumer Product Safety Authority also intercepted potatoes from S. American origin. In both cases these samples were subjected to HTS and viral contigs most similar to torradoviruses were identified. These contigs showed

high similarity to an isolate of an undescribed isometric virus, coded SB26/29, which has been previously associated with a disease named potato rugose stunting in southern Peru. Tentatively the virus was named potato rugose stunting virus (PotRSV). Additional sequences obtained from cultivated potatoes in Peru, as part of the CIP potato virome project confirmed that all of these isolates from US, Peru, and the Netherlands belong to PotRSV. PotRSV like other torradoviruses has two polyadenylated RNA segments. RNA1 ranges between 7,086-7,089 nt and RNA2 from 5,228 to 5230 nt. The closest torradovirus species to PotRSV is squash chlorotic leaf spot virus sharing 41% (query coverage 48%) identity at the amino acid level. The prepublication data sharing enabled us to combine our efforts and with compiled data we tracked potential high risk virus movement out of its exotic range. The benefit is a better understanding of the virus genomic diversity and the ability to develop reliable detection assays for this new virus.

AUTOMATED AND REAL-TIME PROFILING OF PLANT VIRUS INFECTIONS TO SUPPORT DIAGNOSTICS AND QUICK RESPONSE IN OUTBREAK EVENTS

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Text

High-throughput sequencing (HTS) technologies are transforming our means to detect pathogens and perform disease diagnosis. Recent advances in genomic sequencing, as the ones pioneered by Oxford Nanopore Technologies (ONT), are enabling extremely rapid and effective detection, and quick transition from research to adoption in diagnostics settings is already occurring. The pocket-size of the sequencer, ease of library preparation, low sequencing costs and (long) read data generated in a short turnaround time are key characteristics of the ONT platforms, with substantial benefits for point-of-care testing and on-time reactions in response to a disease. Here we present the application of ONT for rapid, semi-automated profiling of (multiple) virus infections in plant samples. An amplicon-sequencing based approach was established by combining VolTRAX, a compact USB-powered device for automated sample and library preparation, with MinION sequencing and subsequent real-time analysis of the sequence reads. We demonstrate that the workflow – from RT-PCR amplification of total RNA to taxonomic assessment of pathogen sequences – can be accomplished in less than 5 hours, with a minimal need of human intervention, enabling a sensitive and rapid identification of viruses in diseased plant material.

FULL-LENGTH SEQUENCING OF CONCATENATED CIRCULAR DNA VIRUS GENOMES USING ROLLING CIRCLE AMPLIFICATION AND OXFORD NANOPORE SEQUENCING

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Text
Geminiviruses are plant-infecting viruses with a mono- or bipartite single-stranded DNA genome, approximately 2.5-3.1 kb in size. Reliable and accurate detection of virus species and subspecies level sequence types can be performed by modern sequencing methodologies, however the percentage of virus-derived reads is often very low. Here we present a species-independent method to enrich viral sequence data and to generate a consensus sequence from Oxford Nanopore Technologies sequencing reads. Rolling Circle Amplification based on the phi29 polymerase was used to generate concatemers of the circular virus genomes in DNA samples from various infected crop plants, including melon, pepper, eggplant, tomato and okra. Multiplexed sequencing of debranched and linearized amplification products displayed an enrichment of the viral sequence content. Some samples showed up to 83% of the read data derived from Begomoviruses, far more than the values found in non-enriched samples: typically 0-2% of the read data. Moreover, the concatenated DNA molecules generated during the Rolling Circle Amplification step resulted in concatemer reads, ranging from 3 to 20+ fold, that allowed generation of a reliable consensus sequence of the viral genome from a single Nanopore read. Cost-effective and reliable detection, description and sequence typing of geminiviruses and other pathogenic viruses with a circular ssDNA genome can be performed very efficiently and reliably with this method.

APPLICATION OF HIGH-THROUGHPUT SEQUENCING (HTS) FOR ROUTINE PLANT VIRUS DETECTION IN THE SOUTH AFRICAN CITRUS IMPROVEMENT SCHEME

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Text
The South African Citrus Improvement Scheme (CIS) is responsible for the production of pathogen-free propagation material for the southern African citrus industry. The CIS provides certified budwood and seed to local and international nurseries to produce trees. Budwood are produced under strict phytosanitary guidelines and multiplication trees are regularly re-tested. Before admission into the CIS new accessions are subjected to pathogen elimination (heat therapy and shoot-tip grafting) and rigorous testing for viruses and viroids. To reduce the timeline without an increase in risk, a high-throughput sequencing detection assay was validated for implementation in the CIS. The credibility of HTS as pathogen detection assay was measured using specific parameters, including repeatability, specificity, sensitivity, and reproducibility. The sensitivity of the HTS assay was compared to routinely used RT-PCR assays in a time course experiment. Controls were introduced at sampling and data analyses levels. Expectedly, both extraction method and sequencing platform resulted in significant differences between the data sets. However, even though the limit of detection of HTS was influenced by pathogen concentration, sample processing method and sequencing depth, HTS detection in this study was found to be equivalent or more sensitive than RT-PCR.

VALIDATION OF A HIGH THROUGHPUT SEQUENCING TEST WITHIN AN ISO17025 ACCREDITED PLANT HEALTH LABORATORY

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Text

The use of High Throughput Sequencing (HTS) for the detection and identification of plant viruses and viroids is widely used. So far, validation of HTS mainly focussed on the bioinformatics pipeline. We described and validated an HTS test from biological material till the reporting of the identified viruses and viroids within an ISO 17025 diagnostic framework. This includes the analysis of multiple first line controls and visual assessment of assemblies to further substantiate the reliability of the results.
Performance criteria analytical sensitivity, repeatability and reproducibility were determined similarly as for traditional molecular methods using tomato brown rugose fruit virus in infected tomato leaves as a model system. Analytical specificity, selectivity and robustness were assessed in a more generic way. This allowed addition of the detection and identification of viruses by HTS to the scope of accreditation. Using this validation approach, the HTS test was demonstrated to be fit for purpose for at least 180 viruses and viroids, including viruses with both DNA and RNA genomes, in a variety of hosts. The ISO 17025 accredited HTS test allows us to detect and identify potentially all EU-regulated viruses and viroids, instead of implementing and validating specific tests (ELISAs, PCRs) for each regulated pest.
We will present challenges and practical solutions in implementing and validating an HTS test within an ISO 17025 accredited plant health laboratory.

ADVANCES IN HIGH-THROUGHPUT SEQUENCING GIVE NEW OPPORTUNITIES IN DISCOVERY, DIAGNOSTICS AND BIOLOGY OF PLANT VIROMES; RESULTS GENERATED WITHIN INEXTVIR PROJECT

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Text
High-throughput sequencing (HTS) based methods have revolutionised plant virus discovery and virome studies. To facilitate a wide employment of the methods, build capacity and bring new advances to the field we joined forces within a Marie Sklodowska-Curie Innovative Training Network (MSCA-ITN) called INEXTVIR (Innovative Network for Next Generation Training and Sequencing of Virome). Fourteen PhD students, from 11 countries, were trained within the project over 12 European academic and industrial partners from 5 countries. Within INEXTVIR we aimed to generate a better understanding of viral communities and their role in agricultural ecosystems by using the latest advances in HTS technologies coupled with modern big data analytical approaches and socioeconomic analysis. The outputs of the project, which will be presented bring new advances overarching several natural and social science fields, including: comprehensive virome studies with detection and discovery of numerous new viruses in diverse crops (e.g., tomato, carrots, cucurbits, fruit trees); characterization of newly discovered viruses; insights into the interactions between virome and the environment (e.g., effects of habitat biodiversity); development of novel approaches for bioinformatics analysis of HTS-derived virome data; and societal perceptions of the risks and benefits related to the virome in agriculture.

WHAT CAN VIRAL METAGENOMICS BRING TO OUR UNDERSTANDING OF THE DIVERSITY AND ECOLOGY OF PLANT VIRUSES IN AGRO-ECOLOGICAL LANDSCAPES ?

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Text
High throughput sequencing (HTS) gave access for the first time to the viral metagenome or virome, by allowing to characterize, without a priori, all or nearly all viruses in a given sample. This broad-spectrum capability of HTS is raising a growing interest to study the diversity and ecology of plant viruses, in particular the richness and composition of viral communities at the agro-ecosystem scale, as well as the virus circulation among host reservoirs and the discovery of new and emerging viruses.
Recent viromics studies revealed diversified and largely unknown phytoviromes in natural ecosystems, with high rates of co-infection and the abundance of so-called persistent (or cryptic) viruses representing more than half of the viruses identified in wild plants. The influence of plant traits (e.g., lifespan, height, occurrence) on the virome richness, and the relationships between host-pathogen richness in cultivated and non-cultivated plant communities were also unravelled. In particular, the richness and diversity of plant communities appeared as influencing the richness and composition of phytoviromes, especially the distribution of persistent and acute viruses. Other results demonstrated the stability of virome richness over time but the large viral intraspecific variability within and among plant communities. Thus, HTS technologies have highlighted and will continue to serve the exploration of the complex network of viral communities in nature for the times to come...

DATA MINING-BASED DISCOVERY OF NOVEL TOBAMOVIRUSES AND VIRUSES ASSOCIATED WITH MACROPHYTES

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Text

The search for new viral sequences provides an opportunity to improve early detection of pathogens, and to predict viral hosts and environmental reservoirs. Accelerated use of high-throughput sequencing has led to an increased amount of publically available sequencing data, which often remains unexplored. Here we present two distinct examples of how data mining can be used either to search for sequences belonging to a group of viruses in various samples or to identify novel viruses associated with a certain group of host organisms. Firstly, we searched for known and novel tobamoviral sequences in a set of published sequence datasets, selected based on Serratus infrastructure. Preliminary results show that novel tobamovirus-like sequences can be found in diverse sets of environments. For novel tobamovirus-like sequences, we presented associations with source environments (e.g., wastewater, human gut) and viral hosts (e.g., sugarcane) and performed phylogenetic analyses. Secondly, we mined publically available transcriptomes of aquatic plants (macrophytes) for viral sequences, utilizing data from the 1000 Plant Transcriptomes Initiative (1KP). Similar experiments have previously revealed the presence of new viral species in a water moss, and a perennial creeping herb. Likewise, we have found that many of the analysed macrophyte transcriptome data sets contain known and novel plant viral sequences, including crop pathogens.

DISCOVERY OF RINGSPOT VIRUSES IN NEW HOSTS BY HTS

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Text

Columbine (Aquilegia sp.) and violet (Viola sp.) are landscape perennial plants. Columbine and violet plants with ringspots and mosaic symptoms were observed in a garden in Syracuse, NY. A combined sample was used for RNA extraction and high throughput sequencing (HTS). BLASTN analysis of seven contigs spanning between 3877 and 7480 bp revealed similarity to tobacco ringspot virus (TRSV) (94-96%), and to tomato ringspot virus (ToRSV) (93-97%). HTS results were confirmed by RT-PCRs using RNA from each sample and primers specific for TRSV and ToRSV. Columbine tested positive for ToRSV and TRSV, while violet tested positive for TRSV. Dagger nematode (Xiphinema sp.) was found in soil samples. ToRSV has been reported infecting columbine in Lithuania. Here we show evidence of ToRSV infecting columbine for first time in the U.S. Columbine and violet are new host for TRSV worldwide.

TOWARDS THE INCORPORATION OF HTS IN THE CONFIRMATORY DIAGNOSTICS PROCESS FOR QUARANTINE PLANT VIRUSES IN THE US

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Text

High-throughput sequencing (HTS) has advanced from research use to a powerful diagnostic tool for plant viruses. Efforts to develop and improve validation metrics, interlaboratory comparability studies, and quality measures established in the diagnostics workflow have facilitated its acceptance in diagnostics. The USDA APHIS PPQ, Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL), an ISO17025 certified facility, provides the official federal diagnostic determination for regulated plant pathogens in the United States. PPCDL relies on various molecular technologies for the detection and identification of plant pathogens. PPCDL scientists have joined national and international efforts to optimize and validate HTS processes for diagnostics and incorporate this technology in the diagnostics workflow. The PPCDL has used HTS to confirm the presence of quarantine viruses in plant samples, examine the genome-wide diversity of emerging plant viruses and detect novel viruses. This presentation will discuss the progress to incorporate HTS as a diagnostic tool that include optimization and validation of processes, and the results of its incorporation for the detection of emerging plant viruses in the United States.

INCIDENCE OF VIRUS INFECTING TOMATO AND PEPPER PLANTS AND SEEDS IN SOUTHEAST ASIAN COUNTRIES BY METATRANSCRIPTOMICS

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Text

Virus infection caused by insect vector from Southeast to East Asia is one of important constraints to the production of solanaceous crops, such as tomato (*Solanum lycopersicum*) and peppers (*Capsicum* spp.). However, limited information is available for incidences of viruses in vegetable crops in many Southeast Asian countries. Here, we report metatranscriptomics using RNA sequencing followed by bioinformatics analyses to determine viruses and viroids in solanaceous plants and/or seeds from Asian countries, including

Vietnam, Cambodia, Laos, Thailand, and Indonesia. We prepared a total of 21 libraries from virus infected pepper and tomato plants derived from different geographical regions in Vietnam, Lao PDR, and Cambodia. Also, we collected commercial or informal tomato and pepper seeds from several Asian countries and prepared 17 libraries in this study. We identified a total of 1,008 virus-associated contigs, which were assigned to 33 different virus species belonging to 13 different viral genera. In commercial pepper seeds, pepper cryptic virus 2 and pepper vein yellows virus were frequently detected. Multiple viral infections were detected in common in both plants, moreover, geographical region and host plant were two major factors to determine viral populations in Vietnam samples. Thus, our results provide the comprehensive overview of viral pathogens infecting two economically important plants in the family *Solanaceae* grown in Asian countries.

EPIDEMIOLOGICAL INSIGHTS WITH NEXTSTRAIN: LESSONS LEARNED FROM APPLICATION IN PLANT HEALTH AND FUTURE PERSPECTIVES

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Text
With new high-throughput sequencing techniques, assembling complete plant pest genomes is becoming the new standard. Particularly for plant viruses this is the case. When faced with outbreaks of priority regulated pests, pest genomes and associated metadata can be put to work to gain insights in the diversity, spread and epidemiology. Managing the large amount of various data and interpreting their interconnectedness is a grand challenge. At present, typically a plethora of static phylogenic trees is produced focusing on a limited set of traits.

Nextstrain is a collection of bioinformatic tools that creates an interactive webpage enabling interactive visual analytic. Genomic diversity of the pest under investigation is presented in the context of geographic distribution and associated multi-layered epidemiological traits. We present three cases in which Nextstrain has been used at the Dutch National Plant Protection Organization to better understand novel findings of plant pests: Tomato brown rugose fruit virus outbreaks, *Thaumatotibia leucotreta* host range expansions and the occurrence of *Synchytrium endobioticum* strains with increased virulence.

Lessons learned from these cases, which are relevant for all organism groups, and future perspectives to increase the impact of the Nextstrain tool will be presented.

NANOPORE SEQUENCING FOR THE ANALYSIS OF OFFICIAL SAMPLES FOR THE PRESENCE OF (QUARANTINE) PLANT VIRUSES

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Text

Climate changes and increased international trade are accelerating the spread of plant viruses, increasing the chance of introduction of new pathogens into new areas, and their persistence in new host species. Accurate identification of plant viruses is crucial for planning an effective prevention of new disease spread and its eradication. Targeted diagnostic tests are not available for many viruses, including some on quarantine lists. Development and validation of individual targeted tests could be time-consuming and costly. In this case, the use of the generic high-throughput sequencing (HTS) approach is sensible. HTS, using Illumina platform, has been used in our laboratory for selected official samples since 2017, resulting in detection of several new viruses for Slovenia. Recently, we introduced nanopore sequencing for the same aim, and shown that it gives comparable results to Illumina sequencing. Comparative studies were performed for detection of plant viruses in bulk plant samples, detection of various begomoviruses, and detection of unexpected plant viruses. Nanopore sequencing has been found to provide comparable results to Illumina sequencing, while being faster and better suited for small laboratories. Thus, nanopore technology was selected in our laboratory for a full validation according to EPPO standard PM 7/98, considering the specific guidelines of EPPO PM 7/151. The output of this process and our experiences obtained during it will be presented.

BIOVALON: A BIOINFORMATICS PIPELINE ADAPTATION AND VALIDATION FOR PLANT VIRUS DETECTION USING HTS METHOD.

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Text

Due to important and rather frequent EU and French regulation evolution, the Anses Quarantine Unit needs to develop targeted and non-targeted tests to detect plants pests. The BioValON project aims to develop a bioinformatics pipeline, part of a HTS test, to detect simultaneously all viruses and viroids in plants in quarantine.
Datasets obtained from total ribodepleted RNAs using Illumina sequencers were used: serial dilutions of virus-infected potatoes (analyzed with another pipeline in 2018) and potentially healthy or known to be infected Citrus, Prunus and Vitis.
The pipeline, resulting from the PrepMedVet project (Anses- Ploufragan), is divided into five steps: quality control, plant’s sequences subtraction, regulated pests detection, other pests detection and unassigned reads identification.
To validate the pipeline, five objectives were foreseen: successive and correct execution of the different steps, similar detection level obtained with data processed in 2018, negligible plant host remaining reads, validated interpretation criteria for viruses and viroids and identification confirmation of all expected pests.
The three first objectives have been achieved. For the analyzed samples, the expected pests (viruses and viroids) were detected and identified reliably. The interpretation criteria establishment and validation is still underway, notably to evaluate cross contaminations. The results are promising and give hope in the pipeline validation.

DISCOVERY OF THREE NOVEL SPECIES OF CARLAVIRUS AND ONE POTEXVIRUS IN MIXED INFECTION IN HIBISCUS ROSA-SINENSIS IN COLOMBIA USING HIGH THROUGHPUT SEQUENCING

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Text

The genus Hibiscus consists of numerous species of herbs, shrubs, and trees. Although hibiscus is not native to Colombia, it is well suited to its arid soil and dry climates which makes it ideal for cultivating in gardens and grows wild in tropical rainforests. Plant viruses in the genera Betacarmovirus, Begomovirus, Cilevirus, Higrevirus, Ilarvirus, Soymovirus, and Tobamovirus have been reported to infect hibiscus. Hibiscus is a well-known host for Brevipalpus transmitted viruses (BTVs) and, during surveys in the citrus growing regions in Colombia, several hibiscus samples were collected for virus testing. One of the hibiscus leaf samples from Risaralda, showing black spots on upper and lower sides, was selected for virome analysis using High-throughput sequencing (HTS) followed by bioinformatic analysis. BLASTn/BLASTx searches of assembled contigs revealed three novel sequences resembling carlaviruses and one presumed potexvirus. In addition, several contigs of Betacarmovirus, Cilevirus, Nepovirus, and Tobamovirus were also identified. All four novel viruses shared less than 70% nucleotide and 50% amino acid identities with each other and virus sequences available in the GenBank. To confirm the presence of Nepovirus and novel species of carla- and potexviruses in the same sample, RT-PCR specific primers were designed, and amplified products were sequenced. To our knowledge, this is the first report of carla-, nepo-, and potexvirus infection in hibiscus.

A NOVEL AUTOMATED WORKFLOW FOR PLANT VIRUS DIAGNOSTICS FROM HIGH-THROUGHPUT SEQUENCING DATA

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Text

Several workflows have been developed for the diagnostic testing of plant viruses using high-throughput sequencing methods. Most of these workflows require considerable expertise and input from the analyst to perform and interpret the data when deciding on a plant's disease status. The most common detection methods use workflows based on de novo assembly and/or read mapping. Existing virus detection software mainly uses simple deterministic rules for decision-making, requiring a certain level of understanding of virology when interpreting the results. This can also result in inconsistencies in data interpretation between analysts which can have serious ramifications. To combat these challenges, we developed an

automated workflow using machine-learning methods, which can decrease human interaction while increasing sensitivity, specificity, and consistency. Our workflow involves three steps: sequence data mapping, feature extraction, and machine learning model training. Using real data, we compared performance of our method with other popular approaches, and show our approach increases sensitivity and specificity while decreasing the detection time for most types of sequencing data.

APPLICATION OF HIGH THROUGHPUT SEQUENCING METHODS FOR A GRAPEVINE IMPORT, EXPORT AND DOMESTIC CLEAN PLANT PROGRAMS IN CANADA

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Text

Virus disease in Canadian vineyards has become a serious problem, resulting in decreased quality and yield of grapes. The only effective solution is the removal and replacement of infected plants. Growers propagating their own grapevine material can perpetuate the problem, with some recent planting testing 100% positive for unwanted virus diseases. Imported and domestically produced grapevine cuttings for planting are primarily check visually for disease which can be unreliable. Audit testing shows that this material can be infected. Growers can request lab testing, but this is expensive and/or limited and the Canadian Food Inspection Agency also has a limited capacity for audit testing of imported material. To improve testing of planting material to ensure a disease free status, rapid and comprehensive testing methods need to be developed. A collaborative project between Canadian universities, government and industry, with funding coming from Genome Canada, is developing methods, based on high throughput sequencing, for large scale testing to ensure that new grapevine planting material are free of harmful viruses. An update on this project will be presented.

REVISITING HIGH THROUGHPUT SEQUENCING DATA USED FOR PLANT VIRUS DETECTION IN ORDER TO FIND EVIDENCE OF NON-VIRAL PLANT PATHOGENS AND PESTS

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Text

High-throughput sequencing (HTS), more specifically RNA-seq of plant tissue, has become an indispensable tool for plant virologists to detect and identify plant viruses. During the data analysis step, plant virologists typically compare the obtained sequences to reference virus databases only, which lead to our hypothesis that they might be missing possible traces of other pathogens in the data. In this study, we set up a community effort to re-analyze existing RNA-seq datasets used for virus detection to check for the potential presence of non-viral pathogens or pests. In total 101 datasets from 15 participants derived from 51 different plant species were re-analyzed, of which 37 were selected for subsequent in-depth analyses. In 29 of the 37 selected samples (78%), we found convincing traces of non-viral plant pathogens or pests (>100 reads per million). The most observed organism categories were fungi (15/37 datasets), insects (13/37) and mites (9/37). Nematodes were not observed and only a few samples showed the presence of plant pathogenic phytoplasmas (1/37), bacteria (3/37) and oomycetes (4/37).
In conclusion, we were able to show that it is possible to detect non-viral pathogens or pests in these metatranscriptomics datasets, in this case primarily fungi, insects and mites. With this study, we hope to raise awareness among plant virologists that their data might be useful for fellow plant pathologists in other disciplines (bacteriology, mycology, entomology) as well.

IDENTIFICATION AND CHARACTERIZATION OF RESISTANCE-BREAKING BARLEY YELLOW MOSAIC VIRUS AND BARELY MILD MOSAIC VIRUS ISOLATES IN GERMANY

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Text
Barley yellow mosaic virus and barley mild mosaic virus cause the yellowing disease in barley and lead to yield losses of 50% in infested fields. The soil-borne vector *Polymyxa graminis* transmits both viral species and forms resting spores containing infectious virus particles. Noteworthy, against *P. graminis* no environmental or economical useful treatment is known. To avoid crop losses due to the two virus species, resistant barley cultivars are used by farmers. Interestingly, even by using cultivars carrying known resistant genes, plants can be found to show virus symptoms and are tested positive in ELISA for either one or both viruses. The aim of this newly started project is to identify resistance-breaking virus-isolates by using field trials and then to characterize the virus isolates by high throughput sequencing. We hope to find characteristic changes in the virus-isolates and want to use this information to further monitor the presence of these resistant breaking virus-isolates in fields in Germany.

HIGH THROUGHPUT SEQUENCING: RESEARCH TO REALITY – THE AUSTRALIAN POST ENTRY QUARANTINE JOURNEY.

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Text
The ability to detect all viruses and viroids present in plants undergoing post-entry quarantine is the ‘holy grail’ for both plant health scientists and regulators alike. For the Australian Government Post Entry Quarantine (PEQ) facility, the aspiration has become a reality after a near-decade long journey from proof-of-concept and validation through to operationalisation. In December 2022, small RNA sequencing and VirReport bioinformatics were deployed as the primary screening tool for virus and viroid detection in imported Prunus, Rubus, Fragaria and clonal grass species at PEQ. The outcomes from this deployment include the potential to reduce quarantine lag times, thousands fewer PCR tests per year, reduced use of biological indexing, and increased capacity for higher volumes of plant imports as a result of increased availability of glasshouse bench space. We anticipate that adopting high throughput sequencing (HTS) will enable plant industries to remain competitive and access more rapidly emerging high-value market opportunities.

EXPLORING PRACTICAL APPLICATIONS OF METABARCODING WITH MINION TO SUPPORT THE SURVEILLANCE OF THE PHLOEM BACTERIA “CANDIDATUS PHYTOPLASMA” AND “CANDIDATUS LIBERIBACTER”

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Text
“*Candidatus* Phytoplasma” and “*Candidatus* Liberibacter” are non-culturable, wall-less phloem bacteria linked to various plant diseases. Some species are quarantine pathogens

while others are regulated non-quarantine pathogens (RNQPs). Hence, a fast detection and correct identification is key for both farmers and authorities. In currently available methods, after detection, additional molecular analyses are needed to identify the correct species and its haplotype or (sub)group. Moreover, both pathogens are known to occur together sometimes making detection and identification dependent on several PCR assays.
In the METAMINSURV project, MinION metabarcoding targeting multiple loci will be evaluated to combine broad detection and identification of both types of phloem bacteria in one test. In silico analyses will be done to evaluate and select barcode(s) and primers to be able to target and distinguish as many species, haplotypes and/or (sub)groups as possible. Mock and spike communities will be prepared by mixing DNA, plant or insect samples with gBLOCKs from known species in different concentrations. MinION metabarcoding protocols and data analysis pipelines will be optimized. Besides phloem bacteria, another case study on fungal forest pathogens from spore traps or seeds will be evaluated. METAMINSURV will hence provide insights in the usefulness of MinION metabarcoding for the detection and identification of these pathogens in terms of cost, speed, sensitivity and specificity.

APPLICATION OF NANOPORE SEQUENCING AS A VIRAL DIAGNOSTIC TOOL: THREE ACCESSIONS, THREE OUTCOMES

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Text
Traded plants for planting must be free from harmful systemic pathogens which may determine economic damages in the considered species or behave as a reservoir for further host change and crop infection.
In this context, we evaluated the sanitary status of a *Jasminum officinalis*, a *Ficus carica* and a *Solanum lycopersicum* using the nanopore MinION device (Oxford Nanopore Technologies). A cDNA-PCR, cDNA and RNA-direct sequencing protocols were used to sequence dsRNA or rRNA-depleted total RNA extracted from leaf tissues from *Jasminum* and fig. In the case of tomato, a targeted approach, with the use of virus complementary primers for the cDNA synthesis was tested. The ONT produced long-reads allowing the identification of a carlavirus (Jasmine virus C), two closteroviruses (fig virus A and fig virus B) for *Jasminum* and fig, respectively, while the full-length genome reconstruction of tomato brown rugose fruit virus and pepino mosaic virus was possible for tomato. As per other high-throughput sequencing technology, the ONT demonstrated to be suitable as an early and generic diagnostic tool before symptom onset or in symptomless plants. However, the different approaches tested produced non-comparable results in terms of coverage and number of viral reads, suggesting the need of further improving the available protocols to be more suitable for plant specimens.

DEVELOPMENT OF PCR-BASED ASSAYS FOR GRAPEVINE BADNAVIRUS 1 USING HTS DATA OBTAINED FROM INFECTED GRAPEVINES

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Text

Grapevine badnavirus 1 (GBV-1; family *Calimoviridae*, genus *Badnavirus*) was detected for the first time in 2018 by high-throughput sequencing (HTS) from Croatian autochthonous grapevine varieties 'Ljutun' and 'Vlaška'. Recently, a screening of the grapevine virus collection (University of Zagreb Faculty of Agriculture) by HTS confirmed the presence of GBV-1 in four additional grapevine accessions. The collected HTS data were used to select the genomic region for primers and probe construction and to develope robust detection assays based on end-point polymerase chain reaction (PCR) and real-time PCR (qPCR). The qPCR showed 100-fold higher sensitivity compared to end-point PCR, regardless of the method of nucleic acids isolation (column-based from Qiagen or GES). Both detection methods (qPCR detectability down to a dilution of 1:10,000,000 and end-point PCR of 1:10,000) were more sensitive with nucleic acids isolation by column-based method than with GES (1:100,000 and 1:100, respectively). Using GES GBV-1 was efficiently detected by qPCR throughout dormancy and most of the growing season, with false negatives at the beginning of vegetation (April/May). The survey, conducted in 93 commercial vineyards and five grapevine collections on 4,327 vines, found that GBV-1 infections occurred only in autochthonous grapevine varieties with an overall infection rate of 13.4% and infections at specific sites/vineyards ranging from 1.9% to 96%.

QUADS INTER-LABORATORY EVALUATION OF HIGH THROUGHPUT SEQUENCING METHODS FOR PLANT VIRUS DETECTION

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Text

Members of the Quadrilateral (QUAD) Working Group for Diagnostic Tools Collaboration, organized an inter-laboratory exchange to evaluate high throughput sequencing (HTS) methods for plant virus detection. A set of Prunus and Malus freeze-dried leaf samples were sent to each participating laboratory. Participants performed multiple nucleic acid extractions and sequencing of each sample using method(s) of their choice. Total, small and double stranded RNA extraction methods were represented. The datasets were evaluated for reproducibility and accuracy using several bioinformatic workflows. The individual laboratories analysed the datasets using their own bioinformatic workflows, then all four country data sets were analysed using an independent bioinformatic workflow. While each sequencing method was able to detect the viruses and viroids present, indicating that HTS is an effective screening method for plant virus diagnostics, some differences were observed and noted. While the amount of sample background or contamination was not fully evaluated, it was present to various degrees in many of the data sets and the patterns were unique to each laboratory. The exception, however, was a consistent low level of contamination in one sample detected across all the labs. These results indicate the importance of appropriate QC standards (control) and protocol validation and confirmation of HTS virus detections with a secondary test method.

TRANSCRIPTOME SEQUENCING ANALYSIS AND FUNCTIONAL VERIFICATION REVEALED THE ROLE OF EXOGENOUS MAGNESIUM IN TOBACCO ANTI-PVY INFECTION

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Text

Potato virus Y (PVY) infection causes necrosis and curling of leaves, which seriously affect the yield and quality of Solanaceous crops. The role of nutrient elements in the regulation of plant resistance to virus infection has been widely reported. Previous studies in our laboratory have demonstrated that foliar spraying of MgSO4 could induce Nicotiana tabacum resistance to PVY infection by increasing the activity of defense-related enzymes. Consistent with the results, we found that exogenous magnesium (Mg) had a certain effect on Nicotiana tabacum anti-PVY infection. Meanwhile, Illumina RNA sequencing revealed that Mg induced resistance to PVY infection mainly by regulating carbohydrate metabolism and transportation, nitrogen metabolism, Ca2+ signal transduction and oxidative phosphorylation. Further more, we used TRV vector to verify the function of homologs of five Nicotiana tabacum genes involved in above pathways in Nicotiana benthamiana. The results showed that NtTPS and NtGBE were the key genes related to PVY infection, NtPPases and NtNiR were related to resistance to PVY infection, while NtCML36 did not play a role in PVY infection. Our transcriptome database and candidate genes functional verification suggested a novel strategy for resistance to PVY infection and provided a theoretical basis for virus-resistance breeding.

CHARACTERIZATION AND FUNCTION ANALYSIS OF SCMV-DERIVED VSIRNAS IN MAIZE RESISTANT AND SUSCEPTIBLE INBRED LINES

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Text

RNA silencing plays an important role in plant antiviral responses, which triggered production of virus-derived small interfering RNAs (vsiRNAs). Sugarcane mosaic virus (SCMV) infection causes serious economic losses worldwide in maize (*Zea mays* L.). In this study, the maize inbred lines Chang7-2 (resistant to SCMV) and Mo17 (susceptible to SCMV) were inoculated with SCMV (SC, SM) and phosphate buffer (MC, MM), respectively. The systemically infected leaves were harvested to perform whole-transcriptome RNA-seq and degradome sequencing. The results showed that the distribution of vsiRNAs on the sense strand of SCMV genome was higher than that on the antisense strand in both SC and SM libraries. The accumulation level of 21-nt vsiRNAs was higher in SM libraries, while more 22-nt vsiRNAs were accumulated in SC libraries. Through degradome sequencing, we identified 706 transcripts targeted by 204 vsiRNAs. The competing endogenous RNA (ceRNA) networks in SCMV-infected Chang7-2 and Mo17 were constructed and verified. Our results also showed that the transcripts of *DCLs*, *AGOs* and *RDRs* were differentially accumulated in resistant and susceptible maize plants after SCMV infection, which were associated with the production and function of vsiRNAs. These findings provide novel insights into the relationship between SCMV-derived vsiRNAs and potential ceRNA networks in resistant and susceptible maize materials.

TRANSCRIPTOMIC AND BISULFITE SEQUENCING IN PAPAYA PLANTS INFECTED WITH BABACO MOSAIC VIRUS (BABMV)

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Text

Babaco mosaic virus (BabMV) infects babaco plants (Carica pentagona). Due to genetic similarities with babaco, papaya plants (Carica papaya) were used to identify the transcriptomic and methylation responses occurring after BabMV infection. We performed RNA and Bisulfite sequencing of 32 samples of papaya leaves from plants infected with the BabMV evaluated at -2, -10, 15- and 30-days post-infection. Illumina reads quality for each sample was checked using FASTQC in Galaxy and mapping to papaya genome was performed with HiSat2 for transcriptomic reads and Bissmark tool for mapping and global cytosine methylation level for DNA sequences. Statistical analysis was carried out by multi-contrast test using Deseq2 for the transcriptomic analysis and logistic regression using the Methylkit package in Rstudio for the DNA reads, For the differential analysis FDR corrected p value <0.05 and log2 fold change > 0.5 criteria were used to select the differentially expressed genes and regions per CpG sites. In addition QTC clusters were constructed to

identified the gene patterns in both analyses. 1585 genes were differentially expressed in the RNA and 508 methylation sites in the DNA samples, GO overrepresentation analysis indicate changes in protein synthesis, oxidative stress and polysaccharide metabolism at 15- and 30-dpi. The use of both next generation sequencing techniques represents an important contribution to understanding the response of the plant to the virus infection.

How to combine remote sensing with epidemiological modelling to improve plant disease management?

CAN REMOTE SENSING DATA IMPROVE MATHEMATICAL MODELS FOR TACTICAL DECISION MAKING AND FUNGICIDE APPLICATION?

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Text

Remote sensing has supported applications on crop fertilization and irrigation, weed management and yield estimates. Applications for disease control, however, are still at small-scale, pilot development. How to use remote sensing data in decision making for tactical crop protection needs further investigation. These tactical decisions include (i) whether and when a fungicide application is needed, (ii) which fungicide(s) should be used, and (iii) at what dosage, which are supported by disease and fungicide models. Disease models provide information on infection events and their severity, and on the length of incubation and latent periods, so they guide fungicide timing and the activity the fungicide may have (pre- or post-infection, pre- or post-symptoms). Fungicide models define the degree and the duration of the fungicide activity; these models can be complemented by plant growth models that help estimating the fungicide dilution after application. Remote sensing data have the potential to supplement disease, plant and fungicide models in order to improve models' outputs and, finally, decision making. For instance, early detection of infection can be used to validate disease model predictions and support pre-symptom application of fungicides. Assessment of plant growth and development through vegetational indexes can improve the accuracy of fungicide models and support better (and spatially variable) definition of the fungicide dosage. Examples are provided for vineyards.

PAIRING HIGH RESOLUTION SATELLITE IMAGERY AND TERRESTRIAL ROBOTICS TO DETECT AND MONITOR GRAPEVINE DOWNY MILDEW EPIDEMICS

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Text

Grapevine downy mildew (GDM), caused by the oomycete *Plasmopara viticola*, plagues humid production regions and can cause 100% yield loss and vine death under conducive conditions. Growers currently rely on frequent fungicide applications for control, but this practice has led to widespread resistance. Rapid remote detection and mapping of GDM outbreaks would enable precision pesticide applications to target high performing but resistance-prone fungicides where and when most needed, while relying on less resistance-prone protectants elsewhere. To actualize this vision, we investigated two platforms for GDM surveillance: high resolution, multispectral satellite imagery and a terrestrial robotic imaging system at the Cornell Pathology Vineyard in Geneva, New York. We evaluated several supervised and unsupervised methods to predict disease severity using satellite spectral features as input. Spectra and vegetation health indices derived from Planet Labs SkySat imagery (50cm pixel size) could differentiate between healthy and diseased vines even at low GDM severity (10% symptomatic leaf area). Automated severity ratings derived from rover-based imagery also correlated well with human scout ratings ($r > 0.75$). Our next step is to integrate the two systems by training satellite imagery on rover generated severity maps. Our results thus far indicate that both satellite and terrestrial robotic platforms are promising methods for mapping GDM incidence and severity.

INTEGRATING REINFORCEMENT LEARNING AND EPIDEMIOLOGICAL MODELS FOR DISEASE CONTROL OPTIMISATION WITH LIMITED INFORMATION

TRIMBLE Rachel. (1), CUNNIFFE Nik. (1)

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Text

Epidemiological modelling is well established as a method to evaluate options for control of epidemics across human, animal and plant health. Those advising stakeholders may simulate the model to trial management strategies or use analytical techniques to optimise control. However, trial and error analysis risks selecting suboptimal strategies and analytical models require significant approximations to be tractable for realistic systems. Reinforcement learning is an approach to optimise sequential decision making which has been used in fields as wide ranging as chess, robotics and control of fusion reactors. The target problem is framed as interactions between an agent and an environment (in this case, a simulated epidemic) and the algorithm learns to optimise the outcome (e.g. the number of plants lost to disease) by targeted exploration of the state space. A key challenge for any control optimisation is acting in situations with partial observability — where parts of the system can be observed but the underlying state of the system is not known. This work applies reinforcement learning to a model of landscape level plant disease spread and breaks down how different elements of model observability and environment formulation make the learning more or less effective. In the context of remote sensing, this work may provide insights into how improving different types of observability in landscape scale epidemic control can improve our ability to optimise epidemic outcomes.

INTEGRATING REINFORCEMENT LEARNING AND EPIDEMIOLOGICAL MODELS FOR OPTIMISATION OF INVASIVE PLANT DISEASE CONTROL

TRIMBLE Rachel. (1), CUNNIFFE Nik. (1)

(1) University of Cambridge, Cambridge, UNITED KINGDOM

Text
Epidemiological modelling is well established as a method to evaluate options for control of epidemics across human, animal and plant health. Those advising stakeholders may simulate the model to trial management strategies or use analytical techniques to optimise control. However, trial and error analysis risks selecting suboptimal strategies and analytical models require significant approximations to be tractable for realistic systems. Reinforcement learning is an approach to optimise sequential decision making which has been used in fields as wide ranging as chess, robotics and control of fusion reactors. The target problem is framed as interactions between an agent and an environment (in this case, a simulated epidemic) and the algorithm learns to optimise the outcome (e.g. the number of plants lost to disease) by targeted exploration of the state space. A key challenge for any control optimisation is acting in situations with partial observability — where parts of the system can be observed but the underlying state of the system is not known. This work applies reinforcement learning to a model of landscape level plant disease spread and breaks down how different elements of model observability and environment formulation make the learning more or less effective. In the context of remote sensing, this work may provide insights into how improving different types of observability in landscape scale epidemic control can improve our ability to optimise epidemic outcomes.

COMBINING IMAGING AND SPATIALLY-EXPLICIT MODELLING FOR THE STUDY OF PLANT DISEASES : LESSONS LEARNED FROM THE STUDY OF PLANT PATHOGEN LESIONS

LECLERC Melen. (1)

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Text
The use of mathematical models offers a mean to analyse epidemiological data. Despites the recent development of imaging technologies to monitor plant diseases at various scales, these new data are still seldom used to fit mechanistic models and estimate key parameters.

In this presentation we present how combining image-based phenotyping with reaction-diffusion models provides new insights into the spread of plant pathogen lesions. The proposed approach consists in i) monitoring inoculated leaflets through imaging, ii) using computer vision methods to align images to each other and segment symptomatic tissues, and iii) fitting a reaction-diffusion model to image sequences with a variational data assimilation approach. This modelling framework was used to analyse data obtained on several pathosystems for a range of pathogen isolates and plant cultivars. It enables one to disentangle the processes involved in host-pathogen interactions and gives new quantitative traits for assessing host resistance and/or pathogen aggressiveness.

Similar approaches may be implemented by plant disease epidemiologists to understand the

spread of pathogens at larger spatial scales using remote sensing data. Besides the methods used for processing images produced by remote sensing, modellers will have to integrate image-data assimilation methods and perhaps, take into account the errors produced by computer vision algorithms to better estimate the parameters of mechansitic models.

PREDICTING STRESS CAUSED BY GRAPEVINE POWDERY MILDEW WITH NASA AIRBORNE IMAGING SPECTROSCOPY IN NAPA VALLEY, CALIFORNIA

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Text

Powdery mildews cause $42.8B in damage annually and are notoriously ubiquitous with 10,000+ host species on all crop producing continents. Grapevine (Vitis vinifera) Powdery Mildew (GPM; Erysiphe necator) is responsible for >90% of negative environmental consequences associated with vineyard management globally as effective control necessitates high frequency fungicide application. While mechanistic models to predict GPM incidence and spread exist, their accuracy is limited by uncertainty in underlying initial disease distribution. The overall goal of this work is to develop a quantitative index for GPM rooted in disease physiology that can be used to parameterize epidemiological models with NASA Airborne Visible and Infrared Imaging Spectrometer Next Generation (AVIRIS-NG) hyperspectral imagery collected over Napa Valley, CA. We compared Normalized Difference Vegetation Index (NDVI), Red-Edge NDVI (NDVIre), Plant Senescence Reflectance Index (PSRI), and others as viable early indicators of GPM-induced stress and compared their accuracy when derived from hyperspectral and multispectral (Sentinel-2) sources. We found NDVIre derived from AVIRIS-NG to be the most accurate indicator of grapevine health and vigor as relates to potential GPM-stress, especially once vines have amassed significant foliar chlorophyll. The next step for this work is to compare the distribution yielded by our quantitative index to simulations from the Gubler-Thomas.

COMBINING REMOTE SENSING AND EPIDEMIOLOGICAL MODELLING TO IMPROVE MANAGEMENT OF RED NEEDLE CAST OF RADIATA PINE IN NEW ZEALAND

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Text

Red needle cast (RNC), primarily caused by Phytophthora pluvialis, is one of the most

important diseases of Pinus radiata (radiata pine) in New Zealand. The disease causes reddening and premature cast of needles, leading to growth loss. The disease predominantly affects the lower crown but extends to the whole of the crown in severe cases. Disease expression generally begins in autumn, with disease severity developing rapidly and peaking in winter or spring. Remote Sensing approaches are being used to investigate several aspects of RNC, including epidemiology, growth impacts, and control options, with an aim to support the development of management options. High resolution imagery from fixed cameras, UAV, fixed-wing aircraft, and satellites is used to manually score trials in mature forests. A combination of terrestrial and aerial LiDAR has been used to map mature trees to allow aerial assessments of individual trees to be combined with on-ground weather, disease, and growth data. Frameworks have also been developed for the use of high- and low- resolution satellite imagery to automatically map and monitor outbreaks of RNC. Simultaneously, data on the environmental tolerances of the different pathogen life stages are being used to develop a process-based infection risk model, for which a range of remote sensing data will be used for calibration and validation. The benefits of this multi-disciplinary approach and the specific challenges of this system will be discussed.

DETECTION OF CERCOSPORA LEAF SPOT DISEASE IN TABLE BEETS FROM UAS MULTISPECTRAL IMAGES

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Text

Cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola* Sacc., is a severe foliar disease that affects the health of table beet crops. To effectively combat CLS, remote sensing techniques, specifically unmanned aerial systems (UAS), could offer a valuable solution for early detection and efficient fungicide application. Between June and August of 2021 and 2022, we conducted UAS flights over C. beticola inoculated table beet plots at Cornell Agritech in Geneva, NY, USA. Five bands (475, 560, 668, 717 and 840 nm) multispectral images were collected at a spatial resolution of 1 cm. CLS severity was assessed based on the percentage area of leaves affected by the disease. The severity level was evaluated by manually inspecting 20 random leaves from each plot. Vegetation indices, derived from multispectral imagery, are known to be effective in assessing plant health and disease determination. Texture features also have been reported to be effective in determining plant traits since they provide additional spatial information to the model. An initial analysis of a subsample of 2021 data produced an R2 = 0.71 using a combination of vegetation indices, reflectance values, and texture features, hinting at the possibility of CLS severity estimation from UAS multispectral images. We aim to further analyze and scrutinize our results by incorporating four flights' worth of data collected at various stages of disease severity.

HIGH RESOLUTION MULTISPECTRAL UAV IMAGERY FOR DISEASE QUANTIFICATION: AN ALTERNATIVE FOR LEAF DISEASE MANAGEMENT IN SUGAR BEET

BARRETO ALCANTARA Abel Andree. (1), ISPIZUA YAMATI Facundo Ramon. (1), MAHLEIN Anne-Katrin. (1)

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Text

In sugar beet production, managing leaf diseases is one of the most relevant activities to safeguard yield. To initiate a control measure, exhausting visual scoring activities must be conducted to release a warning signal based on disease quantification parameters such as disease incidence (DI). By the end of the growing season, a georeferenced estimation of disease severity (DS) can deliver spatial information on yield and pathogen distribution; this last aspect is crucial to track epidemics in the following years. Unmanned aerial vehicle (UAV), multispectral imagery, and deep instance segmentation networks enable an in-field detection and quantification of plant disease. The extraction parameters improve the knowledge of temporal and spatial development of disease. For determining DI and DS, the concept of scoring unit was transferred from practical use to an image-based perspective to analyze recorded fields at leaf and plot level. The results give an overview of how the accuracy of deep learning models and image-based “decision-making” criteria affects the performance of DI. Moreover, a better understanding of the disease spread is available by analyzing various metrics of DS, such as number of clusters, cluster area, and ratio of damaged leaf regions. The results of this work will deliver a possible solution to reduce the so far very laborious work of visual disease assessments in the field and thereby automate warning systems for disease management itself

CLOUD-NATIVE, MACHINE LEARNING BASED DETECTION OF GRAPEVINE LEAFROLL VIRUS IN VITIS VINIFERA WITH NASA IMAGING SPECTROSCOPY IN CALIFORNIA, USA

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Text

Early warning systems for plant disease based on remote sensing can provide rapid and accurate information for efficient resource management, thus reducing losses, expenses, and unintended negative environmental impacts. We previously found that deploying Machine Learning (ML) on spectroscopic imagery (SI) from NASA’s Airborne Visible and Infrared Imaging Spectrometer Next Generation (AVIRIS-NG) yields accurate maps of grapevine leafroll-associated virus 3 (GLRaV-3) at multiple spatial resolutions. Providing these maps to agricultural stakeholders would reduce time, expenses, and uncertainty associated with management, however, both storing SI and training/deploying ML models require significant computing and storage resources. This challenge will magnify tenfold as global SI from the forthcoming satellite Surface Biology & Geology satellite becomes available. We present a cloud-native architecture for plant disease detection to address this challenge using SI from NASA's AVIRIS-NG with GLRaV-3 as a model system. Our system processes SI into disease incidence maps using simple ML (Random Forest, optimized through SMOTE) and easily accommodates new additions and improvements, as well as shifting data modalities, without retaining potentially proprietary stakeholder information. We present an innovative system that empowers stakeholders to make data-driven plant disease

management decisions informed by cutting-edge SI while preserving reproducibility and user privacy.

MAPPING GLOBAL RISK OF FUSARIUM WILT IN A CHANGING CLIMATE WITH REMOTE SENSING AND AEROSOL TRANSPORT MODELING

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Text

Fusarium oxysporum (*Fo*) is a ubiquitous soilborne fungus that can cause Fusarium wilt (FW) in 100+ crops. Uncertainties in aspects of its epidemiology and a lack of global distribution data have historically challenged monitoring and containment efforts. Our NASA Interdisciplinary Sciences project seeks to address this need by integrating remote sensing, aerosol transport modeling, and comparative genomics to build a global disease surveillance system for FW incidence and *Fo* dispersal risk in aerosolized agricultural dust. As foundation, we released an interactive, global web map documenting 4500+ FW incidences reported in peer-reviewed literature. Here, we developed a global susceptibility assessment that integrates all three aspects of the disease triangle. We identified agricultural production zones conducive to FW, noting subsets capable of serving as dust sources, by overlapping the MODIS Deep Blue algorithm with a Landsat-based cropland product. We then restricted this assessment to only regions with reported *Fo* in the past 30 years. Conducive disease environment was modeled using multiple satellite-derived products with species distribution modeling. Results from this assessment along with aerosol transport modeling can inform how related incidence sites on opposite ends of dust events may be. This integrated approach to disease surveillance can provide key insights about drivers for current and future FW distribution and the spread of *Fo* on global dust currents.

ASSESSING LONG-DISTANCE, TRANSOCEANIC AND INTERCONTINENTAL ATMOSPHERIC TRANSPORT OF SOILBORNE PLANT PATHOGENS ENTRAINED WITH AEROSOLIZED AGRICULTURAL DUST

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Text

Soilborne pathogenic fungi are a leading cause of crop disease and are primarily spread through microscopic, durable spores adapted differentially for both persistence and dispersal via soil, animals, water and atmosphere. While intracontinental aerial dispersion of soilborne fungal spores has been well established, transoceanic and intercontinental atmospheric transport of these spores entrained with aerosolized agricultural dust is understudied and may contribute to disease spread. Our NASA ROSES project seeks to address this need by integrating remote sensing, aerosol transport and comparative genomics to assess the long-distance atmospheric dispersal of the plant pathogenic, soilborne fungus *Fusarium oxysporum* (*Fo*) on global dust currents. The CAM6-MIMI climate model was modified to incorporate spore traits that influence dispersal and atmospheric survival, and was parameterized using the 2020 Godzilla dust event. We found modeling evidence of transoceanic and intercontinental atmospheric transport of viable *Fo* spores and offered a danger index for *Fo* spore deposition on susceptible agricultural zones. The main long-distance transport of viable spores and the highest danger for deposition on cropland are between the regions of Eurasia, North Africa, and Sub-Saharan Africa. This study provides key insights about Fusarium wilt epidemiology and lays the groundwork to build an operational, real-time global surveillance system of long-distance plant pathogen transport risk.

COMBINING DATA AND KNOWLEDGE FOR DISEASE SPREAD MODELING AND SIMULATION SHOWN FOR CERCOSPORA LEAF SPOT IN SUGAR BEET

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Text

For an efficient and sustainable management of plant diseases, knowledge about their temporal and spatial occurrence and development is crucial.

To enable targeted and site-specific management measures such as site-specific application, the development of models for large-scale fields is necessary. Traditional models are based on environmental parameters and knowledge on the pathogen's epidemiology, integrating management practices. More recent technologies and innovations from the field of optical sensors and artificial intelligence provide the potential to improve data- and knowledge-based models.

In this work, we present a use case of a simulation model for Cercospora Leaf Spot (CLS) in sugar beet, caused by the fungal agent Cercospora beticola. Therefore, we combine data from optical sensors mounted on drones, weather, and environmental data with epidemiological knowledge to establish a simulation model for the occurrence and development of CLS. Furthermore, we integrate and simulate different fungicide applications

and spread scenarios. As an outcome of our study, we are able to model the disease spread by Spatio-temporal Point Processes (STPPs). This approach will support the generation of risk maps and to establish specific fungicide application maps for CLS and other diseases on the field level.

MONITORING BUTT AND ROOT-ROT DISEASES USING UNMANNED AERIAL VEHICLES

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Text

Forest stands are threatened by numerous pathogens and insect pests. However, it is often difficult to estimate the dieback and mortality they cause at the scale of several stands, as well as their progression over time. Mapping of each tree in forest plots impacted by pests is necessary to estimate the epidemiological dynamics over several years. These maps are now possible on several hectares thanks to the use of high resolution aerial pictures obtained by unmanned aerial vehicles. We have developed an automated analysis of these pictures to obtain these maps and the identification of dying or decaying trees. This analysis was applied to maritime pine plots impacted by butt and root rot disease in the Landes de Gascogne massif (southwestern France). These analyses allowed to characterize the associated mortality and to test whether these telluric diseases present on the stands were associated with specific spatio-temporal structures compared to other types of mortality (bark beetles, hydric stress, ...). This method could be used to identify the emergence of new diseases associated with introductions such as pinewood nematode, and to rapidly implement eradication measures in the identified areas.

QUANTITATIVE INVERSION OF WHEAT STRIPE RUST DISEASE INDEX BASED ON UNMANNED AERIAL VEHICLE HYPERSPECTRAL IMAGERY AND PIXEL-LEVEL REGRESSION ALGORITHM

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Text

There have been many research achievements in the detection of wheat stripe rust disease based on UAV multispectral/hyperspectral remote sensing images and deep learning methods. In this study, 1,920 local wheat varieties were selected as experimental materials in Henan Province, with a planting area of 13,350 square meters. High-resolution hyperspectral images were obtained by drones at a height of 100 meters at different infection stages. Deep learning methods were used to achieve end-to-end quantitative inversion of disease index by adding a Sigmoid activation function and using continuous loss functions such as Laplacian Loss. The study also compared the model performance with different loss functions, model architectures, with or without the addition of the PSA module, and different datasets. The results showed that the LaplacianLoss+MSELoss loss function and the

HRNet_W18 algorithm model had the best performance, with an R2 of 0.875 and an mean average error(MAE) of 0.0129 on the test set. After adding the PSA module, the R2 reached 0.880, and the MAE was 0.0123. When using a few feature indices for modeling (such as 6 feature indices modeling), the model recognition effect decreased significantly to 0.829 compared to the full-band modeling. The results showed that end-to-end modeling based on deep learning algorithms can be directly carried out on the full band to reduce data analysis steps and achieve better inversion effects.

CLASSIFICATION OF SOUTHERN CORN RUST SEVERITY BASED ON LEAF-LEVEL HYPERSPECTRAL DATA COLLECTED UNDER SOLAR ILLUMINATION

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Text

Maize is one of the most important crops in China, and it is under a serious, ever-increasing threat from southern corn rust (SCR). SCR has spreaded northward to northeast China, causing severe yield loss. Thus, a cost-effective, real-time detection method is required. The identification of wheat rust based on hyperspectral data has been proved effective. For SCR research, the reliability and usability of spectra collected under solar illumination (SCUSI) need to be explored.In this study, full-range hyperspectral data (350~2500 nm) were collected under solar illumination, and SCUSI were separated into several groups according to the disease severity, measuring height and leaf curvature. Ten indices were selected as candidate indicators for SCR classification, and their sensitivities to the disease severity, measuring height and leaf curvature, were subjected to analysis of variance (ANOVA). The better-performing indices according to the ANOVA test were applied to a random forest classifier, and the classification results were evaluated by using a confusion matrix. The results indicate that the PRI was the optimal index for SCR classification based on the SCUSI, with an overall accuracy of 81.30% for mixed samples. The results lay the foundation for SCR detection in the incubation period and reveal potential for SCR detection based on UAV and satellite imageries, which may provide a rapid, timely and cost-effective detection method for SCR monitoring.

INTEGRATING UAS-BASED MULTISPECTRAL IMAGING AND EPIDEMIOLOGICAL MODELING IN CEREAL CROPS

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Text

Detecting and estimating disease intensity at the plant population level primarily rely on visual sign and symptom assessments. This traditional approach can be reliable if done correctly but labor-intensive, low-throughput, and prone to human subjectivity. Evidence suggests that sensor-based technologies offer a new opportunity to quantify disease intensity. Our group seeks to combine imagery and epidemiological modeling using wheat

blast and corn tar spot as model systems. Through manuscripts published in 2020 and 2021, we demonstrated that the intensity of wheat blast or corn tar spot could be quantified using UAS-based multispectral imagery with varying levels of accuracy ($0.69<\rho_c<0.92$). In 2023, we demonstrated that image-based features and machine learning could be used to estimate tar spot epidemiological parameters from UAS-based multispectral images collected in field efficacy trials. Disease severity was assessed visually at three canopy levels within micro-plots for two years, while aerial images were gathered with UASs equipped with multispectral cameras. The developed models estimated disease severity at distinct canopy levels ($r\geq0.93$; $\rho_c\geq0.97$), and data were used to model disease progression. Parameters y_0 and AUDPC derived from visual and estimated disease severity were similar, but significant differences ($\alpha=0.05$) between K_{max} or r_L were found. Further studies are required to improve or transfer methods.

THE UTILITY OF PROXIMAL SENSING AND DEEP LEARNING IN THE DETECTION AND CHARACTERIZATION OF TAR SPOT EPIDEMICS ON CORN IN THE UNITED STATES

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Text

In recent years, the widespread incorporation of image sensors through proximal or aerial remote sensing has proven their utility as an alternative approach to conventional, human-vision-based disease estimation. Nevertheless, from a disease management standpoint, obtaining objective, accurate, and high-throughput measurements of signs and symptoms during the growing season are critical in sensor-based phenotyping. Since its first identification in 2015 in the United States., tar spot of corn caused by *Phyllachora maydis* has rapidly spread from Illinois and Indiana through the corn belt and south to Florida. The detrimental impact on yield and the polycyclic nature of tar spot epidemics have made this disease one of the most significant emerging diseases of corn in the United States. In my talk, I will share our work towards developing a pipeline consisting of the previously developed Stromata Contour Detection Algorithm (SCDA v1) and the generation of a Convolutional Neural Network (CNN). Our approach allows high-throughput and automated detection and quantification of tar spot stromata in Red-Green-Blue (RGB) images of corn leaves collected at multiple experimental sites in Indiana in 2021 (onset to later stages of tar spot development). Our work will serve as a foundation for building an accurate and reliable standardized approach that can be utilized nationally and internationally for tar spot research, disease management, surveillance, and epidemiological modeling.

QUANTIFYING VCMAX AND PLANT TRAITS TO MONITOR FOREST DECLINE SYMPTOMS BY COUPLING SATELLITE IMAGES AND BIOPHYSICAL MODELS

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Text

As climate change and globalization are changing forest pathogens and pests distributions and dynamics, accurate forest health monitoring (FHM) systems are increasingly sought after by forest managers to detect and prevent forest disturbances. We developed a hybrid machine learning approach that couples the SCOPE radiative transfer model with Sentinel-2 (S2) satellite time series to estimate maximum carboxylation rate (Vcmax) and leaf biochemical constituents. We found that our predictions matched with the estimates of gross primary productivity better in deciduous broadleaf forests than in forests dominated by needle-leaved evergreen trees. To verify the effectiveness of the proposed FHM system, we explored its capacity to estimate key plant physiological traits and red-edge spectral indicators in the Pinus pinea L. (Stone pine) forest stand monitored through the San Rossore 2 ICOS Ecosystem station in Italy, where an outbreak of a parasitic fungal infection occurred in the summer of 2020. The results reveal that the Vcmax, pigments, leaf water content and the red-edge indicator S2 showed to be more effective than conventional indices (e.g., NDVI) for the early detection of this fungal infection. Our work demonstrates the potential of coupling radiative transfer models and S-2 images to monitor plant physiological traits in support of FHM activities, particularly in the context of pest epidemics

REMOTE SENSING IN SUPPORT OF PLANT DISEASE DETECTION AT DIFFERENT SPATIAL SCALES

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Text

For an efficient Integrated Pest Management (IPM), the ability to monitor the plant’s health status to early spot diseases has proven essential. Among the methods to monitor plant diseases, remote sensing (RS) stands out as an excellent tool. However, along with a wide diversity of plant monitoring needs, there is also a wide variety of RS-based plant disease monitoring tools, each tailored to specific needs within the IPM. Within-field monitoring tools, such as sensors mounted on tractors, are critical in many production systems for which disease symptoms cannot be properly detected from a top-view perspective. This has been demonstrated in pear orchards for fire blight detection. Drones on the other hand, provide detailed information at the field level, enabling the monitoring of individual leaves and plants. This has proven to be useful in the detection of, e.g., Powdery Mildew in sugar beets, or Banana Wilt Disease. The downside of this technology is however the revisit time as well as the scalability to the regional level. To this end, satellites have demonstrated their potential in providing frequent and large-scale information on the phenological stages of crops as well as on their general health condition, both essential for a proper disease spread modelling.

Based on VITO's long-standing research in the use of RS technology for disease detection and monitoring, an overview of these technologies will be presented, together with their merits and pitfalls.

SPREAD OF NEOPESTALOTIOPSIS SP. CONIDIA FROM STRAWBERRY UNDER CONTROLLED CONDITIONS

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Text

The Florida strawberry industry has been recently affected by a new species of Neopestalotiopsis that is more aggressive and has caused significant losses. Since the fungus had been considered of secondary importance, little is known about its life cycle. Thus, experiments were set up in a wind tunnel to evaluate the dispersal of the pathogen from symptomatic strawberry leaves, fruit, as well as dried senescent leaves, and inoculated sandy soil. Plates with selective media for Neopestalotiopsis spp. were placed at 0.6, 1, 3, 5, and 7 m away from the inoculum sources, and the following treatments were tested: 5 m/s, 5 m/s + water, 7 m/s, and 7 m/s + water. To describe the dispersal gradients, an exponential model was fitted to the number of colony-forming units of Neopestalotiopsis sp. found on the plates and to the distance from the inoculum source. The exponential model described the dispersal gradient for treatments with water, although a few colonies were found in the treatments without water. The highest number of CFU were found in plates where strawberry fruit and strawberry dried leaves were the inoculum sources. Most inoculum moved less than 1 m, regardless of the inoculum source. The 7 m/s wind + water moved the inoculum further than 5 m/s + water. Our data suggest that Neopestalotiopsis dispersal occurs within short distances, but higher wind speeds, which commonly occur during storms in Florida, may move conidia longer distances.

VOLATILE ORGANIC COMPOUNDS -CHEMICAL SIGNALS TO COMMUNICATE PLANT HEALTH

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Text

Plants and their respective pests, including pathogens, communicate their physio-chemical status to their surroundings by emitting volatile organic compounds (VOCs). The specificity and uniqueness of these VOCs could be utilized as "infochemicals" to detect, identify and monitor diseases. Pre-symptomatic detection would allow more targeted and less resource intensive pest control strategies to be employed. In addition, the release of pathogen-related VOCs might elicit defense responses in neighboring plants that delay the spread of the disease within the crop stand. As such, the study of VOCs holds an untapped potential in plant pathogen epidemiology and management. We have collected VOCs emitted from wheat grown both in the greenhouse and in the field exposed to different fungal pathogens and identified those compounds emitted from infected plants by GC-MS. Concentrations of VOCs were very low, but the target diseases could be identified based on the VOC profiles of

the infected host plants. The project ‘PurPest- Plant Pest Prevention through technology-guided monitoring and site-specific control', currently funded by EU’s Horizon Europe program, is exploring the most recent sensor technology to detect and identify pathogens and insect pests based on their VOC signature in host plants to limit their spread, target control measures and better understand the drivers of pest invasion.

A MODELLING APPROACH TO MAP THE RISK OF HLB IN THE IBERIAN PENINSULA

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Text

Huanglongbing (HLB), or citrus greening, is a devastating citrus disease, currently found in Asia, Africa and North and South America. At present, no cases of HLB have been found in Europe, but in the past decade one of the disease vectors, the African citrus Psyllid (AfCP), has been found in several locations in North-Western Spain and Portugal. The presence of an established vector population means there is a high risk of transmission between citrus if HLB is subsequently introduced.

We present the findings of a 1 km^2 computational model of vector and pathogen spread in the Iberian Peninsula. The density of citrus in residential areas and commercial orchards, as well as climate suitability, influence the pattern of spread. The majority of vectors disperse locally and are dependent on the availability of citrus plants, but we also account for long-distance dispersal via mechanisms such as wind or human transportation. Using the current estimated distribution of AfCP as an initial condition, results often show a pattern of slow growth of the psyllid in the North-West. However, once long distance dispersal or new introduction of psyllid into the densely populated commercial citrus regions in the South or East of Spain occurs, the population quickly increases. There is subsequently a high risk of rapid spread of HLB upon the introduction of an infected plant in this region.

AN EPIDEMIOLOGICAL MODEL TO ASSESS THE EFFICACY OF MONITORING TECHNOLOGIES FOR EARLY DETECTION OF TREE PESTS AND PATHOGENS AT LOCAL AND TREESCAPE LEVELS

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Text

Trees are an essential natural resource that stores carbon, provides habitats and food for wildlife and is an important ecosystem service. Nevertheless, they are under increasing threat from pests and pathogens. Efficient monitoring of tree conditions is needed to respond

efficiently to plant health threats and minimise the risk of new outbreaks. Monitoring of tree health through visual inspection alone is prone to error and bias and consumes time, monetary and human resources. Thus, it would be in the interest of stakeholders if tree health could be assessed and monitored rapidly while utilising sensors and technologies, from Internet of Things (IoT) devices through drones and satellite imagery which in addition offer continuous monitoring. However, each of the technologies comes with limitations and costs. We assess their monitoring potential with a spatially-extended metapopulation model of a landscape consisting of woodland parcels, incorporating both local and long-distance spread. We include the IoT/visual inspections, which are potentially able to detect low levels of stress but at a high cost and low frequency. We contrast this approach with satellite imagery which offers continuous monitoring over large areas, but with a significantly lower resolution. The detection process is quantified by the time from the start of the epidemic to the first detection. We then relate the efficacy to the total cost of the monitoring programme.

ESTIMATING BEET YELLOWS SEVERITY AT PLOT RESOLUTION WITH SATELLITE OBSERVATIONS

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Text

Beet yellows is now perceived as a major problem in plant health in Europe due to the modification of the regulations concerning the use of phytosanitary products. An agro-ecological approach to this issue requires actions at multiple levels, via prophylaxis, treatments and insurance systems in particular. To implement these actions effectively, we need an increased level of information. With this in mind, we are interested in a way to estimate the severity of beet yellows at plot resolution over large territories. We have thus developed an approach combining field observations (precise but partial) and satellite observations (less precise but with a high coverage rate). We tested different supervised approaches exploiting raw images or indices calculated on the basis of images, as well as a post hoc approach allowing to integrate multi-scale dependencies between observations.

A COMPARTMENTAL MATHEMATICAL MODEL BASED ON APHID FEEDING BEHAVIOURS ALLOWS MORE REALISTIC MODELLING OF NON-PERSISTENTLY TRANSMITTED PLANT VIRUSES

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Text

Plant viruses threaten global food security and are often transmitted by arthropod vectors. Non-persistently transmitted (NPT) plant viruses are characterised by a very short virus retention time in the vector and are transmitted almost exclusively by aphids. Compartmental models using ordinary differential equations to capture the course of an epidemic have been used in plant virus epidemiology for decades. However, the underlying model structure, in

which the infective period of vectors is fixed, omits a key feature of non-persistent transmission: probing or feeding on a plant is often what causes an aphid to lose its infectivity. A recent model by Donnelly et al. (2019) captures this behaviour via a Markov chain that tracks the behaviour of individual aphids. We introduce a new compartmental model which replicates this model, while allowing the easy extensibility characteristic of compartmental models. It is comprised of linked Susceptible-Infected models for the plants and aphids, where loss of aphid infectivity is conditioned upon its probing and feeding behaviour, rather than occurring at a fixed rate. This additional biological realism means our model behaves differently to previous compartmental models of NPT viruses, therefore allowing us to more accurately investigate virus transmission dynamics for all NPT systems. We focus on the case of viral manipulation of host plant phenotype, which changes aphid landing and feeding behaviour to enhance virus spread.

DISEASE CLIMATIC RISK MODEL INTERPRETATIONS AT MULTIPLE SPATIAL SCALES

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Text

Climatic risk models are often used to help understand the areas and seasons where invading organisms will have the greatest risk of establishing and causing a negative impact. These risk models often make predictions for a large spatial scale using inputs of time-averaged climatic data (e.g. annual). However, many organisms respond to, or have mechanisms influenced by, much shorter time scales (e.g., diurnal effects) and finer spatial scales (e.g., microclimate effects). Large-scale averaging may hide important information and could lead to misrepresentation of the dynamics of pathogen and pest populations and their spread in time and space. This risks misinterpretation of risk and errors in surveillance and management efforts. We use the existing myrtle rust (*Austropuccinia psidii*) climatic risk model in conjunction with field data, as an example to explore the scale of variability in microclimate variables across forest edges and what that could mean for risk model interpretations. These are compared to risk predictions from the national forecast grid, regional weather stations and local weather stations. We discuss the implications of these comparisons to risk predictions for future incursion responses, for example *Xylella fastidiosa*, and what resolution is ‘good enough’ for what particular purpose.

MEASURING PLANT STRUCTURE AND FUNCTION USING OPTICAL REMOTE SENSING – CURRENT STATUS AND RECENT ADVANTAGES OF AIRBORNE AND SATELLITE REMOTE SENSING AND THEIR POTENTIAL FOR DISEASE DETECTION

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Text

We have experienced a great increase of earth observation satellites in the past years and

nowadays a large number of satellite earth observation products are available on user-friendly plattforms. Despite this large number of remote sensing products it remains a challenge to early detect plant diseases from satellite data. The main challenges are related to the small symptoms that often are hidden in the large satellite imagery and insufficient revisiting time of many satelllite platforms.
In this didactical presentation an introduction on the basis of optical remote sensing is given and recent advances in Earth Observation will be highlighted. Existing multi-spectral mission, recently launched hyper-spectral mission concepts, as well as fluorescence and thermal approaches will be reviewed on their potential for disease detection.

HIGH-RESOLUTION HYPERSPECTRAL AND THERMAL IMAGING FOR THE EARLY DETECTION OF PLANT DISEASES. PROSPECTS AND LIMITATIONS.

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Text
Progress in the last 20 years in thermal and imaging spectroscopy has advanced tremendously, allowing the large-scale monitoring of crop physiological processes. Successes have been obtained in biotic and abiotic stress detection, particularly through sensor miniaturization and innovative physically- and artificial intelligence-driven modelling techniques. These developments have enabled the screening of subtle physiological changes through spectral analysis. Remote sensing efforts as part of European initiatives (POnTE, XF-ACTORS and recently BeXyl), and through regional programs have focused on the development of algorithms for the early detection of Xylella fastidiosa and Verticillium dahliae -induced symptoms. These studies have shown that using specific spectral plant traits successfully reveals infections at early / pre-visual stages. Nevertheless, several issues remain to be addressed, such as i) lack of high spatial resolution hyperspectral satellite images to detect subtle physiological changes on individual tree crowns; ii) lack of high-resolution thermal imagery to detect changes linked to transpiration reduction in infected vegetation; iii) lack of suitable multispectral bandsets in commercial satellite sensors; and iv) limited scale coverage of airborne hyperspectral and thermal sensors (i.e. drones and piloted platforms). These aspects will be discussed in the context of global detection and monitoring of harmful organisms causing plant diseases.

OMGN: Oomycete Molecular Genetics Network Annual Meeting

WHOLE GENOME SEQUENCING AND PHYLOGENOMIC ANALYSIS SHOW SUPPORT FOR THE SPLITTING OF GENUS PYTHIUM

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Text

The genus *Pythium* (nom. cons.) sensu lato (s.l.) is composed of many important species of plant pathogens. Early molecular phylogenetic studies suggested paraphyly of *Pythium*, which led to a formal proposal by Uzuhashi and colleagues in 2010 to split the genus into *Pythium* sensu stricto (s.s.), *Elongisporangium*, *Globisporangium*, *Ovatisporangium* (= *Phytopythium*), and *Pilasporangium* using morphological characters and phylogenies of cox2 and 28S rDNA. Although the split was fairly justified by the delineating morphological characters, there were weaknesses in the molecular analyses, which created reluctance in the scientific community to adopt these new genera for the description of new species. In this study, this issue was addressed using phylogenomics. Whole genomes of 109 strains of *Pythium* and close relatives were sequenced, assembled, and annotated. Phylogenomic analyses were performed with 148 single-copy genes represented in at least 90% of the taxa in the data set. The results showed support for the division of *Pythium* s.l. The status of alternative generic names that have been used for species of *Pythium* in the past (e.g., *Artotrogus*, *Cystosiphon*, *Eupythium*, *Nematosporangium*, *Rheosporangium*, *Sphaerosporangium*) was investigated. Based on our molecular analyses and review of the *Pythium* generic concepts, we urge the scientific community to adopt the concepts proposed by Uzuhashi and colleagues in 2010 in their work going forward.

AN OPEN-ACCESS T-BAS PHYLOGENY FOR EMERGING PHYTOPHTHORA SPECIES

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Text

Phytophthora species cause severe diseases on food, forest, and ornamental crops. We developed an open access phylogenetic tool using the Tree-Based Alignment Selector Toolkit (T-BAS) for 192 formally described species of Phytophthora and 33 informal taxa in the genus *Phytophthora*. The phylogenetic tree uses sequences of eight nuclear genes and was inferred using the RAxML maximum likelihood program. A search engine was developed to identify microsatellite genotypes of *P. infestans* based on genetic distance to known lineages. The T-BAS tool provides a visualization framework allowing users to place unknown isolates on a curated phylogeny of all *Phytophthora* species. The tree can be updated in real-time as new species are described. The tool contains metadata including clade, host species, substrate, sexual characteristics, distribution, and reference literature, which can be visualized on the tree and downloaded for other uses. This phylogenetic resource will allow data sharing among the global *Phytophthora* community and the database will enable users to upload sequences and determine the phylogenetic placement of an isolate within the larger phylogeny and download sequence data and metadata. The database will be curated by *Phytophthora* researchers and is housed on the T-BAS web portal in the Center for Integrated Fungal Research at NC State. The T-BAS web tool can be

leveraged to create similar metadata enhanced phylogenies for other Oomycete, bacterial or fungal pathogens.

DNA METABARCODING AS A SUPPORT TOOL OF TRADITIONAL ISOLATION METHODS TO DESCRIBE THE COMPLEXITY OF PHYTOPHTHORA COMMUNITIES

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Text

With the advent of the new millennium, traditional PCR has become the most reliable tool for the identification of cultured microorganisms. Recently, advancements in molecular technologies paved the way to 'omic sciences', new disciplines leading to the description and interpretation of communities of microorganisms in complex biological samples. Among these sciences, the DNA metabarcoding is emerging as the best support tool for the surveillance of *Phytophthora* communities within environmental samples carried out by traditional isolation. In this study, leaf baiting isolation and DNA metabarcoding were used to describe *Phytophthora* communities from soils of a nature reserve, a botanical garden and a citrus orchard. Overall, 155 baited isolates and the 32 metabarcoding-ASVs leaded to the identification of 21 *Phytophthora* taxa, including species exclusively recorded by baiting (*P. bilorbang*, *P. cryptogea*, *P. gonapodyides*, *P. parvispora* and *P. pseudocryptogea*), species exclusively detected by metabarcoding (*P. asparagi*, *P. occultans*, *P. psycrophila*, *P. syringae*, *P. aleatoria*/*P. cactorum*, *P. castanetorum*/*P. quercina*, *P. iranica*-like, and 5 unknown *Phytophthora* taxa) and species in common with both techniques (*P. citrophthora*, *P. multivora*, *P. nicotianae* and *P. plurivora*). Results suggested that the combination of leaf baiting and metabarcoding is the best approach to gain the most comprehensive diversity of *Phytophthora* communities in soil samples from different environments.

HEAVY RAINFALL HAS GIVEN RISE TO SEVERE CROP DISEASES CAUSED BY PHYTOPHTHORA SPP. IN TAIWAN

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(1) Taiwan Agricultural Research Institute, Taichung City ,, TAIWAN

Text

In Taiwan, heavy rainfall has been the most typical severe weather event in summer, causing various crop diseases. Most diseases were caused by *Phytophthora* spp., including Phytophthora blight of Welsh onion, Phytophthora blight of cucurbits, Phytophthora heart rot of pineapple, Phytophthora blight of orchids, Phytophthora blight of passion fruit, Phytophthora leaf blight, and fruit and root rot of papaya, and so on. Since 2009, continuous heavy rainfall has been a big problem in the summer in Taiwan, often causing flooding in the

fields. It usually led to poor growth, the death of large areas of Welsh onion, as well as severe fruit rot of melon. The crop yield loss was initially thought to be the poor water tolerance of the cultivars in the rainy season. Later studies proved it was a severe disease caused by *Phytophthora nicotianae*, *P. melonis*, and other *Phytophthora* spp. induced by continuous heavy rainfall. Because Phytophthora blight develops fast and can cause severe infections in several days, it will be too late to apply pesticides after the rainy season. Field trial results show that the regular application of phosphite weeks before the rainy season or/and the precise application of fungicides before and during the rainy season could significantly reduce the disease severity. In addition, healthy seedlings and weather forecasting are also crucial for disease prevention.

A QTL MAPPING APPROACH LEADS TO THE IDENTIFICATION OF CANDIDATE AVIRULENCE GENES OF GRAPEVINE DOWNY MILDEW

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Text

Plasmopara viticola is a biotrophic oomycete responsible for grapevine downy mildew, one of the most destructive diseases in viticulture. Breeding efforts for new resistant cultivars are based on the introgression of *Resistance to Plasmopara viticola* (*Rpv*) factors from wild grape species. However, in recent years, a number of isolates able to overcome the resistance conferred by different *Rpv* genes have been reported. The risk of rapid breakdown of resistance makes it urgent to understand the genetic factors underlying the virulence of the pathogen.

We carried out a QTL mapping study focused on *P. viticola* adaptation to three major resistance genes: *Rpv3*, *Rpv10* and *Rpv12*. Sexually compatible strains were crossed to generate two F1 progenies, on which targeted genotyping-by-sequencing was performed. We built a set of unprecedented linkage maps of the *P. viticola* genome, with a consistent number of seventeen linkage groups that likely correspond to chromosomes.

Each offspring was cross-inoculated on a panel of grapevine cultivars carrying one of the aforementioned *Rpv* genes or none. Linkage analysis shows that resistance breakdown is under the control of one major QTL for each *Rpv* gene. These QTLs map to regions enriched in predicted secreted effectors, in which large deletions are observed for strains virulent on *Rpv3* and *Rpv12*. These results pave the way for the functional validation of the interaction between *Rpv* genes and candidate P. viticola avirulence genes.

A CULTUROMICS APPROACH IDENTIFIES RHIZOSPHERIC BACTERIAL STRAINS INVOLVED IN LEGUMES PROTECTION AGAINST THE ROOT ROT AGENT APHANOMYCES EUTEICHES

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Text

In recent years, using a consortium of selected bacteria from the root microbiota, also known as a "synthetic community" (SynCOM), to promote plant health has become increasingly common. The objective of this study is to elaborate a model SynCOM from the rhizosphere of Medicago truncatula (Mt) to unravel the role of root microbiota in mediating plant-microbe interactions in the context of biotic stress caused by Aphanomyces euteiches, a devastating oomycete that causes root rot in legume plants. A high throughput culturomics protocol was used to obtain 1364 isolates from the rhizosphere of Mt. The collection of isolated bacteria was genotyped using Illumina 16S metabarcoding sequencing. The UCLAST algorithm was employed with a 97% identity to select 812 pure isolates with 79 unique OTUs. The relative abundance analysis of the collection showed that the uppermost taxa were similar to those observed in molecular identification obtained from soil DNA. Among the selected 812 pure isolate 12 were found to inhibit A. euteiches growth in a dual culture assay. In planta testing, only one strain of Pseudomonas sp. showed significant difference from the untreated control. This collection of 79 unique OTUs was used to constitute a synthetic community as a model of the root microbiota of Medicago plants. A multi-omics approach will be used to analyze the behavior of this SynCOM in a gnotobiotic system to study its role in plant microbe interactions.

INSIGHTS INTO THE GENOME OF PHYTOPHTHORA AGATHIDICIDA, THE KAURI DIEBACK PATHOGEN

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Text

Phytophthora agathidicida is the main causal agent of a devastating dieback disease that kills iconic and ancient kauri (*Agathis australis*) in New Zealand. Like for so many other *Phytophthora* pathogens, there is no simple way to control or eradicate it. Studying the genomes and genes of species such as *P. agathidicida* will provide a much deeper understanding of how *Phytophthora* pathogens work and, ultimately, how we might combat them. We recently assembled the genome sequence of *P. agathidicida* to chromosome level - one of the first for any *Phytophthora* species (Cox et al. 2022 Frontiers in Microbiology 13: 1038444). The complete genome sequence shows the extent of duplication and diversification of genes such as effectors that are predicted to have roles in virulence. Here we describe recent progress in analysing the *P. agathidicida* genome and assessing effector gene expression *in planta*, as well as functional studies of both cytoplasmic and apoplastic effectors. The *P. agathidicida* genome sequence shows many similar genomic and gene features to those of other sequenced *Phytophthora* species. Because of this, it is anticipated that this genome sequence will be a useful resource for the broader *Phytophthora* research community as we make a collective global effort to control these plant killers.

A MULTIPURPOSE TOOLKIT OFFERED PRACTICAL ASSISTANCE TO ADVANCED FUNCTIONAL ANALYSIS OF PHYTOPHTHORA SOJAE GENES

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Text

Oomycetes, represented by *Phytophthora*, have a threat of serious injury to natural and farm ecosystems due to complex pathogenic mechanism. Recently, CRISPR/Cas9-based gene-editing strategy has been established in *Phytophthora sojae*, becoming a powerful tool for oomycete functional gene research. However, an integrated gene research system needs functional complementation and reintroduction of target gene(s). Currently, lacking efficient selection marker for complementation becomes the short board of gene function research. Here, we report that the gene *NAT1* (GenBank: CAA51674.1), which encodes Nourseothricin acetyltransferase and confers resistance to antibiotic Nourseothricin, can be used as a selection marker for Phytophthora transformation. Therefore, a new genetic manipulation toolkit is developed based on vectors containing NAT1 or NPT II, offered practical assistance to advanced functional analysis of *P. sojae* avirulence genes. In this study, we demonstrated that the NAT gene can be used as a screening marker and constructed a complete functional genetic research system in *P. sojae*. This report will greatly accelerate the functional genomics of oomycetes.

A PHYTOPHTHORA SOJAE RXLR EFFECTOR IMPACT HOST DEFENSE-ORIENTED TRANSCRIPTOME REPROGRAMMING BY TARGETING SOYBEAN MEDIATOR SUBUNIT 21

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Text

Upon pathogen infection, plant-activated defense systems undergo dramatic transcriptome reprogramming. In eukaryotes, conserved mediator complex bridges transcription factors and RNA polymerase II to regulate the transcription of specific genes. Mediator complex affects almost all stages of transcription and plays an essential role in the transition from normal development to immune response. However, pathogens may manipulate host transcription by delivering effectors into plant cells. Here, we report that the nucleus-localized RxLR effector PsAvh109 of *Phytophthora sojae* regulates plant immunity by interacting with the soybean (*Glycine max*) mediator subunit 21 (GmMED21). We further showed that GmMED21 is a positive regulator of plant resistance to pathogen. Silencing of *MED21* in *Nicotiana benthamiana* suppresses the expression of salicylic acid (SA) signaling pathway genes, leading to increased pathogen infestation. Consistent with this, over-expression of *PsAvh109* in soybean also suppressed the genes response to SA signaling pathway and significantly enhanced the invasion by *P. sojae*. In addition, we found that the nuclear localization of the effector PsAvh109 is crucial for its action. The further research reveals Avh109 blocks the function of host core Mediator. As a result, our study identifies a regulatory mechanism by which pathogen effectors target the mediator complex to regulate the transcription of plant defense genes.

UNDERSTANDING THE EARLY EVENTS OF PLANT INFECTIONS BY OOMYCETES, AT NEW SPATIO-TEMPORAL SCALES: FROM ATTRACTION AND AGGREGATION OF ZOOSPORES TO HOST PENETRATION

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Text

Plant pathogens have evolved a wide range of strategies enabling surface colonization and invasion of host despite the plant defense mechanisms. Current knowledge of small spatio-temporal scales on mechanisms allowing attraction toward hosts and progression across each first plant cell layer remains sparse. To characterize which host signals and plant cell functions regulate zoospore attraction and penetration, we developed a multidisciplinary study of the rhizospheric dialogue between the telluric oomycete, *Phytophthora parasitica* and Arabidopsis. On the one hand, we generated new phenotyping tools dedicated to the short time-scale quantification of both zoospores behavior swimming in the presence of ionic signals and aggregation on the root surface. On the other hand, we defined the transcriptome of roots and zoospores during attraction of *P. parasitica* and the translatome of each root cell layer during the penetration of zoospores. Thus, we showed that (1) the zoospores aggregated on root in the first minute after inoculation, (2) both roots and zoospores stimulated transcriptomic changes during attraction, and (3) when *P. parasitica* penetrated the rhizodermis, the translatomes were also modulated beyond in the cortex, the endodermis and the stele while these cell layers are not yet colonized. The implication of these results in understanding the early stages of infection, at short spatio-temporal scales, and their use for disease control will be discussed

REVEALING PRINCIPLES OF PHYTOPHTHORA ZOOSPORES SENSING AND MOTION PROPERTIES THROUGH A BIO-PHYSICAL APPROACH

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Text

In the soil, early infection events of *Phytophthora* species are mediated by sensory and propulsion capabilities of biflagellate unicellular zoospores, orchestrated by rhizospheric

guidance factors. Lack of detailed information on zoospores plasma membrane proteins prevents a comprehensive understanding of how they contribute to the perception of rhizospheric environment, particularly during migration toward host plant. A bio-physical approach was developed to identify the molecular key-players mediating host-driven taxis. At first, the membrane protein repertoire of *Phytophthora parasitica* zoospores was investigated through LC-MS/MS approach, resulting in a distinct peptide signature between zoospores cell body and flagella plasma membranes. Then, using a microfluidic set-up, functional biomechanics analyses were developed to quantify both zoospore motion (velocity, trajectory and cell rotation) and flagella beating (frequencies and oscillation amplitude) in response to distinct rhizospheric stimuli. The set-up further enabled to discriminate zoospores specific stimuli response among other rhizospheric microbial species. Altogether, the obtained results contribute to elucidate the mechanism of protein-mediated sensing and motion response of *Phytophthora* zoospores and improve the understanding of the complex rhizospheric interaction network driving oomycete dispersal.

GENOMIC INVESTIGATIONS REVEAL ATYPICAL DYNAMIC MITOTIC VARIATION CAN RAPIDLY DRIVE DIVERSITY IN SPINACH DOWNY MILDEW

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Text

Spinach (*Spinacia oleraceae*) downy mildew (SDM), caused by the oomycete pathogen *Peronospora effusa*, is the most important disease of spinach worldwide. Breeding resistant spinach cultivars is a critical management strategy. Race typing of SDM has been an important approach for tracking the evolution of the pathogen. Our objective was to investigate plasticity of the whole genome under controlled and field conditions for multiple race types. Genomic DNA was extracted from putative single lesions and PCR-free libraries were sequenced using Illumina HiSeqX in a 2x150bp configuration. Data were processed and heterozygous allele frequencies estimated using BWA and GATK as well as CLC Genomics Workbench. Heterozygous allele frequencies were visualized at the chromosome level. Most heterozygous allele frequencies were inconsistent with the expectations for a diploid organism and proved unstable between isolates as well as locations in the genome. Race type did not correlate with genotype and the SDM pathosystem appears to be dynamic. The plasticity of the genome has broad implications.

NUCLEOTIDE-SUGAR BIOSYNTHESIS PATHWAY IN OOMYCETES: FROM GENOME TO FUNCTION

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Text

Oomycetes are fungi-like microorganisms and pathogenic to many plant and animal species. Our study evaluates the distribution and molecular level diversity of the nucleotide-sugar (NTS) biosynthetic enzymes within the oomycetes domain. The NTS are essential polymerizing units of carbohydrates and their conjugates in all living organisms. A comprehensive phylogenetic analysis and metabolic pathway reconstruction were performed based on the sequence-structure-functional analysis and genome mining approaches. The phylogenetic result shows that a total of 24 unique NTS-related enzymes producing different types of nucleotide-sugar units are present within oomycetes. The overall phylogenetic analysis demonstrated that each clade represents a particular enzyme family. Moreover, sequence diversity was found between the members of each clade with sequence identities from 25-99.99 %. Here we postulate that one clade can represent one enzyme family associated with a particular function. The paraphyletic clade in the phylogenetic tree indicates the presence of either isoform or species/strain-specific enzyme. The complete NTS pathway reconstruction provides detailed information about the presence and absence of NTS enzymes or an alternate route for synthesizing activated sugar nucleotide units. This information will help to understand the NTS enzymes and their role in the composition of the oomycetes cell wall, which can further assist in developing anti-oomycetes agents.

PHASE-SPECIFIC TRANSCRIPTIONAL PATTERNS OF THE OOMYCETE PATHOGEN PHYTOPHTHORA SOJAE UNRAVEL GENES ESSENTIAL FOR ASEXUAL DEVELOPMENT AND PATHOGENIC PROCESSES

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Text

Oomycetes are filamentous microorganisms easily mistaken as fungi but vastly differ in physiology, biochemistry, and genetics. This commonly-held misconception lead to a reduced effectiveness by using conventional fungicides to control oomycetes, thus it demands the identification of novel functional genes as target for precisely design oomycetes-specific microbicide. The present study initially analyzed the available transcriptome data of Phytophthora sojae and constructed an expression matrix of 10,953 genes across the stages of asexual development and host infection. Hierarchical clustering, specificity, and diversity analyses revealed a more pronounced transcriptional plasticity during the stages of asexual development than that in host infection, which drew our attention by particularly focusing on transcripts in asexual development stage to eventually clustered them into 6 phase-specific expression modules. Three of which respectively possessing a serine/threonine phosphatase expressed during the mycelial and sporangium stages, a histidine kinase expressed during the zoospore and cyst stages, and a bZIP transcription factor exclusive to the cyst germination stage were selected for down-stream functional validation. In this way, we demonstrated that PP2C, HK, and bZIP32 play

significant roles in P. sojae asexual development and virulence. Thus, these findings provide a foundation for further gene functional annotation in oomycetes and crop disease management.

SCREENING OF ALFALFA VARIETIES RESISTANT TO PHYTOPHTHORA CACTORUM AND RELATED RESISTANCE MECHANISM

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Text

Alfalfa is one of the most important legume forages in the world. Root rot caused by soil-borne pathogens severely restricts the production of alfalfa. The knowledge of the interaction between alfalfa and root rot-pathogens is still lacking in China. Phytophthora cactorum was isolated from symptomatic seedlings of an alfalfa field in Nanjing with high levels of damping-off. We observed the different infection stages of P. cactorum on alfalfa, and found that the purified P. cactorum strain was aggressive in causing alfalfa seed and root rot. By evaluating the resistance of 37 alfalfa cultivars from different countries to P. cactorum, we found Weston is a resistant variety, while Longdong is a susceptible variety. We further compared the activities of various enzymes in the plant antioxidant enzyme system between Weston and Longdong during P. cactorum infection, as well as gene expression associated with plant hormone biosynthesis and response pathways. The results showed that the disease-resistant variety Weston has stronger antioxidant enzyme activity and high levels of SA-responsive PR genes, when compared to the susceptible variety Longdong. These findings highlighted the process of interaction between P. cactorum and alfalfa, as well as the mechanism of alfalfa resistance to P. cactorum, which provides an important foundation for breeding resistant alfalfa varieties, as well as managing Phytophthora-caused alfalfa root rot.

PECTIN METHYLESTERASES INHIBITOR MODULATE PLANT HOMOGALACTURONAN STATUS IN DEFENSES AGAINST THE PHYTOPHTHORA SOJAE

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Text

Pectin Methylesterases Inhibitor Modulate Plant Homogalacturonan Status in Defenses against the *Phytophthora sojae*

Hosts and pathogens are engaged in a continuous struggle for physiological dominance that drives the evolution and specialization of key defense and virulence proteins. A major site on the struggle is the plant cell wall. Here, we show the involvement of the dynamic remodeling pectin methylesterification of cell wall in the co-evolutionary struggle between host and microbe. Pathogen-secreted apoplastic pectin methylesterases, PsPME1, that loosening the plant cell wall and synergizing the activity of pathogen secreted endo-polygalacturonases by decreased the degree of pectin methylesterification. However, GmPMEI, a soybean

produced pectin methylesterases inhibitor protein, expression controlled by PME-related damage-associated molecular patterns that binds to both soybean and P. sojae pectin methylesterases and inhibits them enzyme activity to remodeling the pectin to high methylesterification status for protecting themselves from enzymatic degradation. Totally, our work highlights that plants exploit induced defense mechanisms based on biochemical modification on the cell wall in shaping the balance of the arms race in the co-evolutionary conflict between host and microbe.

THE SOYBEAN (GLYCINE MAX) LYSM RECEPTOR KINASES GMNFR5A AND GMCERK1 MEDIATE CHITOOLIGOSACCHARIDES-TRIGGERED IMMUNITY

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Text
Chitin is a major component of fungal cell walls and serves as a molecular pattern for the recognition of potential pathogens in the innate immune systems of plants. Previous research suggested that chitin has different immune signaling pathways in Arabidopsis and rice, including extracellular receptor recognition and intracellular signal transduction. The mechanism of induced resistance of chitin oligosaccharide (COSNAC) and its deacetylated product chitosan oligosaccharide (COS), collectively referred to as chitooligosaccharides, is not clear in soybean. Herein, we report that chitooligosaccharides trigger immune responses and plant disease resistance in soybean. GmNRF5a and GmCERK1 are required for chitooligosaccharides recognition in soybean. Unexpectedly, COSNAC is directly recognized by GmNFR5a and GmCERK1, whereas COS only binds GmNFR5a. In addition, we confirmed that GmCERK1 and GmRLCK5 transduce intracellular signals of chitooligosaccharides through proteins interaction and phosphorylation. Taken together, our results suggest GmNFR5a and GmCERK1 play a key role in the perception of chitooligosaccharides elicitors, and the existence of a complete phospho-signaling transduction pathway from GmNFR5a and GmCERK1 mediated chitooligosaccharides recognition to GmRLCK5 activation in soybean.

ABPP-MS METHOD IDENTIFIES ORIGINAL MODULAR EXTRACELLULAR PROTEASES FROM A. EUTEICHES IN PEA APOPLAST DURING INFECTION

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Text
In the field *A. euteiches* causes the root rot disease of legume crops as pea and alfalfa and interacts in the lab with the legume model *Medicago truncatula.* During infection, *A.*

euteiches secrete myriad of proteins to both combat the host plant defense mechanisms and to survive in adverse environmental conditions [1]. Microbial proteases are predicted to be crucial components of these systems. Here, we identified an overrepresentation of tandemly repeated proteases within *A. euteiches* genome, which are upregulated during host infection. We developed an Activity Based Protein Profiling and mass spectrometry (ABPP-MS) approach [2,3] on apoplastic fluids isolated from pea roots infected by the pathogen. We characterized 35 active extracellular microbial proteases, which represents around 30% of the genes expressed encoding serine and cysteine proteases during infection. Notably, eight of the detected active secreted proteases carry an additional C-terminal domain [4]. This work demonstrates ABPP-MS as an efficient tool to quickly substantiate genomics prediction of oomycete pathogenicity factors. This system can be easily translated to other pathosystems and will facilitate the selection of microbial candidate genes for functional analysis.

[1] Camborde et al., New Phytol 2022.
[2] Morimoto and van der Hoorn, Plant Cell Physiol. 2016.
[3] Kaschani, et al., 2009. Molecular & Cellular Proteomics 2009.
[4] Kiselev et al., Frontiers in Plant Science, 2023. In press

PHYTOPHTHORA DIX DOMAIN-CONTAINING PROTEINS: AN ENERGY DISTRIBUTION PLATFORM?

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Text

In animals, the DIshevelled and aXin (DIX) domain favours the aggregation of essential regulators of the Wnt-beta-catenin signalling cascade. DIX domains also occur in SOSEKI proteins that localize to specific corners of plant cells. Interestingly, oomycete genomes encode DIX-containing proteins with unique combinations of functional domains, suggesting their role differs from those identified in animals and plants. Using the cacao killer *Phytophthora palmivora* as a model system, we investigate the contribution of DIX domain-containing proteins in oomycete plant pathogens. *In vivo* imaging and interaction studies suggest these proteins contribute to energy distribution in hyphae. Our work aims to exploit oomycete genetic specificities to provide inroads for crop protection against this class of plant pathogens.

A LAB-ON-A-CHIP DEVICE TO STUDY THE GROWTH OF OOMYCETES IN O2 GRADIENTS

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Text
Diseases caused by oomycetes impact ecosystems with loss of biodiversity, and in the agricultural, horticultural, forestry and aquaculture sectors cause huge economic losses. It is

thus important to understand how they grow and infect their hosts. The energy required for growth and infection is presumed to come from oxidative respiration, despite the fact that the infection structures and hyphae may be exposed to oxygen concentrations as low as 1 – 2% in and around the host. Pathogenic fungi, which utilize similar infective strategies, have been shown to sense and adapt to these hypoxic conditions, significantly altering their gene expression. As far as we are aware, there are as yet no reports of how oomycetes respond to differing oxygen concentrations. With a view to investigating this, we are developing oxygen sensor Lab-on-a-Chip (LOC) devices that expose oomycetes to oxygen gradients. Made from gas-permeable polydimethylsiloxan (PDMS), the devices comprise a central channel along which hyphae can grow and two side channels, one on each side of the central channel. These are filled with oxygen or nitrogen that diffuses through the PDMS. This creates an oxygen gradient in the central channel that can be measured with the hypoxia-sensing dye Pt(II) meso-tetrakis(pentafluorophenyl)porphine (PtTFPP) which is embedded in PDMS. I will describe experiments using these devices and the tropic responses of several oomycete species, including Phytophthora and Achlya.

PERFORMANCE OF COMMERCIAL VARIETIES AGAINST PHYTOPHTHORA SOJAE

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Text

Phytophthora stem and root rot of soybean, caused by Phytophthora sojae, is mainly managed with single or stacked qualitative disease resistance genes. As pathotype complexity of the population increases, management through the tolerance of the commercial varieties would be advisable. The goal of this study was to evaluate the response of commercial genotypes against the pathogen. We used the hypocotyl inoculation and infected rice techniques. Six commercial genotypes were used together with “Sloan” variety as susceptible control, and 3 pathotypes of P. sojae (which differed in virulence on 1 to 6 Rps genes and are the most representative on the pampeana region). In the first technique, the response of the genotypes was identified as susceptible (70 % or more seedlings killed) or resistant (30% or less seedlings killed). The infected rice technique was carried out in a Randomized Design with 3 repetitions per treatment. The total length of the roots of the surviving plants in the pots was evaluated 21 days after sowing. The data obtained were subjected to ANAVA and Fisher's LSD test ($p<0.05$). The variety identified as commercial 4 is the only one that presented significant differences compared to the control and the rest of the varieties. It was classified as resistant and reached 316% more growth in length. We conclude that it could be possible to use this variety as a tolerant control for future field resistance trials against Phytophthora sojae in Argentina.

GENOME-WIDE ASSOCIATION STUDIES IDENTIFY THE OOMYCETE MATING-TYPE LOCUS SEQUENCE AND AVIRULENCE CANDIDATE GENES IN GRAPEVINE DOWNY MILDEW (PLASMOPARA VITICOLA)

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Text
The availability of high-quality reference assemblies for oomycetes enables genome-wide association studies (GWAS) to link genotype and phenotype variation, identifying the genomic architecture of pathogens' life-history traits. In P. viticola, mating can occur only between individuals of different mating types (heterothallism). Using GWAS, we identified a genomic region of 570 kb associated with the mating-type phenotype (Dussert et al. 2020). P2 individuals were homozygous for the MAT-a allele at the mating-type locus, whereas P1 individuals were heterozygous, carrying the MAT-a and MAT-b alleles. The mating-type region features a gene that encodes a transmembrane protein that might act as a hormone receptor; this is noteworthy since hormones have previously been identified as mating-type factors in Phytophthora spp. Our subsequent research delved into the genomic factors that drive the breakdown of grapevine's partial resistance to downy mildew, specifically focusing on Rpv3. Using GWAS, we discovered a distinct structural variation exclusively present in the genomes of strains that are virulent on grapevines carrying the Rpv3 locus. The structural variation consisted of in a deletion of 30 kb encompassing two closely-related genes that encode proteins of 800-900 amino acids with a signal peptide. The predicted structures of both proteins contain repeats that form structural elements typical of the LWY-fold, a conserved structural module in oomycete effectors.

TWO INDEPENDENT CLEAVAGE EVENTS ARE INVOLVED IN RXLR-EER EFFECTOR PROCESSING

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Text
Phytophthora plant pathogens are a serious and continuing threat to agriculture and the natural environment. Secreted effectors are critical to their infection success and the translocated RXLR class are a key set of these. We have shown that RXLR effectors are secreted from *Phytophthora* in a non-conventional fashion, despite having typical signal peptides. We have been investigating what this secretory pathway involves. It was shown, first with Avr3a, and subsequently for other RXLRs, that there is cleavage at the RXLR motif during secretion from the pathogen. Using a range of RXLR effector variants and mutations we show consistent cleavage at the RXLR, including at some degenerated motifs. Furthermore, we reveal that the EER motif represents a second cleavage site. We propose a model for how the RXLR motif may function in the secretory pathway selection.
Session 3

TARGETED CRISPR-CAS9-BASED GENE KNOCKOUTS IN APHANOMYCES EUTEICHES

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Text

Oomycete genome editing using CRISPR/Cas9 system represents the most promising and powerful technique to study genes involved in pathogenicity such as effectors. However, most of the reported successful studies have been obtained on *Phytophthora sp*. using polyethylene glycol-mediated protoplast transformation to insert Cas9 DNA plasmids for gene disruption. This method presents some disadvantages. Firstly, PEG-mediated transformation protocol needs strain adaptation and includes difficulty in obtaining high concentrations of viable protoplasts, high transformation efficiency or stable transformants. Secondly, the random integration of foreign DNA can lead to undesired gene disruption or reduced transcription rates. Furthermore, the transgene overexpression of Cas9 can result in off-target cleavage or toxicity. Here, we propose to circumvent these problems by adapting a protocol previously described on brown algae. By combining microprojectile bombardment for delivery of ribonucleoprotein complexes, we successfully transformed for the first time *Aphanomyces euteiches*, the root rot pathogen of legumes. We report that mutations at specific target sites are generated following the introduction of CRISPR-Cas9 ribonucleoproteins into *A. euteiches* cells. By transposing the positive selection system described for algae, we next propose a double mutation approach on a selected effector gene. Potentially, this method should be readily transferable to other oomycete species.

MITOCHONDRIAL GENOMICS – A SYSTEMATIC APPROACH FOR PHYLOGENETICS, TAXONOMY AND DEVELOPMENT OF DIAGNOSTIC MARKERS

MARTIN Frank. (1)

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Text

A database of approximately 800 oomycete mitochondrial genomes representing 260 species in 19 genera has been assembled and used in a systematic approach for phylogenetic studies, design of a new barcode locus for species identification and development of diagnostic assays. Extracting the same gene from a broad range of taxa helps identify the most useful loci for phylogenetic analysis; the ability to examine flanking regions from a range of taxa for genes of interest also helps in the design of conserved amplification primers. Comparison of gene order differences facilitated development of a new barcode locus for species identification. In oomycetes the *rps10* gene is flanked by a unique order of tRNAs that enabled design of conserved amplification primers. Since this gene order is not observed in plants or Eumycotan fungi there are low levels of nonspecific amplification from environmental samples. Comparison of *rps10* with *cox1* sequences from the same isolates indicate an equal level of discrimination among taxa. The Grunwald lab developed a metabarcoding approach for oomycetes using this locus (Phytobiomes 6:214-226). Unique gene order differences among genera have also proved useful for design of diagnostic assays for *Phytophthora, Plasmopara, Aphanomyces* and current work with *Pythium* spp. For some taxa the identification of unique putative open reading frames by BLAST analysis of the entire database were useful for development of diagnostic assays.

AN AGO PROTEIN IS REQUIRED FOR AVIRULENCE GENE SILENCING IN AN OOMYCETE PLANT PATHOGEN

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Text

Successful pathogens can rapidly overcome host resistance through epigenetic silencing, but the underlying mechanisms of epigenetic variation are largely elusive. Based on genome-wide association study, we identified a natural allele of an Argonaute protein in Phytophthora sojae that confers adaptability to resistance soybean cultivar. Knockout of PsAGO2 impaired avirulence gene Avr1b silencing and the psago2 mutants were recognized by soybean cultivar carrying Rps1b. Further data revealed that PsAGO2 can bind 24-26 nt sRNAs and recruit the histone methyltransferase complex PRC2 to establish H3K27me3 at Avr1b loci. Our finding supports a model in which H3K27me3 formation is mediated by sRNA in oomycete, highlighting the role of a new function of AGO protein in epigenetic gene silencing in a plant pathogen.

XEG1: A CASE STUDY OF MICROBIAL ATTACK AND PLANT IMMUNITY IN THE APOPLAST

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Text

The apoplast constitutes a major interaction niche in plant-microbe interactions. During infection, microbial pathogens secrete a large repertoire of effectors that act in the apoplast to modulate host conditions for infection. Plants respond to microbial attack via perception of conserved molecular patterns or apoplastic effectors using cell surface immune receptors to mount defense. The apoplastic effector XEG1 is a glycoside hydrolase 12 protein secreted by the soybean root rot pathogen Phytophthora sojae. XEG1 displays hydrolase activity toward xyloglucans and essential for Phytophthora infection. As a countermeasure, soybean secretes the inhibitor GmGIP1, which binds directly to XEG1 and inhibits its hydrolase activity, to increase soybean resistance. P. sojae secretes a paralogous XEG1-like protein, XLP1, with no enzyme activity. XLP1 binds GmGIP1 more tightly than XEG1, and acts as a decoy protecting XEG1 from the inhibitor GmGIP1. XEG1 is degraded by host aspartic protease GmAP5 in the apoplast. However, XEG1 undergoes N-glycosylation, which protects XEG1 from GmAP5 degradation. In addition, XEG1 can be recognized by a plant membrane-localized receptor-like protein RXEG1 to mount defense. Structural analyses revealed that RXEG1 inhibits the hydrolase activity of XEG1 and plays a dual immunogenic role in plant defense. Together, these studies revealed that co-evolutionary arms race tailored the multi-layered defense and counter-defense in plant-microbe interactions.

DETECTION AND MANAGEMENT OF APHANOMYCES ROOT ROT OF SUGAR BEET

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Text

Aphanomyces cochlioides is the causal agent of seedling damping-off and Aphanomyces root rot (ARR) of sugar beet. The pathogen can persist in the soil as dormant oospores. It is essential for the growers to know the risk of Aphanomyces prior to planting so that they can develop a suitable disease management approach to mitigate yield losses. Aphanomyces root rot can look very similar to root rots and seedling diseases caused by other fungi and oomycetes, so developing a diagnostic assay would be of great value to the sugar beet industry. A specific quantitative PCR (qPCR) assay targeting mitochondrial DNA was developed to detect and quantify *A. cochlioides* DNA in infested field soils and infected sugar beet samples. The assay has a detention limit of 0.1 pg of pathogen DNA and was able to detect A. cochlioides in naturally infected sugar beet root samples. Currently seed treatments offer protection during the first few weeks after planting. However, if the soil moisture remains high later in the season, significant yield losses can occur due to chronic root rot. Precipitated calcium carbonate (PCC), a byproduct of the sugar beet factories is very effective in protecting plant stands and reducing ARR severity up to 12 years after a single application. Use of PCC is widely adopted by the growers in Minnesota and North Dakota. A strong positive correlation between soil extractable calcium (SEC) and root yield suggesting that calcium is playing a vital role in reducing ARR.

SERENDIPITOUS OBSERVATION LED TO PRACTICE OF USING PRECIPITATED CALCIUM CARBONATE IN CONTROLLING APHANOMYCES COCHLIOIDES IN SUGAR BEET

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Text

Sugar beet (*Beta vulgaris* L.) major crop worldwide for sugar production. Minnesota and North Dakota, adjoining states in the US produce 57% of the US sugar beet production that results in $5 billion in total economic activity. One of the major limiting factors for sugar beet production is *Aphanomyes cochlioides* that causes seedling damping off and Aphanomyces root rot. Tachigaren/hymexazol as a seed treatment is the only fungicide labeled for and widely used on sugar beet for protecting seedlings from *A. cochlioides*. In the 2003, research conducted in Minnesota to determine whether precipitated calcium carbonate (CaCo3) could help to reduce the time that sugar beet could be grown in fields with carryover herbicide showed that the CaCo3 reduced the impact of root with symptoms of Aphanomyces root rot. Further research using 11 to 44 tonnes per hectare of precipitated CaCo3 significantly reduced damage caused by *A. cochlioides.* As a result, sugar beet growers in the USA with fields identified with A. cochliodes have used the practice of applying 7 to 16 tonnes per hectare of CaCo3 before planting sugar beet for a high quality crop. Research further showed that the CaCo3 provided protection from *A. cochlioides* for over 12 years.

CONSERVATION AND DIVERGENCE OF RAR1-MEDIATED NONHOST RESISTANCE DURING LAND PLANT EVOLUTION

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Text
Session 4
Over 450 million years of co-evolution, plants and pathogens have developed sophisticated strategies to manipulate one another. In plants, nonhost resistance (NHR) describes a collection of molecular and cellular mechanisms that neutralize non-adapted pathogens. Although NHR has been extensively studied in angiosperms, the origin and evolution of NHR in land plants is largely unknown. Here, we demonstrate the conservation of a NHR mechanism mediated by the RAR1-SGT1-HSP90 chaperone complex and identify lineage-specific divergence in terrestrial ferns. The RAR1-SGT1 interaction is highly conserved among lineages and even occurs between distantly related ortholog pairs. Intriguingly, we identified a single exception in the C-fern, whose homologs were incapable of interactions outside of its lineage. We hypothesize that lineage-specific differences in the SGT1-interacting CHORD2 domain determines this specificity, which is supported by protein-modeling studies. To determine a role for RAR1-mediated NHR in divergent lineages, we generated a liverwort (*Marchantia polymorpha*) *Mprar1* mutant and screened it against 28 diverse *Phytophthora* isolates. Excitingly, the *Mprar1* mutant exhibited significant defects in NHR to candidate pathogens that we are now examining in more detail. Overall, our findings suggest that the core mechanism of RAR1-mediated NHR is conserved in land plants, while plant lineages have fine-tuned the system during their evolutionary histories.

POPULATION STRUCTURE OF CACAO PATHOGEN PHYTOPHTHORA MEGAKARYA

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Text
Phytophthora megakarya is an aggressive and extremely destructive pathogen that causes black pod disease of cacao, significantly limiting yield in the world's leading cacao producing region in West and Central Africa. To effectively use genetic breeding to improve cacao resistance to black pod disease, the genetic diversity of both host and pathogen populations must be considered. We examined genetic diversity and population structure of *P. megakarya* using genomic data from 166 isolates collected from Cameroon, Nigeria, and Ghana. We used reads from genotyping by sequencing of 150 isolates and from published whole genome sequences of 15 isolates to call 2,644 high quality SNPs relative to the reference genome Pm1/GH34 from Ghana. Isolates could be assigned to one of two major clades. One clade contained isolates from Nigeria and Ghana and the other contained isolates collected in all three countries. The two major clades showed differing degrees of genetic variation among isolates and heterozygosity of SNPs. Genomic data will be integrated with isolate phenotypes determined using experimental inoculations of cacao pods to evaluate variation in genetic determinants of virulence in *P. megakarya*.

POTENTIAL ANTI-PYTHIUM INSIDIOSUM THERAPEUTICS IDENTIFIED THROUGH SCREENING OF AGRICULTURAL FUNGICIDES

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Text

Pythiosis is a life-threatening infectious disease of humans and animals caused by the oomycete microorganism Pythium insidiosum. Clinical manifestations of pythiosis include blood vessel, eye, skin, or gastrointestinal tract infections. According to geographical distribution analysis, pythiosis has been increasingly reported in 23 countries across the world, with an overall mortality rate of 28%. Pythiosis is treated with a combination of surgery, immunotherapy, and antimicrobial drugs. Radical surgery, which usually results in a handicap, is often required to save patients' lives due to the limited efficacy of conventional antimicrobial drugs. Immunotherapy could reduce the need for surgeries and improve recovery rates in a few cases. However, new and effective medical treatments are urgently needed for pythiosis. This study aims to find potential anti-P. insidiosum agents by screening 17 agricultural fungicides that inhibit plant-pathogenic oomycetes and validating their efficacy and safety. We found that cyazofamid, fenamidone, and fluopicolide could effectively inhibit P. insidiosum and showed relatively low toxicity to human cells. Cyazofamid was the most promising chemical with the highest calculated therapeutic ratio, and its mode of action may involve binding cytochrome c reductase of P. insidiosum. In conclusion, pythiosis still lacks effective treatment. This study demonstrates some agricultural fungicides that could be repurposed for treating pythiosis.

KIWIFRUIT VINE DECLINE SYNDROME: ARE WE CLOSING THE CIRCLE?

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Text

The Kiwifruit Vine Decline Syndrome (KVDS) is a disease that severely affects the root system of kiwifruit plants, causing their sudden collapse. While the causes behind KVDS are still being debated, previous work shows that this syndrome has a biotic origin, and it is potentially induced by oomycetes. In this context, our work focused on clarifying causes and mechanisms behind KVDS. Our field surveys identified three major sites where we isolated several oomycetes identified as Phytophtora spp., Pythium spp., and Phytopythium spp. Amplicon metagenomics focused on bacterial, fungal, and oomycete communities show marginal differences in the diversity and structure of microbial communities between

symptomatic and asymptomatic plants. However, more detailed analyses showed a clear presence of Phytopythium sp. only in presence of KVDS symptoms. Furthermore, Phytopythium vexans was the most frequently isolated pathogen using a baiting approach. Then, we performed the high-throughput isolation of potential biocontrol agents, which yielded a pool of bacterial isolates with a strong antagonistic activity against representative isolates of Phytophtora sp., Pythium sp. and Phytopythium sp.. Collectively, our results strongly support the biotic origin of KVDS, potentially caused by Phytopythium spp. through a complex interaction with the environment, the plant, and the plant microbiome, building up the base for sustainable control strategies.

INVESTIGATION OF THE ROLE IN VIRULENCE OF PHYTOPHTHORA INFESTANS EFFECTOR PI06099

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Text
Plant pathogens secrete many effector proteins which are translocated inside plant cells and act to suppress host defences and promote pathogen colonisation. The RxLR effector Pi06099 from potato late blight pathogen *Phytophthora infestans* interacts with the plant red light receptor Phytochrome B (PhyB). Red light promotes plant immunity by accelerating cell death in response to the *P. infestans* MAMP INF1. Silencing the *Pi06099* effector using Host Induced Gene Silencing (HIGS) and stable RNAi transgenic lines demonstrated that it contributes to the virulence of *P. infestans* on Potato and *Nicotiana benthamiana*. Furthermore, domain swapping and mutagenesis of Pi06099 has been used to investigate the disruption of the interaction with PhyB and phenotypes associated with effector virulence.

DISCOVERY OF PROTEIN MARKERS OF OOMYCETE EV'S

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Text
Session 1: Cell Biology, Signaling and Metabolism

The movement of effector proteins and RNAs from pathogen into host cells during infection is known to occur. However, the exact mechanisms facilitating this movement are still being widely studied. One possible delivery route involves the secretion and uptake of extracellular vesicles (EVs) between organisms.

In this study we isolated EVs from the oomycete Phytophthora infestans, cause of potato late blight, with the aim of identifying oomycete-associated EV markers and investigating the cargo of these bodies. This is being achieved by a proteomics approach to identify both

secreted and vesicular proteins during in vitro growth. We have identified some known EV proteins found widely in EV proteomes, supporting our methodology and approach. Additionally, we have identified some oomycete-specific proteins that have as yet unknown functions but appear to be transmembrane proteins, including TMP1 (Trans-Membrane Protein 1). TMP1 accumulates in the same density fraction in sucrose gradients as the RXLR effector protein, PITG_04314, during EV isolation implying they could be associated with the same EV. The overall aim of this work is to find markers of EVs that we can use to determine how these EVs are secreted and taken up into the plant cell and whether this is a mode of transport for pathogenicity factors such as RXLR effectors.

NEW APPROACHES TO EXPAND OUR UNDERSTANDING OF CRYPTIC OOMYCETE ELICITIN PROTEINS

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Text

Phytophthora cinnamomi, one of the most devastating plant pathogens, poses a serious threat to the biodiversity and has a host range of almost 5000 plant species. Belonging to the class Oomycetes, *P. cinnamomi* is a sterol auxotroph and relies on external sterol sources for completion of its life cycle. Our recent review paper on elicitins of *Phytophthora* outlined the importance of these highly conserved proteins in recruiting sterols and providing it to the pathogen, however, the process is largely unknown. Research on the dependency of *P. cinnamomi* on plants for sterols, the process following sterol recruitment and thereafter, sterol signalling, will help us better understand the importance of sterols for oomycete survival. Thus, through a targeted metabolomics technique, we explored the dynamics of sterol acquisition by *P. cinnamomi*. We have also begun to explore gene editing which, in a limited *Phytophthora* species has assisted in gene functionalisation. Thus, to expand the possibilities of gene editing in *P. cinnamomi*, we have optimized a protoplast isolation process from the multinucleated hyphae of this pathogen. To understand the cell biology of the protoplasts, we utilized various vital stains to visualize internal structures, along with fluorescent dye to quantify viable protoplasts. These methodologies provide an opportunity to establish CRISPR/Cas-mediated gene editing of elicitins in *P. cinnamomi* to expand our fundamental knowledge of these proteins.

IDENTIFYING PROTEIN-PROTEIN INTERACTIONS WITH TURBOID IN PHYTOPHTHORA INFESTANS

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Text

Determining how proteins function and interact with one another is a key step in understanding how cellular systems function. There are a variety of methods for determining protein-protein interactions, such as co-immunoprecipitation, yeast-2-hybrid, and proximity labelling methods. One of the most recent developments is TurboID, a proximity labelling

method that uses a high efficiency biotin ligase to tag interacting or nearby proteins with biotin, followed by streptavidin purification and mass spectrometry. We have adapted TurboID for use in the potato late blight pathogen, Phytophthora infestans, with the aim of identifying proteins interacting with effectors as they are secreted. We have generated transgenic lines of P. infestans expressing effector-TurboID fusions. These have been assessed for correct protein localisation, conditions for biotin labelling, and protein purification. Progress towards identifying proteins involved in secretory pathways will be presented.

RE-EMERGENCE OF THE POTATO LATE BLIGHT THREAT IN EUROPE DRIVEN BY AN EVOLVING POPULATION OF PHYTOPHTHORA INFESTANS

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Text

Successful integrated pest management (IPM) is dependent on knowledge of the pathogen, its host and the environment and how each influences management practices. Late blight, caused by Phytophthora infestans, continues to threaten potato and tomato crops on a global scale. An evolving pathogen population and increasingly rigorous regulations on chemical use are driving a transition from prophylactic fungicide use to more integrated approaches combining host resistance, knowledge of pathogen spread and infection risk with smarter use of fungicides and biological products. The evolving population of P. infestans prompted the EuroBlight consortium to collect data on the diversity of P. infestans, analysed with simple sequence repeat genetic markers. Surveys of late blight infected crops by many collaborators from 2013–2022 has resulted in over 16 thousand genotyped samples from across Europe held in an isolate database with associated analysis tools and a mapping interface. The population is dominated by relatively few clonal lineages that we have tracked over time and space (www.euroblight.net). We have identified traits such as fungicide resistance that drive the emergence, evolution and spread of some clones and share the knowledge with the industry to tailor IPM practices. In contrast to the clones, 20-30% of the European population comprises genetically diverse strains consistent with oospore-derived sexual populations and their evolving traits are challenging to predict.

COMPARATIVE GENOMICS OF EUROPEAN APHANOMYCES EUTEICHES STRAINS

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Text
The plant pathogen Aphanomyces euteiches causes root rot in its broad host range of various legume species. The homothallic oomycete is known for its low genetic diversity due to predominant clonal reproduction. In a recent study using short sequence repeat markers, three genetically distinct groups were identified in pea-infecting A. euteiches strains from Europe. A central European population differed significantly from genetically distinct groups comprising strains from the most northern (eastern Sweden and Finland) and most southern (Italy) sampling regions. From this strain collection, 69 strains representing the three groups were genome sequenced and the genomes assembled and annotated. Initially, a genealogical concordance phylogenetic species recognition analysis will be performed to establish the species status of the northern and southern groups. We will further perform a comparative genomics analysis of A. euteiches with focus on gene content and gene family evolution. Effectors, CAZymes and proteases have been shown to play important roles in oomycete virulence, and these gene families will be characterised in detail. Further, the data set offers the possibility for scanning the genomes for signatures of positive selection using selective sweep analysis. This project will improve our understanding of the genetics underlying diversity and virulence in European A. euteiches populations. The annotated genomes will be made available through AphanoDB

EVOLUTION OF LWY EFFECTOR REPERTOIRE IN PHYTOPHTHORA

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Text
Effectors are deployed to manipulate host cellular processes and promote infection by pathogens. Many *Phytophthora* effectors consist of tandem repeats of (L)WY motifs, each containing a conserved 3-5 α-helical bundle. Some of these (L)WY effectors have the WY1-(LWY)n arrangement in which neighboring (L)WY-LWY units are concatenated by a conserved linkage, resulting in an overall non-globular shape. Despite the structural conservation, the (L)WY units show divergence in surface-exposed residues, leading to the hypothesis that the shuffling of (L)WY units may contribute to the diversification of *Phytophthora* effector repertoire. To investigate potential (L)WY shuffling, we developed a bioinformatic pipeline, which identified 74-155 LWY sequences from five *Phytophthora* genomes. Many of these genes are arranged in multi-LWY gene clusters in the genome, which may serve as hotspots for effector evolution. Interestingly, up to ~64% of the LWY genes encode putative proteins without the canonical N-terminal secretion Signal Peptide, indicating the presence of a dynamic sequence reservoir that could promote effector evolution. Comparison of sister *Phytophthora* species revealed LWY effectors as recombined products, lending support to a recombination-based mechanism that could contribute to the birth of novel virulence activities. This study offers important insight into the functional diversification of an effector repertoire driven by modular protein architecture.

A PHYTOPHTHORA INFESTANS MYB TRANSCRIPTION FACTOR INVOLVED IN SPORULATION AND HOST PENETRATION

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Text

Completion of the *Phytophthora* lifecycle involves the precise expression of thousands of genes, typically regulated by the action of transcription factors. We aimed to identify transcription factors that bound to conserved sequence motifs in the promotors of infection-regulated genes from the potato late blight pathogen, *Phytophthora infestans*. Using a motif found in the promotors of many effector coding genes, we conducted a yeast-1-hybrid screen, which identified a single *P. infestans* candidate MYB transcription factor. Silencing of this transcription factor revealed that it regulated sporulation and pathogenicity phenotypes. In particular, silenced lines were unable to penetrate intact leaves, but could colonise wounded leaves. Transcriptome analysis of silenced lines, compared to wild type, identified over 1000 differentially expressed genes, including 42 RXLR effectors and 79 carbohydrate active (CAZy) proteins. Additional transcription factors and kinases were also discovered and, when silenced, also led to loss of pathogenicity.

CONTROL OF PYTHIUM PATHOGENS IN HYDROPONIC GREENHOUSES

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Text

Hydroponic greenhouses are more efficient in their use of water than conventional soil-based systems. With the use of energy efficient light, and elevated CO2 conditions, these production systems enable the year-round production of leafy greens in northern climate zones. However, this novel environment is also ideally suited for the spread of pythium pathogens in the recirculating water. These pathogens can be introduced via air dust, contaminated water, insects, foot traffic, contaminated soil etc. In the US, Pythium pathogens have emerged as particularly economically impactful pathogens of spinach, and other leafy greens. Our research has identified a group of Pseudomonas fluorescens strains capable of contact-dependent killing of isolates obtained from greenhouse operations from California, Indiana, Ohio, and New Jersey. To identify species-specific virulence factors, we have implemented a sequential strategy of bioinformatic analysis; DNA synthesis of boundary regions containing in-frame deletions of target genes, electroporation of the vector containing gene KOs; selection of vector and assays of virulence following operon disruption by integration of vector; counter-selection for removal of vector; and finally, verification of gene deletion events and changes in gene virulence.

GENOMIC BIOSURVEILLANCE OF SUDDEN OAK DEATH PATHOGEN PHYTOPHTHORA RAMORUM REVEALS VARIANTS, HYBRIDS AND SUPER-SPREADER EVENTS

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Text

Invasive alien tree pathogens often have complex invasion histories. Understanding the source and pathways of invasion is crucial to improve prevention. Genomic biosurveillance can help untangle the invasion history. The BioSAFE project sequenced and analysed more than 500 genomes of a global collection of the pathogen responsible for the sudden oak death, the sudden larch death and Ramorum blight (*Phytophthora ramorum*). Variants within clonal lineages of P. ramorum were often geographically and/or chronologically restricted. We detected a shift in variants of the EU1 and NA2 lineages of *P. ramorum* in nurseries in British Columbia, Canada. One of the EU1 variants replaced all previous variants and spread to many nurseries, a signature of a potential super-spreader event. We also identified interlineage hybrids that are F1 progenies. They produce viable sporangia and chlamydospores and are infectious to rhododendron, a common host. Comparison of variant composition in nurseries, following treatment, revealed instances of eradication success and failure. For rapid and high-throughput biosurveillance, we have developed SODseq, a tool that generates high-throughput sequence data for 355 informative amplicons that recapitulate the patterns obtained with whole genome sequencing. This tool can be used with DNA extracted from cultures or directly from environmental samples or infected host tissues and provide useful genomic data that can inform mitigation approaches.

THE COEVOLUTIONARY RACE BETWEEN HYALOPERONOSPORA ARABIDOPSIDIS AND ARABIDOPSIS THALIANA AT A TRANSCONTINENTAL SCALE

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Text

Plants and their pathogens are locked in a perpetual coevolutionary battle for survival. We present a transcontinental investigation of coevolution in the Hyaloperonospora arabidopsidis – Arabidopsis thaliana pathosystem. We generate whole genome sequences of over 400 host-pathogen pairs from natural infections collected throughout both the native eurasian range and the human-commensal colonisation of North America, as well as new near-complete long-read genome assemblies with evidence-based annotation. We investigate the demographic history of both host and pathogen, examine coevolution both generally and of individual gene pairs, and describe variation in the genetic networks of interacting host and pathogen genes. Our results show that the negative-frequency dependent selection on both the pathogen and host genomes leads to the presence of balanced polymorphisms in the wild pathosystem, in contrast to the directional selection generally experienced by pathogens of crop pathosystems.

SYNCHROSPORA GEN. NOV., A NEW PERONOSPORACEAE GENUS WITH AERIAL LIFESTYLE FROM A NATURAL CLOUD FOREST IN PANAMA

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Text

During a survey of *Phytophthora* diversity in Panama fast growing oomycete isolates were obtained from naturally fallen leaves of an unidentified tree species in a tropical cloud forest. Phylogenetic analyses of ITS, LSU, beta–tubulin, cox1 and cox2 sequences revealed they belong to a new species of a new genus, officially described here as *Synchrospora* gen. nov., which resided as basal genus within the *Peronosporaceae*. The type species *S. medusiformis* has unique morphological characters. The sporangiophores show de-terminate growth, multifurcating at the end forming a stunted, candelabra-like apex from which multiple (8 to >100) long, curved pedicels are growing simultaneously in a medusa-like way. The caducous papillate sporangia mature and are shed synchronously. The breeding system is homothallic, hence more inbreeding than outcrossing, with smooth-walled oogonia, plerotic oospores and paragynous antheridia. Optimum and maximum temperatures for growth are 22.5 and 25–27.5 °C, consistent with its natural cloud forest habitat. It is concluded that *S. medusiformis* is adapted to a lifestyle as canopy-dwelling leaf pathogen in tropical cloud forests. More oomycete explorations in the canopies of tropical rainforests and cloud forests are needed to elucidate the diversity, host associations and ecological roles of oomycetes and, in particular, *S. medusiformis* and possibly other *Synchrospora* taxa in this as yet under-explored habitat.

SESSION 7:Taxonomy, Nomenclature, New Taxa

MICROTUBULE-MEDIATED NUCLEAR POSITIONING IN P. PALMIVORA

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Text

Oomycetes of the genus Phytophthora are filamentous plant pathogens. They are coenocytes, possessing multiple nuclei in a single cytoplasm. Like other tip-growing

organisms, proper nuclear organization is likely to be paramount in maintaining hyphal growth, a process in which the microtubule cytoskeleton is often strongly involved. Using fluorescent reporters, we investigate microtubule dynamics in relation to nuclear positioning in the broad range plant pathogen Phytophthora palmivora. We previously found clues for microtubule-mediated force generation, and now show how microtubules associated with nuclei impose strict inter-nuclear distancing. Oryzalin-mediated microtubule depolymerization shows how nuclear disorganization leads to growth deficiencies, and we explore how microtubule overlaps could provide the force required to distance nuclei.

BIOCONTROL OF POTATO LATE BLIGHT BY NATURAL COMPOUNDS FROM TRAMETES VERSICOLOR WITH POTENTIAL ANTIMICROBIAL AND BIOSTIMULANT EFFECTS

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Text

Potato late blight is a devastating disease infecting cultivated potato (*Solanum tuberosum*), caused by the oomycete *Phytophthora infestans*. The use of biocontrol agents and plant resistance inducers are sustainable and alternative ways to fungicides to contrast the disease in field. These can include microorganisms capable to contain the pathogen or compounds derived from them that act as plant defense biostimulants. In this study we tested the effect of natural compounds derived from the liquid culture of the fungus *Trametes versicolor*, known for antioxidant and antimicrobial properties, against potato late blight. For this purpose we conduced both *in vitro* and *in planta* assays in which we also tested chitosan and ß-aminobutyric acid (BABA), known respectively as antimicrobial against *P. infestans* and biostimulant on potato. In the first assay, the oomycete growth was analyzed via spectrophotometer and microscopy, whereas the *in planta* effect was investigated via gene expression and mass spectrometry. We showed that the cultural filtrate (CF) of *T. versicolor* inhibits the growth of *P. infestans* with an effect comparable to chitosan. Moreover, when sprayed on potato leaves, both CF and BABA are capable to enhance plant defense hormones. Further studies will focus on the mode of action of CF based on its chemical components (mainly polysaccharides and peptides) and the use of natural compounds are promising for small-scale field trials in order to control potato late blight.

PHYTOPHTHORA: TAXONOMY AND PHYLOGENY AND ASPECTS OF MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION BASED ON THE TYPES

ABAD Z. Gloria. (1), BURGESS Treena. (1), REDFORD Amanda. (1), BIENAPFL John. (1), BOURRET Tyler. (1), BENSCH Konstanze. (1), CACCIOLA Santa O. (1), SCANU Bruno. (1), MATHEW Reny. (1), KASIBORSKI Beth. (1), SRIVASTAVA Subodh. (1), KAGEYAMA Koji. (1), VERKLEIJ Gerard. (1), BRODERS Kirk. (1), WHITE Diane. (1), BRANDANO Andrea. (1), ALOI Francesco. (1), LA SPADA Federico. (1), RIOLO Mario. (1), SCHENA Leonardo. (1)

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Text
Phytophthora with 212 species contains many members that cause diseases of economic and environmental impact in nurseries, horticulture, agricultural and natural ecosystems; many species are of regulatory concern. This status requires the implementation of robust tools for morphological and molecular identification based on the reference specimen (type), which has been designated to represent the name for each described species. Since 2014, members of the USDA S&T Plant Pathogen Confirmatory Diagnostics Laboratory and Pest Identification Technology Laboratory have been collaborating with national and international experts to implement robust systems for species identification. In September 2019, the "*IDphy*: morphological and molecular identification based on the types" international resource with Lucid and Tabular Keys was launched online to cover the 161 species described until 2018 and in 2021 the *IDphy* app with Lucid key for morphological characterization. In 2023, we are working on *IDphy* version 2 to cover the 212 species described, and the innovative molecular toolbox with seven genes, and databases for Sanger sequencing and metabarcoding high-throughput sequencing technologies. In addition, a manuscript for the Revision of the Taxonomy and Phylogeny of *Phytophthora* based on the types is in progress and implemented to operate in conjunction with *IDphy* V2. We expect that these resources will be of great benefit for the international community working with *Phytophthora*.

WORLDWIDE FOREST SURVEYS REVEAL FORTY NEW PHYTOPHTHORA CLADE 2 SPECIES WITH FUNDAMENTAL IMPLICATIONS FOR PHYTOPHTHORA BIODIVERSITY, BIOGEOGRAPHY AND EVOLUTION AND GLOBAL FOREST BIOSECURITY

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Text

During surveys of global *Phytophthora* diversity 40 new species were detected mainly in natural forests and streams in Europe, Asia and the Americas and assigned to the five subclades of *Phytophthora* Clade 2 based on a multigene phylogeny of nine nuclear and three mitochondrial gene regions. The evolutionary history of the Clade appears to have involved the pre-Gondwanan divergence of three extant subclades, 2c, 2d and 2e, all having disjunct natural distributions on separate continents and comprising species with a soilborne and aquatic lifestyle and a few partially aerial species in Clade 2c; and the post-Gondwanan evolution of subclades 2a and 2b in South-/East Asia and South America, respectively. Clade 2b comprises soil inhabiting and aerial species whereas Clade 2a has evolved further towards an aerial lifestyle comprising only species which are predominantly or partially airborne. The 74 described Clade 2 species result from both allopatric non-adaptive and sympatric adaptive radiations. They represent most morphological and physiological characters, breeding systems, lifestyles and forms of host specialism found in the genus *Phytophthora* demonstrating the strong biological cohesiveness of the genus. The finding of 40 previously unknown species from a single *Phytophthora* clade highlights a critical lack of information on the scale of the unknown pathogen threats to forests demonstrating the anachronism of plant biosecurity protocols based on lists of named organisms.

SEVERE DIEBACK AND MORTALITY OF WILD OLIVE TREES ASSOCIATED WITH PHYTOPHTHORA SPECIES IN ITALY

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Text

Wild olive (*Olea europaea* var. *sylvestris*) is a drought-tolerant and disease-resistant tree, often used as rootstock on which cultivated olive varieties are grafted. However, since 2022, extensive dieback and mortality of wild olives trees have been reported in an area of ca 4000 ha in central Sardinia (Italy). Infected trees showed severe leaf chlorosis, wilting or

defoliation often associated with root rot and necrotic lesions on the feeder roots. In the investigated areas, estimated tree mortality and disease incidence were 60 and 80%, respectively. Rhizosphere soil was sampled from over hundred unhealthy trees and subjected to the baiting technique, using fresh Ceratonia siliqua leaves as baits and SMA as *Phytophthora* selective medium. The isolates obtained were identified by analyses of their morphological traits and sequences of ITS and cox1 genes. Five homothallic *Phytophthora* species from Clades 2 and 6 were consistently isolated from symptomatic trees. Their pathogenicity was tested using 6-month-old wild olive seedlings and the soil infestation method. These findings highlight as *Phytophthora* may represent an emerging pathogen to the genus Olea. Investigations are ongoing to better understand the epidemiology of the disease and other possible factors involved in the outbreak. In addition to the ecological impact, a consequence of this damaging new disease is that further use of wild olive as a source of rootstock for grafting olive varieties could be limited.

PHYLOGENETIC DIVERSITY OF THE OAK PATHOGEN PHYTOPHTHORA QUERCINA FROM EUROPE AND NORTH AFRICA

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Text

Phytophthora quercina was first reported causing root rot on oak trees in central Europe. Since then, this oak-specific pathogen has spread across Europe mainly associated with chronic decline on Quercus cerris, Q. faginea, Q. ilex, Q. petrea, Q. pubescens and Q. robur, and more recently Q. suber. Since distinct phenotypes were observed amongst the isolates detected from oak trees, thus morphological and phylogenetical analyses were conducted in this thesis to assess the intraspecific variability of P. quercina. Overall, 64 different isolates of P. quercina from several European countries and oak species were analysed. Colony morphology and growth rates were compared, and nuclear and mitochondrial DNA sequences were investigated by phylogenetical analyses. The results show a high variability within the P. quercina population both in terms of colony features and growth rates. The phylogenetical analyses (Bayesian Inference and Maximum Likelihood) have enabled discrimination of 12 different lineages into the European P. quercina population, with clear association between host and geographic area. In addition, a putative new species, closely related to P. quercina, was isolated from Chamaecyparis lawsoniana var. globosa in Croatia.

EXPLORING NUTRITIONAL RELATIONSHIPS BETWEEN PLANTS AND PATHOGENIC OOMYCETES

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Text
All plant pathogens obtain nutrients from the plant host to fuel their own growth. At the same time, plants must reprogram their own metabolic pathways to fuel their immune responses and withhold nutrients from pathogens. These critically important functions are accomplished in environments where key nutrients are either limiting, sufficient, or in excess. Physiological experiments have demonstrated that changes in host nutrient status either promotes or retards pathogen growth or plant resistance. However, these data are fragmentary, particularly for plant interactions with oomycetes (e.g., Phytophthora species, downy mildews) that account for tens of billions of dollars in annual crop losses. This presentation will focus on the interaction between Arabidopsis and the oomycete pathogen Hyaloperonospora arabidopsidis (Hpa). Hpa is an obligate biotroph and establishes putative feeding structures, called haustoria, in plant mesophyll cells. We have developed a new system for transcriptomics, based on Translating Ribosome Affinity Purification, in which we can analyze the transcriptome of haustoriated plant cells to understand how their metabolism and immune responses are altered by the direct interaction with Hpa haustoria. We will describe this system and summarize the initial insights into the biology of plant cells that are actively parasitized by oomycetes.

THE TELOMERE-TO-TELOMERE REVOLUTION: UNVEILING OOMYCETE GENOMES

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Text
Oomycetes include diverse pathogens that include obligate biotrophs, hemibiotrophs, and necrotrophs that result in foliar and root diseases. Technological advances in genome sequencing have enabled the generation of near complete genome assemblies of diverse species; these are foundational resources that can be used to study how the pathogen rapidly adapts to evade control. We have generated telomere-to-telomere assemblies from several oomycete genera, focusing on the obligately-biotrophic oomycetes that cause downy mildew diseases as well as Phytophthora and Pythium spp. Comparative genomics determined that downy mildew causing oomycetes are polyphyletic and share a 17-chromosome ancestral state along with many Phytophthora clades. Annotation of these assemblies has identified horizontal gene acquisition events from phytopathogenic fungi and defined high-identity gene clusters that encode putative effectors. Sequencing of multiple isolates revealed hallmarks of allo- and auto-heterokaryosis. Alloheterokaryons are believed to arise by somatic fusions of distinct genotypes, while autoheterokaryons are believed to arise via somatic mutations in a single founding genotype; both result in distinct genotypes sharing a common cytoplasm. Heterokaryosis increases the adaptability of the pathogen and has consequences for determining virulence pathotypes and population genomic inferences.

THE ROLE OF THE OOMYCETE PHYTOPYTHIUM VEXANS AS A BIOTIC STRESS COMPONENT OF KIWIFRUIT VINE DECLINE SYNDROME IN ITALY

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Text
Several soilborne oomycetes belonging to the genus Phytopythium have been associated to kiwifruit vine decline syndrome (KVDS) which is defined as a multifactorial syndrome where both abiotic and biotic stressors are involved. Phytopythium vexans is one of the main species isolated in affected orchards also revealing several interactions with other taxonomic groups composing the microbiome associated to the syndrome. Microbiome network analysis between taxa revealed strain-specific interactions, requiring further studies on the genomics of involved oomycetes. Furthermore, plant response to the presence Phytopythium vexans in Actinidia deliciosa roots, characterized through gene expression analysis approach, revealed an upregulation of ROS scavenging pathways and hormonal stress signaling in response to the pathogen presence and flooding at specific time points. Further transcriptomic studies on Actinidia roots will reveal a major understanding of all the involved pathways in oomycete pathogenesis on fruit trees. The model of KVDS as a multifactorial syndrome, where climate change plays an important role in defining the onset of the syndrome, requires the application and combination of different omics techniques for reaching a wider comprehension of oomycete pathogenesis in complex systems.

Pectobacteriaceae: soft rot pathogenesis and symbiosis

INTRODUCTION TO CLASNIP (WWW.CLASNIP.COM), A WEB-BASED APPLICATION FOR IDENTIFICATION AND CLASSIFICATION OF DICKEYA AND PECTOBACTERIUM SPP.

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Text
Bioinformatic approaches for the identification of microorganisms have evolved rapidly, but existing methods are either time-consuming, complicated or expensive for massive screening of pathogens and their non-pathogenic relatives. A rapid and reliable classification at interspecies and intraspecies levels can facilitate the early detection of plant pathogens, and is important for disease containment and cost effective, timely action for outbreaks or invasions. Clasnip (www.clasnip.com) is a web-based platform, initially designed for haplotyping potato zebra chip disease caused by 'Candidatus Liberibacter solanacearum'. The platform integrates a state-of-art classification algorithm based on Hidden Markov Model (HMM) and maximum likelihood. It has curated databases for the classification of bacteria ring rot pathogen (Clavibacter sepedonicus) and associated Clavibacter spp, soft rot and blackleg pathogens such as Dickeya and Pectobacterium spp, and potato virus Y phylogroups for the classification and similarity evaluation of closely related microorganisms

at interspecies and intraspecies levels. In this presentation, classification of Pectobacterium spp from pure culture and environment samples were tested with 100% accuracy based on sanger sequence, genome sequence, and metagenomic NGS data. In addition, users with less bioinformatics training can build custom databases for their own applications under the Clasnip system. Detailed applications on Clasnip will be discussed.

RELATION BETWEEN TUBERS TESTING FOR SOFT ROT PECTOBACTERIACEA AND THE BLACKLEG EXPRESSION IN SUBSEQUENT FIELDS

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Text

Blackleg is one of the major potato diseases subjected to strict tolerances for seed production. Its presence may result in downgrading or rejection of seed potato fields. Voluntary seed tubers detection tests of latent infections by soft rot *Pectobacteriacae* prior to planting have recently increased. However, the correlation between the predictive tests with blackleg expression is poorly documented. Herein, the aim was to determine if evaluated latent infections in tuber could predict the risk of blackleg in the following year. Quantification of *Pectobacterium atrosepticum*, *P. parmentieri*, *P. brasiliense*, *Dickeya dianthicola* and *D. solani* in lots of 200 tubers, was performed thanks to inov3PT qPCR Taqman assays specific. Lots were then distributed in fields and blackleg incidence was monitored by inspectors during growing season. Then, relationships between estimated level of latent infections in seed lots and blackleg incidence in field were studied. Results revealed that latent infection levels could not unequivocally predict the occurrence of blackleg in the field. The predictive value of latent infection on blackleg expression differs with the bacterial species. *Dickeya* latent infection is significantly related with increased expression of symptom. Conversely, for *Pectobacterium*, which is frequently detected, no correlation was shown. That's why tuber testing has to be integrated in a global blackleg risk management as disease emergence depends on several factors.

PECULIAR EVOLUTION OF TWO PECTOBACTERIUM SPECIES, P. AQUATICUM AND P. QUASIAQUATICUM, SUGGESTING AN ADAPTATION TO A NEW ENVIRONMENTAL NICHE.

BEN MOUSSA Hajar. (1), PEDRON Jacques. (1), HUGOUVIEUX-COTTE-PATTAT Nicole. (2), BARNY Marie-Anne. (1)

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Text

Historically, research on Soft Rot Pectobacteriacea (SRP) has focused on economically important crops and ornamentals and knowledge of these bacteria outside the plant context remains poorly investigated. Recently, two closely related species Pectobacterium

aquaticum and Pectobacterium quasiaquaticum were isolated from water and have not been isolated from any plant yet. To identify the distinctive characteristics of these two species, we performed a comparative genomic analysis of 80 genomes representing 19 Pectobacterium species and performed an evolutionary reconstruction. Both water species underwent a reduction in genome size associated with a high pseudogene content. A high gene loss was predicted at the emergence of both species. Among the 199 gene families missing from both P. aquaticum and P. quasiaquaticum genomes but present in at least 80% of other Pectobacterium genomes, COG analysis identified many genes involved in nutrient transport systems. In addition, many type II secreted proteins were also missing in both species. Phenotypic analysis revealed that both species had a reduced pectinolytic activity and a biofilm formation defect associated with a reduced virulence on several plants. These genomic and phenotypic data suggest that the ecological niche of P. aquaticum and P. quasiaquaticum may differ from that of other Pectobacterium species.

INTRASPECIES VARIATION AND MALDI-TOF MS-BASED PHYLOPROTEOMIC STUDY OF AN ECONOMICALLY IMPORTANT PLANT PATHOGEN DICKEYA SOLANI

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Text

Dickeya solani, a pectinolytic phytopathogen from the *Pectobacteriaceae* family, is responsible for soft rot and blackleg diseases threatening global potato production. There are still no effective control methods against this bacterium. After studying and closing the pangenome of *D. solani*, an in-depth phenotypic characterization of 20 *D. solani* strains of various origin and year of isolation was conducted. Two *D. solani* strains, *i.e.* IFB0223 and IFB0455, of diminished abilities to macerate plant tissues, which coincided with their limited cellulolytic activity, lack of proteases action, reduced motility, lower siderophores production and impaired growth *in vitro* in contrast to the other *D. solani* isolates, were described. Notably, the pectinases action of IFB0223 and IFB0455 was only slightly reduced. Next, phyloproteomic analysis on 449 MALDI-TOF MS spectra recorded for 20 strains disclosed variation in the *D. solani* population with the low virulent strains IFB0223 and IFB0455 grouping together. Some similarities in the topologies between phyloproteomic- and core genome-based dendrograms were furthermore revealed. In addition, we disclosed four representative MALDI-TOF MS spectra for this species. The presented insight into biodiversity of *D. solani* adds to knowledge on this economically-significant pathogen and suggests that rather than genomes, the proteomes should be searched for explanations of intraspecies variation in the pathogenic potential of *D. solani*.

DOES THE MICROBIOME OF SOIL INFLUENCE DEVELOPMENT OF BLACKLEG AND SOFT ROT DISEASES CAUSED BY PECTOBACTERIACEAE?

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Text

In the recent years, scientists started to suspect that development of disease symptoms in crops is related to the so-called suppressiveness of the soil. In this regard, suppressive and non-suppressive soils are distinguished. Their microbiological composition either inhibit or favor development of certain disease symptoms on the there-grown plants. Therefore, the aim of this project was to investigate whether there is a relation between the soil type, composition of soil microbiota and progression of the *Pectobacteriaceae*-driven infections.

The herein studied suppressive and non-suppressive soils were selected basing on long-term monitoring data on the occurrence of *Pectobacteriaceae*-triggered infections on potato fields in Poland, collected since 1996, in addition to the current blackleg and soft rot incidences from 2021. The insight into the soil microbiome was achieved by analysis of 16S rDNA gene amplicons sequenced on the MiSeq machine, with a paired-end approach using the Illumina v3 set. Based on the metagenomic approach relying on R programming language including the DESeq2 package, it turned out that *Bacillus, Acidobacterium* and *Actinobacterium* spp. were more frequently identified in the suppressive than the non-suppressive soil samples.

This research provided evidence that the composition of soil microbiota is an important factor determining the prevalence of blackleg and soft rot diseases in potato fields.
Funding: NCN, Preludium21 2022/45/N/NZ9/01923

AGGRESSIVENESS AND BEHAVIOR OF DIFFERENT PECTOBACTERIUM AND DICKEYA SPECIES INVOLVED IN POTATO BLACKLEG DISEASE

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Text

Blackleg causes high economic losses for the seed potato industry worldwide. The disease is caused by bacteria belonging to the genera Pectobacterium or Dickeya. Recent developments in sequencing technology led to refine their taxonomy. Since 2016, the number of described species has increased from 12 to 33, highlighting their great genetic diversity. To date, few data are available about their specific behavior on potato host.
In order to compare the aggressiveness of 5 different Pectobacterium and 2 Dickeya species, we inoculated the pathogens on tubers just before plantation in trial fields. Each inoculum consisted in a mix of 5 strains belonging to a same species. Then, different parameters reflecting the aggressiveness and fitness of the inoculated strains were observed, as blackleg expression or vertical transmission in harvested tubers.
The results showed differences between species for all the parameters studied, highlighting different colonization strategies on potato host. Moreover, the qPCR analysis of blackleg symptoms obtained after inoculation with a mix of strains belonging to two different species can reveal a possible antagonistic relation between pectinolytic bacteria species association. Finally, a metabarcoding sequencing approach performed to monitor each inoculated strain revealed the predominance of one strain in each analyzed symptom, not always the same,

highlighting a pioneer effect during the symptom development.

T3SS INHIBITORS AS ANTIBIOTIC ALTERNATIVES IN FIRE BLIGHT MANAGEMENT

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Text

Fire blight, caused by Erwinia amylovora, is the top two most devastating diseases of apples and pears. Floral spray of antibiotics such as streptomycin is one of the few options that can effectively suppress blossom infection of fire blight in commercial orchards. However, the long-term use of streptomycin facilitated the evolution of the pathogen to develop resistance. Since the initial report in 1971, streptomycin resistance has been constantly observed in almost all apple-producing regions in the U.S. We identified a series of small phenolic compounds that can potently inhibit the expression of key type III secretion system (T3SS, a major pathogenicity factor) genes in the fire blight pathogen without affecting its growth. We proved that virulence suppression could lead to effective control of fire blight in our pilot field study. Most importantly, as the virulence inhibitors do not impose selective pressure, we anticipate less chance for the pathogen to mutate to develop resistance to the virulence inhibitors as they do to antibiotics.

INSIGHTS FROM THE INTERACTION OF BACTERIOPHAGES AND SOFT ROT PECTOBACTERIACEAE BACTERIA

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Text

Pectinolytic Soft Rot Pectobacteriaceae (SRP – *Pectobacterium* spp. and *Dickeya* spp.) cause soft rot diseases on various crops and ornamentals worldwide, leading to losses of up to 250 million Euro annually in crop production. Lytic bacteriophages able to infect and kill SRP bacteria can be readily isolated from virtually all SRP-containing environments, yet little is known about the selective pressure those viruses exert on their hosts. This presentation will cover the topic of receptors used by lytic bacteriophages to interact with *Pectobacterium* spp. and *Dickeya* spp. cells, spontaneous phage-resistance building up in the phage-full environment and the role of prophages (viral sequences) present in the SRP genomes in the ecology and environmental fitness of these bacteria. With these independent approaches, we investigated a trade-off hypothesis that phage infections may cause fitness advantages/disadvantages for the SRP bacteria in the environment.

This research was financially supported by the National Science Center, Poland (Narodowe Centrum Nauki, Polska) via a research grant OPUS 13 (2017/25/B/NZ9/00036) and grant

SONATA BIS 10 (UMO-2020/38/E/NZ9/00007) to Robert Czajkowski.

TN-SEQ REVEALED DISSIMILAR GENE REPERTOIRES OF DICKEYA SOLANI INVOLVED IN COLONIZATION OF LESIONS AND ROOTS OF SOLANUM TUBEROSUM

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Text

Dickeya and Pectobacterium species are necrotrophic pathogens that macerate stems (blackleg disease) and tubers (soft rot disease) of Solanum tuberosum. They proliferate by exploiting plant cell remains. They also colonize roots, even if no symptoms are observed. The genes involved in pre-symptomatic root colonization are poorly understood. Here, transposon-sequencing (Tn-seq) analysis of D. solani living in macerated tissues revealed 126 genes important for competitive colonization of tuber lesions and 207 for stem lesions, including 96 genes common to both conditions. In root colonization, Tn-seq highlighted 83 genes, all different from those in maceration conditions. They encode the exploitation of organic and mineral nutrients, including glucuronate, and synthesis of metabolites: cellulose, aryl polyene and oocydin. Overall, this work distinguished two metabolic networks supporting either an oligotrophic lifestyle on roots or a copiotrophic lifestyle in lesions. This work revealed novel traits and pathways important for understanding how the D. solani pathogen efficiently survives on roots, persists in the environment and colonizes progeny tubers.

GENOTYPIC AND PHENOTYPIC ANALYSES ON PECTOBACTERIUM ATROSEPTICUM ASSOCIATED WITH POTATO SOFT ROT AND BLACKLEG DISEASES IN POLAND

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Text

Among the soft rot Pectobacteriaceae (SRP) the P. atrosepticum (Pba) species is responsible for serious damages affecting both potato yield and tuber quality especially under temperate climate condition. As limitation of the spread of SRP is solely prevention-based, we undertook broad characterization on both genotypic and phenotypic levels of the Pba isolates acquired during monitoring of potato fields in Poland in 2013, 2014 and 2016. Of REP, ERIC and BOX-based genomic fingerprinting, the BOX-PCR allowed for classification of 118 Pba strains into six groups. Finally, 23 Pba strains representing all BOX

groups and originating from various growing seasons were selected for genotypic, phylogenetic and phenotypic studies. rpoS-based phylogeny revealed intraspecies variation (16 SNP sites) among the studied Pba strains, in contrast to the analyses relying on gyrA (1 SNP) and recA (0 SNP) sequences. Pba strains showed higher potency to macerate potato tissue at 20°C than 28°C. Characterized isolates exhibited rather uniform production of plant cell wall degrading enzymes (pectinases, cellulases and proteases), lipases, siderophores and biofilm, however in majority of cases inferior in comparison to Dickeya solani and Pectobacterium carotovorum strains.
In conclusion, this study revealed genotypic and phenotypic uniformity in addition to high virulence potential of Pba strains isolated from potato fields during several growing seasons in Poland.

LC-MS-MOLECULAR NETWORKING TO PROFILE THE METABOLOME OF PECTOBACTERIUM BRASILIENSE 1692

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Text

Pectobacterium brasiliense is an economically important pathogen that infects potatoes and causes significant economic loss of crops worldwide. Although metabolites against *P. brasiliense* produced by potato tubers and stems have been identified, information on the metabolites that *Pbr*1692 secretes in the host environment to manipulate host defenses or for cell growth is lacking. Therefore, this study aimed to employ untargeted liquid chromatography coupled with mass spectrometry and molecular networking to understand the cell metabolism of *Pbr*1692. The molecular networking results indicated that *Pbr*1692 produces metabolites, some of which are involved in virulence and cell growth. Among these, the derivative phytotoxin N-coronafacoyl-valine was detected in the exometabolome in the exponential growth phase, suggesting a role of N-coronafacoyl-valine in *Pbr*1692 virulence. Furthermore, the molecule lumichrome was detected at the exponential growth phase of growth in both the exo- and endometabolome suggesting a possible role in cell growth and proliferation. Additionally, siderophores were detected in the endo and exometabolome of the exponential and stationary growth phase, suggesting siderophores are required for the growth of *Pbr*1692 and their production is population density-dependent. This study will assist in further identifying metabolites and their role in *Pbr*1692 growth and virulence.

A NATURAL SINGLE NUCLEOTIDE MUTATION IN THE SMALL REGULATORY RNA ARCZ OF DICKEYA SOLANI SWITCHES OFF THE ANTIMICROBIAL ACTIVITIES AGAINST YEAST AND BACTERIA

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Text

The necrotrophic plant pathogenic bacterium *Dickeya solani* emerged in the potato agrosystem in Europe. All isolated strains of *D. solan*i contain several large polyketide synthase/non-ribosomal peptide synthetase gene clusters. Analogy with genes described in other bacteria suggests that the clusters *ooc* and *zms* are involved in the production of secondary metabolites of the oocydin and zeamine families, respectively. A third cluster, named *sol* produces a new antibiotic, solanimycin, capable of killing many yeasts, including the human pathogen *Candida albicans*. In our study, we analysed the antimicrobial functions of these three PKS/NRPS clusters against bacteria, yeasts or fungi in different wild-type *D. solani* isolates. Phenotyping and comparative genomics revealed that the small regulatory RNA ArcZ plays a major role in the control of the clusters *ssm* and *zms*, but not *ooc*. A single-point mutation, conserved in some *Dickeya* wild-type strains, including the type strain IPO 2222, impairs the ArcZ function by affecting its processing into an active form. Our study showed that single-nucleotide polymorphisms of sRNA encoding genes can have huge impacts on bacterial phenotypes. It is thus critical to pay attention to the allele diversity of sRNA genes.

DYNAMIC AND DIVERSIFIED BACTERIAL COMPLEX CAUSING POTATO BLACKLEG AND SOFT ROT IN THE NORTHEASTERN UNITED STATES

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Text

Potato blackleg and soft rot (BSR) has caused substantial economic losses to potato production in the Northeastern region of the United States since the 2015 outbreak. To understand the disease epidemiology, pathogens were isolated from potato plants and tubers showing BSR symptoms from various locations in multiple years. The isolates were identified using a polymerase chain reaction. Representative bacterial species were compared in infection and pathogenicity using whole genome sequencing analysis. The bacterial complex recovered from single disease lesions was examined using metagenomic sequencing. Dickeya dianthicola genotype I was the predominant species in the outbreak, as observed in the field. Pectobacterium parmentieri has been frequently associated with tuber

damage in storage. In addition, several other bacteria were found associated with BSR including D. polaris, D. dadantii, D. atroseptica, D. zeae, P. brasilliense, and P. versatile. The frequency of the species has changed dynamically. Noticeably, P. versatile is now the most frequently isolated species among all the pathogens in 2022. D. aquatica, isolated from surface water ,caused severe BSR on potatoes, and could be a potential inoculum source. Multiple bacterial species synergistically caused higher disease severity than that caused by any single species. Tubers had a much higher infection incidence when they were contaminated/inoculated at harvesting or before storage than inoculated at planting.

ROLE OF LRP IN DICKEYA DADANTII

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Text

Bacterial pathogenic growth requires a swift coordination of pathogenicity function with various kinds of environmental stress encountered in the course of host infection. Among these, nutritional deficiency is one of the major stresses that pathogenic bacteria must face. The nucleoid Associated protein (NAP) LRP play a key role in bacteria genome regulation in response to nutritional stress. LRP is widely distributed in Bacteria and Archaea and was shown to control, in addition to basic metabolism genes, various adaptation processes, including virulence genes in animal pathogens. No study has been conducted to determine the role of LRP in plant-pathogenic bacteria. Therefore, we investigate the role of LRP in the model phytopathogen *Dickeya dadantii*. We inactivated LRP and determined both phenotypic effects of *lrp* null mutation on *D.dadantii* virulence and the transcriptional response under various conditions of growth. We show that *lrp* null mutation reorganizes the genomic expression affecting the main virulence genes involved in both symptomatic and asymptomatic phases of infection, these regulations being dependent on the availability of nutrients. Thus, LRP allows the bacterium to optimize the use of energy to colonize the host plant.

THE CHROMOSOME-ENCODED TYPE II TOXIN-ANTITOXIN SYSTEMS OF DICKEYA DADANTII 3937.

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Text

Type II toxin-antitoxin (II TA) systems are the intracellular modules consisting of 2 non-secreted proteins: a stable toxin and an antitoxin, which binds the toxin and neutralizes its toxic effect. The disturbance in the intracellular toxin and antitoxin ratio typically leads to inhibition of bacterial growth or even cell death. A number of studies suggests that chromosome-encoded TA modules are engaged in important processes including maintenance of mobile genetic elements and bacteriophage defence, but possibly also biofilm formation and stress response.

The *Dickeya dadantii* 3937 strain serves as a model for pathogens causing the soft-rot disease in a wide range of angiosperm plants. In 2011 Glasner *et al*. published the DNA sequence of *D. dadantii*, and identified in silico over 20 putative II TA systems. However, the computational analysis does not provide proof of a TA systems activity in a living bacterial cell. Therefore, in this study we determined which of II TA systems of *D. dadantii* is functional *in vivo*. We were able to establish that among 24 putative II TA modules, 7 were active in vivo, while the activity of 17 was excluded, either on the basis of DNA sequence analysis or after experimental validation. Among 7 bona fide TA systems, one was described previously as the $ccdAB_{Ech}$ module of *E. chrysanthemii*, while the remaining 6 were newly validated by our group. Among them the *dhiTA* system probably encodes proteins belonging to the novel T/A superfamilies.

CHARACTERIZATION OF A NEW GENUS OF THE PECTOBACTERIACEAE FAMILY AND RECTIFICATION OF THE OUTLINE OF THIS FAMILY

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Text

The bacterial order *Enterobacterales* is currently divided into eight recognized families: *Budviciaceae*, *Enterobacteriaceae*, *Erwiniaceae*, *Hafniaceae*, *Morganellaceae*, *Pectobacteriaceae*, *Thorselliaceae*, *Yersiniaceae*, and the not yet recognized family *Bruguierivoracaceae*. The *Pectobacteriaceae* family comprises plant pathogens able to provoke diverse diseases, including plant maceration due to the production of pectinases disrupting the plant cell wall. To better understand their natural diversity, a survey of pectinolytic bacteria was performed in lakes of the French region La Camargue near the Mediterranean Sea. Sixteen atypical pectinolytic isolates were obtained from brackish water of three lakes. In phylogenomic trees, the novel strains formed a new clade of *Pectobacteriaceae*, separate from the previously described genera of this family: *Affinibrenneria*, *Brenneria*, *Dickeya*, *Lonsdalea*, *Musicola*, *Pectobacterium*, and *Samsonia*. Phylogenomic study of representative members of the order *Enterobacterales* clearly indicated that *Acerihabitans* does not belong to *Pectobacteriaceae* and should be reclassified in the *Bruguierivoracaceae* family. In contrast, the relative position of *Symbiopectobacterium* in the *Enterobacterales* tree supports its appurtenance to *Pectobacteriaceae*. Finaly, based on phenotypic, genomic and phylogenetic characteristics, we propose the creation of a new genus including the sixteen pectinolytic isolates from Camargue brackish lakes.

METALS AT THE BACTERIAL/PLANT INTERFACE, CASE OF A PHYTOPATHOGENIC BACTERIUM AND ZINC

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Text
Metals are increasingly recognized as playing an essential role in bacterial/host interaction, and in particular in pathogenicity phenomena. In this work, we investigated the role of the metalloregulator Zur in the interaction between the plant pathogenic bacterium Dickeya solani and plants. Zur senses zinc and regulates the transcription of genes involved in zinc homeostasis. However, a growing number of studies show that the Zur regulon is not restricted to genes involved in the maintenance of homeostasis but that some of these genes are involved in the pathogenicity of the bacteria. We constructed a mutant deleted from the zur gene in D. solani. As expected, this mutant showed increased sensitivity to Zn. We also showed that deletion of zurcaused an increased sensitivity to polymyxin B, an antimicrobial peptide. Interestingly, the mutant dispalyed a very attenuated virulence. This attenuated virulence was not related to a developmental defect of the mutant in the plant. We also showed that the production of pectate lyases, major virulence factors, was induced by Zn, but that Zur did not directly control these genes. Finally, real-time monitoring of the expression of the znuAgene, encoding a Zn acquisition system, during the infection of the plant, revealed that Zn acquisition is important in the early phases of the infection.

PBR1692 SECRETOME ANALYSES

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Text
Pectobacterium brasiliense (Pbr) are important phytopathogens affecting potato and other crop plants, globally. To enhance our understanding of the biology of P. brasiliense, the Pbr1692 secretomes of outer membrane vesicles (OMVs) and the Type 6 Secretion System (T6SS) were determined using mass spectroscopy. Functional annotation of OMV proteins revealed that OMVs harbour cargo associated with other secretion systems such as phospholipase A1 and A2, polygalacturonase, peptidase, cellulase and Avirulence L (AvrL). Furthermore, the role of Pbr 1692 OMV-mediated competitive fitness against Dickeya dadantii, virulence on potato tubers, and elicitation of a hypersensitive response (HR) in Nicotiana benthamiana leaves was demonstrated. This study further used in silico computational prediction tools to identify T6SS-secreted proteins. To validate these in silico predictions, secretomes of Pbr1692 wild type (T6SS-active strain) and tssBC sheath mutant (T6SS-inactive strain) secretomes were compared to each other as well as to the predicted substrates. A combined total of 449 proteins were secreted by the T6SS-active and inactive Pbr 1692 strains. Of these, a total of 19 proteins were secreted by the T6SS-active Pbr 1692 strain only, suggesting that these proteins are secreted in a T6SS-dependant manner. These included T6SS hallmarks such as VgrG and Hcp, and HNH endonuclease. Overall, our results suggest that OMVs and the T6SS potentially share substrates with each other.

IDENTIFICATION AND CHARACTERIZATION OF BACTERIA FROM THE GENUS PECTOBACTERIUM ISOLATED FROM THE SOIL, WATER, AND INSECTS

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Text

Bacteria of the *Pectobacteriaceae* family are widespread plant pathogens that are being isolated globally from a wide range of plants but also from water, soil, or insect guts. We decided to check whether Soft Rot Pectobacteriaceae strains isolated from soil, water, and insects exhibit equally strong pathogenicity as pectinolytic strains isolated from diseased plants. We isolated 85 pectinolytic strains from 46 water, soil, and insect samples. The strains were identified by species-specific PCR, and *recA* gene sequencing. The isolated bacteria were assigned to the eight species, *P. atrosepticum, P. aquaticum, P. brasiliense, P. carotovorum, P. polonicum, P. quasiaquaticum, P. versatile* and *Dickeya zeae*. The species *P. versatile* was most abundant.

To perform the characteristics of isolated strains, comprehensive biochemical analyzes were carried out, and the adaptive potential to adverse environmental conditions such as high salinity, extreme pH and limited water availability was examined. In addition, susceptibility to selected antibiotics and possible resistance mechanisms were examined.

We have found that *Pectobacterium* strains that do not originate from plants are characterized by a very large adaptation potential to harsh environmental conditions and exhibit equally strong virulence as strains isolated from plants with disease symptoms. Moreover, some strains revealed the ESBL and KPC type of resistance to antibiotics.

Funding OPUS18-2019/35/B/NZ9/01973

CHARACTERIZATION OF BIOLOGICAL AND SYNTHETIC MICROSTRUCTURES AND THEIR INTERACTION WITH PECTOBACTERIUM

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Text

Surface architecture and its effect on bacterial attachment, colonization, and micro-environment, are poorly understood. Our aim is to unravel the complex interaction of leaf surfaces with bacterial cells and explore the mechanism by which microstructures influence bacterial behavior and gene regulation. For that purpose, two closely related ornamental species of the genus Zantedeschia were chosen as model hosts for the soft rot pathogen *Pectobacterium brasilense*. While *Z. aethiopica* (ZA) has a relatively smooth, less competent surface for bacterial cell attachment, the hybrid cultivar 'Captain Romance' (CR) has a rough surface, which is highly compatible with bacterial colonization. Using biomimetic replicas of leaf surfaces, we were able to associate the phytopathogen attachment patterns and the surface microstructure, by this to establish a direct linkage between bacterial behavior and the microstructure. Our hypothesis is that the microstructure also affects the microenvironment and by this differential regulation patterns of the colonizing bacteria. Our research combines multidisciplinary approaches such as biomimetics, microscopy, and

molecular microbiology to study the events that affect microbes' interaction with natural and artificial surfaces.

ROLE OF ANAEROBIC RESPIRATIONS OF CARBON SOURCES ON SURVIVAL AND ECOLOGICAL FITNESS OF DICKEYA GENUS

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Text

Bacteria are ubiquitous and occupy a very wide range of ecological niches where oxygen can be rapidly limited. In this case, bacteria develop flexible metabolic respiration. Therefore, demonstrating the possibility of using carbon sources as alternative terminal electron acceptors (TEAs) and elucidating the underlying genetic pathways has great potential to help understand bacterial strategies to adapt and persist in the environment. This question is more relevant in the case of phytopathogenic bacteria which face many specific challenges to infect plants. It is the case for Dickeya, an emergent pathogen, found in various ecological niches, such as plant apoplast, and responsible for soft rot disease in a wide variety of plants. Understanding anaerobic respiratory pathways involved in Dickeya persistence and colonization will allow us to better understand its pathogenicity, and thus better counter it. In this study, we demonstrated that D. dadantii is able to use malate and asparagine as TEA. By constructing metabolic model of D. dadantii, we predicted pathways involved in anaerobic growth using asparagine and malate. Mutants affected in asparagine pathway were constructed and we evidenced the role of asparagine respiration in D. dadantii virulence. We also tested whether asparagine pathway is conserved among Dickeya and Pectobacterium genus.This study demonstrated that anaerobic respiration is an important trait involved in D. dadantii virulence.

DIVERSITY AND BIOLOGICAL FEATURES OF PECTOBACTERIUM BRASILIENSE, CAUSATIVE AGENT OF POTATO BLACKLEG

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Text

In the last decade, *Pectobacterium brasiliense* has become the dominant blackleg causing soft rot Pectobacteriaceae (BL-SRP) in seed potatoes in the Netherlands and *P. brasiliense* is now responsible for over 90% of all blackleg incidences. The genetic diversity of *P. brasiliense* in potato is high, but only a limited number of haplotypes is able to cause blackleg. Most strains belonging to these virulent haplotypes can cause disease. To detect the various blackleg causing haplotypes of *P. brasiliense*, enrichment TaqMan assays were developed.

Seed potato cultivation starts with minitubers (PB1) which are free of BL-SRP. However, surveys conducted at different Dutch farms, showed that already during the growing season a PB1 crop can become infected with BL-SRP, with incidences of over 40%, depending on

growing season and location. In most cases one of the virulent *P. brasiliense* haplotypes was detected. In surveys performed to identify possible infection sources, BL-SRP were not detected in rain water, but 2.3% of the insects sampled in a potato field carried BL-SRP, in most cases a virulent haplotype. In field experiments, spray-inoculation of haulms of young potato plants with *P. brasiliense* resulted in tuber infections even at a low inoculum density of approximately 100 cfu per plant, indicating the risks of contamination of haulms.
This project was financed by the Foundation TKI Horticulture and Starting Materials (grant nr. TU18028), BO-Akkerbouw, NAO, Agrico, HZPC and NAK

DNA SUPERCOILING AS A GLOBAL TRANSCRIPTIONAL REGULATOR: A COMPLEX MECHANISM INVOLVED IN THE INFECTION PROCESS OF THE PHYTOPATHOGEN DICKEYA DADANTII ?

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Text
Dickeya dadantii is a bacterium causing soft rot disease in a wide range of plant species. During the infection process, D. dadantii undergoes environmental stresses: acidic stress during penetration in the apoplast, oxidative stress due to the plant immune response, and osmotic and oxidative stresses during plant maceration and disease generalization. Interestingly, these stresses affect DNA supercoiling (SC): acid and oxidative stresses lead to DNA relaxation and osmotic stress induces an increase in SC level. Deciphering the mechanisms of SC-related transcriptional regulation in that species is thus crucial for our understanding of the mechanisms of virulence. As far as we know, the SC level is mainly regulated by topoisomerase I and DNA gyrase. Inhibiting either of these enzymes with antibiotics leads to global SC modifications and subsequent changes in global gene expression. We analyzed the first transcriptomic response of a Gram-negative bacterium to topoisomerase I inhibition by an antibiotic. We detected distinct patterns of expression level and spatial organization along the chromosome. Particularly, we found that all SC variations affect the expression of two major virulence genes, encoding pectate lyases (destroyer of the cell wall, causing the soft rot symptom), pelE and pelD.

VIRULENCE REGULATORY NETWORK OF DICKEYA DADANTII: WHAT IS THE ROLE OF POSTTRANSCRIPTIONAL REGULATION?

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Text

The genus *Dickeya* are enterobacterial plant pathogens responsible for soft rot disease in a wide range of plant species, including economically important crops e.g. potato and rice. The infection process is divided into two main phases: the asymptomatic phase where *Dickeya spp.* produce early virulence factors to colonize the apoplastic spaces between plant cells; and the symptomatic phase, which is associated with the secretion of late virulence factors, i.e. plant cell wall degrading enzymes that macerate the plant tissue. Therefore, the spatial and temporal production of the virulence factors must be precisely controlled to ensure the efficient colonization and degradation of the host. While many transcriptional regulators are involved in controlling *Dickeya*'s virulence factors, knowledge of post-transcriptional regulation is still in infancy. Our first results on *Dickeya dadantii* RNA chaperons suggest a post-transcriptional regulation of virulence. Additionally, the obtention of *D. dadantii* transcriptional landscape allowed us to identify RNAs predicted to interact with the mRNAs of virulence factors regulators that play a role in response to oxidative stress and changing metabolic content in the apoplast. Ongoing work aims to establish a link between regulatory RNAs, virulence factors, and environmental changes encountered by bacteria during infection, which will lead to a better comprehension of the complex virulence regulatory network of *D. dadantii*.

COMPARISON OF THE ACTIVITY OF THE VFM QUORUM SENSING SYSTEM IN DIFFERENT STRAINS OF THE GENUS DICKEYA.

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Text

The Vfm Quorum Sensing (QS) system is preponderant for the virulence of different species of phytopathogenic bacteria of the genus *Dickeya*. The *vfm* gene cluster includes 26 genes involved in the biosynthesis, sensing or transduction of the Vfm signal. The transduction of the Vfm signal was shown to result into the activation of the promoter of the gene *vfmE* encoding a transcriptional regulator of the AraC family which itself activates the promoter of genes encoding the plant cell wall degrading enzymes (PCWDEs). The transcriptional regulator VfmE was shown to also activate promoters of the *vfm* gene cluster, allowing an exponential auto-induction of the Vfm QS system. Additionally, the activity of the transcriptional regulator VfmE was shown to also be controlled by Vfm-independent regulatory pathways involving the secondary messenger c-di-GMP and/or transcriptional regulators encoded outside of the *vfm* gene cluster. Here, we used a reporter gene fused to the promoter of the gene vfmE and compared the activity of the *vfmE* promoter among strains of *Dickeya*.

Phytobiomes Research for Plant Health

MODULATION OF PLANT DEFENSE GENE EXPRESSION IN BARLEY BY RUSSIAN WHEAT APHID HONEYDEW AND HONEYDEW-ASSOCIATED BACTERIA

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Text

Plant health outcomes are largely based on the dynamic interactions of multiple organisms in the phytobiome. Our previous work demonstrated that variation in Russian wheat aphid (RWA, *Diuraphis noxia*)-induced chlorosis on wheat is determined, in part, by aphid-associated bacteria. Aphids with high titers of bacteria induced expression of salicylic acid (SA) biosynthetic genes and accumulation of SA, a hormone that inhibits insect resistance, in susceptible wheat. High, sustained expression of SA biosynthetic genes was followed by downregulation of jasmonic acid (JA) biosynthetic genes; JA is associated with insect resistance. These trends in plant defense gene modulation are also evident in barley, an alternate host for RWA with considerable genetic resources. We hypothesize that aphid-associated bacteria contribute to aphid virulence by modulating the plants' insect defense mechanisms. Although saliva from aphids contains culturable bacteria, scanning electron microscopy (SEM) studies did not detect bacteria in high numbers in the aphid salivary glands or foreguts. However, the same genera of bacteria were detected in aphid honeydew as in saliva; thus, we are focusing on the role of honeydew in induction of plant defense. In this presentation, we will report on the impact of honeydew and honeydew-associated bacteria on plant defense response pathways in barley.

UNRAVELLING THE EPIPHYTIC MICROBIOME OF RASPBERRIES: HOW FRUIT AGE AND POLYTUNNEL LOCATION IMPACT PATHOGENIC AND BENEFICIAL FUNGI AND BACTERIA ON THE SURFACE OF RASPBERRIES

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Text

Raspberries are a profitable crop, susceptible to fungal diseases. In the UK, *Cladosporium* fruit rot is becoming more prevalent. For many fungal pathogens, inoculum is spread via airborne spores that land on the surface of plant tissues. *Cladosporium* is reported to be an abundant component of aero-inoculum, and is likely to land on the fruit surface. It is not known if more *Cladosporium* may germinate and grow as the fruit ages, or if beneficial organisms may inhibit such fungal growth. Hence understanding how fruit age impacts the microorganisms on the fruit surface may provide insight into how they cause or prevent disease.

The aero-microbiome is also impacted by air movement, which may be impeded by the presence of polythene plastic. The gradient of temperature and humidity associated with large polythene tunnels may also impact the aero-microbiome composition.

In this study, we sampled green, ripening and ripe fruit at two time points, and two locations

(the outer edge and centre of polytunnels). We then performed metabarcoding to profile the microbiome on the surface of raspberry fruits.
Preliminary results show that sampling date, fruit age, location within the polytunnel and the polytunnel location all have significant effects on the epiphytic microbiome of raspberries. Differential analyses revealed that multiple pathogenic species (including *Cladosporium*) increased and decreased across fruit ages and between fruit sampled in the inner and outer sections of polytunnels.

THE RULES BEHIND PLANT MICROBIOME ASSEMBLY: A BIG DATA APPROACH

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Text
The study of plant microbiomes is increasingly attracting a large interest worldwide, generating groundbreaking knowledge on the composition, function, and origin of the plant-associated microbial communities. However, we are only beginning to understand the rules behind the assembly of plant microbiomes. In this study, we leverage on the massive amount of publicly available data generated by over thousands of studies (timespan 2017-2021), which we collected and re-analyzed under a common framework. Using a variety of metrics and approaches, we tested several questions, including: (i) which are the major factors contributing to the assembly of plant microbiomes? (ii) do specific stressors (e.g., pathogens, drought) generate unique changes in the plant microbiomes? (iii) does plant phylogeny reconciliate with the diversity/structure of plant microbiomes? We also carefully searched each article, highlighting important methodological limitations that need to be considered in the future research endeavors. Our results contribute to a more general understanding of plant-microbiome interactions, which are thought to be key towards eco-sustainable plant protection, ecosystems conservation, and successful ecological restoration

DRY LENTICEL ROT – AN EMERGING POSTHARVEST DISORDER ON APPLES IN NORTHERN ITALY

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Text
Symptoms of dry lenticel rot, also known as Ramularia spots, were first observed 2012 on apples after long-term conservation in the cold storage in South and Tyrol Piedmont (Northern Italy). Since then, only few notifications of this postharvest disorder were made from the fruit growers cooperatives. However, since 2019, a notable increase of dry lenticel rot, has been observed in South Tyrol and recently, occurrence of similar symptoms has been reported from other apple growing areas in Austria and France. A collection of more than 100 fungal isolates from affected fruit and originating from various orchards was established. Subsequent multi locus sequencing analysis of five gene loci identified Ramularia mali as causative agent of this postharvest disease. Nevertheless, isolates of

deviating morphology and genetic variability were identified and thus, their impact on the disorder needs to be investigated. Ramularia mali is able to switch from endophytic to epiphytic lifestyle, however, the trigger for this switch remains to be elucidated. Symptom development might be linked to the apple peels' microbiome composition, thus, metabarcoding of the fruits' endo- and epiphytic microbiome shall provide further information and contribute to the identification of potentially antagonistic or beneficial microorganisms for further applied research.

GENETIC ANALYSIS OF STRIPE RUST RESISTANCE IN CIMMYT COMMON WHEAT LINE KFA/2*KACHU UNDER CHINESE RUST ENVIRONMENT

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Text

Breeding resistant wheat variety is the most economic and efficient way to manage wheat stripe rust, while identification of new stripe rust resistance gene and development the molecular markers will be very useful for wheat breeder in the breeding program. In the present study, a recombinant inbred line (RIL) population derived from a cross of the resistant parent KFA/2*KACHU and susceptible parent Apav#1 was used to map stripe rust resistance loci. In combination of GBS genotyping platform and BSR-Seq method, we mapped a new race-specific stripe rust resistance gene on wheat chromosome 5BL at the seedling stage, temporarily named as *YrK*. *TraesCS5B02G330700* encoded a receptor-like kinase was considered as the key candidate gene of *YrK* based on virus induced gene silencing (VIGS) method. Its expression showed a significant up-regulated at 24h after inoculation and the functional molecular markers were developed based on the polymorphic SNPs in the CDS region. In addition, a total of four adult plant resistance (APR) loci were identified on wheat chromosome 1BL, 2AS, 2BS and 4AL, respectively. Among these, *QYr.hazu-1BL* and *QYr.hazu-2AS* was verified as the known resistance genes *Lr46/Yr29/Pm39* and *Yr17*, respectively. *QYr.hazu-2BS* was a new APR locus and the closely linked SNP markers were converted into breeder-friendly KASP markers.

IS THE PLANT RHIZOBIOME ENGAGED IN INTERACTIONS BETWEEN PECTOBACTERIUM AND PLANTS?

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Text

Phytopathogens, to infect a host, must bypass the host's defence system and the additional barrier their plant microbiome provides. To determine whether the plant microbiome affects

the pathogen and determines its interactions with the plant or vice versa, we investigated the changes in the plant rhizobiome composition during plant-bacteria interactions.
Using the 16S rRNA amplicon sequencing approach, we determined the microbiome composition of soil and rhizosphere of Arabidopsis, Chinese cabbage, Calla lily and Turmeric before and after inoculation with Pectobacterium. First, we counted the number of cultivable bacteria in each sample, and pure bacterial strains were isolated. Then the interaction between the strains from the rhizosphere and *Pectobacterium* was studied.
The sequence analysis of the 16S rRNA gene showed that the biodiversity of the soil microbiome is significantly lower than those observed in the case of plants' rhizosphere. The microbiome of each plant has different species composition.
Interestingly, it was observed that *Pectobacterium* significantly reduced the number of taxa in the rhizosphere of each plant. However, no antagonism between strains isolated from the rhizosphere and *Pectobacterium* was found. The obtained results indicate the negative impact of *Pectobacterium* on the biodiversity of the plant microbiome, which in turn may lead to the breakdown of their protective barrier and the development of disease symptoms.
Funding OPUS18-2019/35/B/NZ9/01973

TRICHODERMA ASPERELLUM SECRETED 6-PENTYL-ALPHA-PYRONE PROTECTS MAIZE PLANTS FROM THE LATE WILT PATHOGEN, MAGNAPORTHIOPSIS MAYDIS

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Text
Late wilt disease, caused by the fungus *M. maydis,* threatens commercial maize production in high-risk areas. Thus, searching for control options against the pathogen is one of the top priorities in Israel, Egypt, and other countries. Disease-resistant maize genotypes can reduce yield loss. Yet, aggressive variants of the fungus can overcome host resistance. The current study aimed at inspecting *Trichoderma asperellum*, a maize endophyte, and its secreted metabolites, particularly the purified 6-pentyl-α-pyrone (6-PP), against the pathogen. First, adding *T. asperellum* directly to seeds with sowing provides significant protection to sprouts (up to 42 days) in a growth room, with more than two-fold growth promotion and reduced pathogen root infection (detected by real-time PCR). The same procedure applied in a commercial field reduced the cobs' symptoms by 11%, resulting in nine-fold lower pathogen DNA levels in the stem tissue. Second, the *T. asperellum* secreted 6-PP compound (30 µg/seed) was used in seed coating and tested against the *T. asperellum* secretory metabolites' crude (diluted to 50%). At the season's end, these treatments improved plant biomass by 90–120% and cob weight by 60%. Moreover, the treatments significantly ($p < 0.05$) reduced the symptoms (up to 20%) and pathogen infection (94–98%). The current study's results reveal the potential of 6-PP as a new fungicide against *M. maydis*. Such a treatment may protect maize plants from other soil diseases.

DECIPHERING THE ACTIVE STRAWBERRY MICROBIOME ASSOCIATED WITH ROOTS AND RHIZOSPHERE SOIL

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Text

Plants recruit diverse microbial communities from the soil. Plant-microbiome interactions confer multiple beneficial impacts on crop plants. Understanding of such interactions in strawberry production systems is still obscure. Using a strawberry crop model, we conducted a 3-year field experiment at Castle Hayne, North Carolina to test the hypothesis that carbon sources (molasses, mustard meal, and mixtures) when used in anaerobic soil disinfestation (ASD) procedures can improve soil health and impact microbial communities. Our results demonstrate that ASD, particularly with the mixture of molasses and mustard meal significantly produced higher marketable yield, and reduced soil-borne pathogens. Microbiome data analysis showed increased relative abundance and structure of both bacterial and fungal communities and influenced the α-diversity and β-diversity compared with the non-treated control. We discovered shifts in the assembly of beneficial microbiome members such as *Arthrobacter, Bacillus*, *Paraburkholderia*, *Bradyrhizobium* and *Trichoderma harzianum*. Collectively, this study adds to our understanding of the eminent roles of beneficial microbiomes and uncovering mechanisms that can lead to potential biologically based solutions for disease resilience in strawberry, and enhanced productivity, consistent with a sustainable agriculture.

Powdery mildews: phylogenetics, phylogenomics, and molecular host-pathogen interactions

AN AVIRULENCE EFFECTOR FROM BARLEY POWDERY MILDEW FUNGUS CAN SUPPRESS MILDEW LOCUS A RESISTANCE.

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Text

The barley powdery mildew fungus *Blumeria hordei* (*Bh*) relies on a huge repertoire of effectors for successful infection and the development of disease symptoms on the barley host. In resistant lines, the *Bh* AVRA effectors are recognised by cognate barley nucleotide-binding leucine-rich repeat receptors encoded at the *Mildew locus A* (MLA NLRs). For example, AVRA13-1 is recognized by MLA13. The effector-receptor interaction activates host cell death, and this terminates powdery mildew disease development. We demonstrate that a virulent form of AVRA13, called AVRA13-V2, escapes MLA13 recognition by substituting a serine for a leucine residue at the C-terminus. Counterintuitively, this substitution in AVRA13-V2 results in an enhanced MLA13 association and prevented the detection of AVRA13-1 by MLA13. Therefore, AVRA13-V2 is a dominant-negative form of AVRA13. Our interaction

analysis involving effector and receptor mutant variants constitute an important step to define intermediate receptor conformations during NLR activation and implies that the dominant-negative function of AVRA13-V2 has contributed to the breakdown of Mla13 resistance.

HOST PHOSPHOLIPIDS ARE INVOLVED IN RESISTANCE AND SUSCEPTIBILITY TO FUNGAL INVASION INTO EPIDERMAL CELLS OF BARLEY

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Text

ROP GTPases regulate cell-polarity in plant growth processes. The barley ROP RACB is involved in susceptibility towards infection by the barley powdery mildew fungus Blumeria hordei. To improve our knowledge on cellular pathways that connect RACB-signaling to disease susceptibility, we used an untargeted CoIP-LC-MS/MS screening from Bh-infected barley plants, that expressed constitutively activated (CA) GFP-RACB. Thereby, we uncovered new RACB interaction partners of plant and fungal origin. At least three of those proteins, a plant phosphoinositide phosphatase, a plant phosphoinositide phospholipase and one putative Bh-effector protein, are involved in the interaction outcome. The candidate interactors and RACB are able to interact with overlapping anionic phospholipid species in vitro, and in case of RACB, this lipid-interaction is mediated by its polybasic region (PBR). In planta, the PBR appears to be essential for RACB's plasma membrane localization and recruitment to the haustorial neck region during Bh-invasion. Other canonical interaction partners of RACB are also targeted to this fungal interface in a RACB-dependent manner. Phosphatidyl-4-phosphate and phosphatidylserine show a distinct enrichment at the haustorial neck region, suggesting a connection to subcellular targeting of RACB at this site. We suggest that the interplay of ROPs with anionic phospholipids steers subcellular enrichment of components that are pivotal for fungal penetration success or failure.

POWDERY MILDEW IN AUSTRALIAN MUNGBEAN CROPS: IDENTIFICATION OF THE PATHOGENS, THEIR HOST RANGE AND EPIDEMIOLOGY

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Text

Since the 1970's, powdery mildew has been a consistent threat to mungbean (Vigna radiata) production across Australia. Powdery mildew can reduce yields by more than 40% without fungicide management. Despite its economic importance, the precise identification of the pathogens and many aspects of their disease has been unclear. This study was designed to validate the identification and taxonomy of the species causing powdery mildew in Australia, and improve our understanding of their host range, virulence and climatic factors that favour

disease. In this study we identified Podosphaera xanthii and Erysiphe vignae, a newly recognised taxon, as the causal agents of the disease based on cross-inoculation studies and the examination of internal transcribed spacer sequences of the nuclear ribosomal DNA and/or morphology of freshly collected and herbarium specimens. Further examination of fresh and historical herbarium specimens was conducted to investigate the host range of these pathogens. Cross-inoculation experiments were performed to determine whether P. xanthii isolates that infect mungbean are different to the P. xanthii isolates that infect cucurbits. We also report on the effect of temperature on conidial germination, infection and sporulation in the two species. The outcomes of this study significantly improve our understanding of powdery mildew in Australian mungbean crops and assist in the development of better control strategies for these diseases.

COORDINATED ROLE OF CHITIN-TRIGGERED IMMUNITY SUPPRESSION MECHANISMS OF PODOSPHAERA XANTHII

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Text

Fungal pathogens are the main destructive microorganisms for terrestrial plants and pose increasing challenges for global agricultural production. Chitin is a vital building block for fungal cell walls and a widely effective inducer for plant immunity that, through chitin-triggered immunity, can defend against fungi attack. That is why the phytopathogenic fungi have developed different virulence factors that allow them to suppress the activation of this defensive response. In this study, the molecular mechanisms of chitin-triggered suppression, previously identified in the cucurbit powdery mildew *Podosphaera xanthii*, have been evaluated in detail. These mechanisms consist of the modification of chitin immunogenic oligomers (CDA), the binding to these oligomers (CHBE) and their degradation (EWCAs). For this, the RNA interference (RNAi) technology, which consists of the application of double-stranded RNA (dsRNA) designed to suppress the expression of target genes, was used. The preliminary results obtained using this strategy significantly reduces the development of the fungus and the symptoms of powdery mildew disease in melon, suggesting that chitin signaling suppression mechanisms are essential for the development of *P. xanthii*.

This work was supported by AEI (PDC2021-121373-C21).

CONSERVED AND NON-ANNOTATED PROTEINS OF PODOSPHAERA XANTHII: NEW TARGET CANDIDATES FOR THE CONTROL OF POWDERY MILDEWS BY SPRAY-INDUCED GENE SILENCING

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Text

One of the most important limiting factors for cucurbit production worldwide is the powdery mildew fungus *Podosphaera xanthii*. Despite the efforts invested in plant breeding programs and chemical companies, effective control of this pathogen remains elusive to growers. In this work, we examined the potential of RNAi technology called spray-induced gene silencing (SIGS) for controlling cucurbit powdery mildew. For that, we first developed a new and simple gene silencing method for *P. xanthii* based on the application of dsRNAs to the plant surface. Moreover, to identify new target candidate genes, we focused on the study of a set of sixty conserved and non-annotated proteins (CNAPs) deduced from the *P. xanthii* transcriptome. After protein modeling and ligand prediction studies, six proteins presumably involved in respiration, glycosylation and efflux transport, were selected. Functional analysis of these CNAP coding genes by dsRNA-induced gene silencing resulted in strong silencing phenotypes with large reductions in fungal growth and disease symptoms. Due to their important contributions to fungal development, three of these *CNAP* genes were selected as targets to conduct SIGS assays under plant growth chamber conditions. The spray application of these dsRNAs induced high levels of disease control, supporting that SIGS could be a promising tool for controlling powdery mildews.

This work has been funded by AEI (AGL2016-76216-C2-1-R; PID2019-107464RB-C21).

IDENTIFICATION, PHYLOGENY AND GEOGRAPHIC AND HOST RANGE EXPANSIONS OF POWDERY MILDEWS INFECTING CROPS AND WILD PLANTS

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Text

Powdery mildew fungi (Erysiphaceae) are widespread obligate biotrophic pathogens of many dicots and monocots, including important agricultural and horticultural crops. Over 900 species representing 19 genera belong to the Erysiphaceae, a monophyletic lineage within Helotiales, Ascomycota. Recent comprehensive phylogenetic analyses based on nuclear ribosomal DNA (nrDNA) sequences and single-copy orthologous amino acid sequences confirmed earlier results on the phylogeny and phenotypic evolution of powdery mildews, which served as the basis for the identification of species and genera. Host range and geographic expansions of some species have also been recently confirmed with genetic and genomic analyses. This presentation will provide an overview of recent advances in understanding the evolutionary history of powdery mildews infecting crops and wild plants in different parts of the world.

CHALLENGES IN WHOLE GENOME SEQUENCING OF POWDERY MILDEW FUNGI AND USE OF PHYLOGENETIC SIGNAL TO ASSESS GENOME QUALITY

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Text

Genome-scale phylogenetic analyses of powdery mildew fungi corroborate earlier phylogenies based on sequences of the nuclear ribosomal DNA (nrDNA) but also reveal several contaminations and mis-assembly issues in publicly available genomes. The obligate biotrophic nature of powdery mildews makes genome sequencing and assembly challenging. This is complicated by the fact that research groups working on genomics of powdery mildew fungi often do not make specimens publicly available, making re-sequencing and further scrutiny of the results difficult. Providing multiple examples from publicly available genome datasets, we demonstrate that, while assessment of Benchmarking Universal Single-Copy Orthologs can provide important information regarding genome quality, it needs to be complemented by additional quality control analyses. We recommend that the presence of nrDNA sequences in whole genome datasets be investigated to evaluate the quality of genome assemblies. We also discuss the importance of depositing specimens in internationally accessible herbaria to facilitate their safeguarding as well as availability to the research community to confirm findings and extend previous studies, especially where detailed plant-pathogen interaction studies and analyses of effector repertoires are conducted without providing phylogenetic analyses to confirm the identity of the sequenced powdery mildew specimens.

NEW INSIGHTS INTO THE GENETIC STRUCTURE OF GRAPE POWDERY MILDEW POPULATIONS IN ISRAEL AND THEIR ECOLOGICAL ATTRIBUTES

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Text

Plant pathogens usually originate and diversify in geographical regions where hosts and pathogens co-evolve. *Erysiphe necator*, the causal agent of grape powdery mildew, is a destructive pathogen of grapevines worldwide. Although Eastern US is considered the center of origin and diversity of *E. necator*, previous reports on resistant native wild and domesticated Asian grapevines suggest Asia as another possible origin of the pathogen. By using multi-locus sequencing, microsatellites, and a novel application of amplicon sequencing (AmpSeq), we show that the population of *E. necator* in Israel is composed of three genetic groups: Groups A and B that are abundant worldwide, and a new group IL, which is genetically different from any known group in Europe and Eastern US. Group IL showed distinguished ecological characteristics: it was dominant on wild and traditional vines (95%); its frequency increased along the season; it was more aggressive than A and B isolates on both wild and domesticated vines; and its mating types ratio was different from

group B ratio. Conidial morphology and adaptation to microclimate were also significantly different across the different genetic groups. The low genetic diversity within group IL suggests that it has invaded Israel from another origin. Therefore, we suggest that the Israeli *E. necator* population was founded by at least two invasions, of which one could be from a non-East American source, possibly from Asian origin.

CRACKING THE CODE OF POWDERY MILDEW ANCIENT DNA

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Text

From the establishment of reliable DNA extraction and sequencing protocols to application – an overview of working with powdery mildew reference collection specimens in a molecular era. Through this research it was recognised that powdery mildew reference collections should be considered as ancient DNA (aDNA) due to the degraded and fragmented nature of the specimen. This work included a methods comparison study in which 13 DNA extraction were tested on 25-year-old powdery mildew specimens to identify a consistent method which can recover high quality aDNA. Following this we investigated standard PCR, alongside next-generation library preparation and sequencing. Our results demonstrate that fragmented DNA library preparation together with Next Generation Sequencing (NGS) to be the best platform for analysing degraded aDNA. These methods were then applied to resolve taxonomic questions in two important horticultural crops for Australian biosecurity. Our findings clarified the pathogen status of these species in Australia and resulted in the description of a new powdery mildew species. Finally, genomic sequence data from powdery mildew specimens over a 116-year period was analysed to understand how powdery mildew DNA degrades over time in plant pathogen reference collections.

MOLECULAR CO-EVOLUTION OF POWDERY MILDEW FUNGI WITH THEIR HOST PLANTS

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Text

The powdery mildew fungi (*Erysiphaceae*) are globally occurring fungal pathogens of more than 10,000 plant species and pose a constant threat for agriculture. These pathogens are intimately connected with their host through their haustoria, where plant-fungus communication presumably takes place. Powdery mildew fungi deliver a range of effector proteins and likely also small interfering RNAs and transfer-RNA fragments to subvert plant immunity. Ubiquitously distributed transposable elements seem to play key roles in powdery mildew evolution. They make up >60% of the genomes of most powdery mildews and thus can be a powerful source of genetic variation and genome instability. Multiple sequence analysis of paralogous transposons in powdery mildew species for which high quality genome assemblies are available revealed recent and lineage-specific bursts of transposon activity. We found that transposable elements are transcriptionally active in the barley

powdery mildew pathogen (*Blumeria hordei*) at specific stages of infection. The pathogen dynamically regulates its abundant transposable elements via epigenetic mechanisms, RNA interference, and antisense lncRNAs. Antisense lncRNAs could be a mechanism to repurpose transposable elements and give rise to novel genes. Thus, transposable elements are the likely key drivers of adaptive evolution in powdery mildew fungi.

GENETIC ANALYSIS OF AVIRULENCE GENES IN POWDERY MILDEW

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Text
Race-specific resistance against wheat powdery mildew depends on the specific recognition of mildew avirulence genes by the host Pm (powdery mildew) resistance genes. To understand the molecular and evolutionary aspects of this interaction, we have isolated in the last years several *AvrPm3* and *Pm3* genes from the pathogen and the host, respectively. We have used map-based cloning in biparental mapping populations of diverse isolates, as well as genome-wide association analysis to isolate Avr genes. Recently, we have established an efficient protocol for UV mutagenesis in mildew. Using host plants with specific resistance genes as selective hosts, loss-of-avirulence mutants were isolated for a number of alleles of the *Pm3* resistance gene. Based on whole-genome sequencing we identified the underlying mutations and we found a diversity of molecular changes, including point mutations, as well as smaller and larger delections. The mutagenesis protocol can now be applied to additional avirulence genes. In particular, we want to study the avirulence components of the powdery mildew pathogen recognized by the recently described race-specific immune receptors which do not belong to the NLR type of proteins.

STANDING GENETIC VARIATION IN AVIRULENCE EFFECTORS UNDERLIES THE RAPID RESISTANCE BREAKDOWN OF TWO INTROGRESSED RYE RESISTANCE GENES IN WHEAT

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Text
Crop wild and domesticated relatives are valuable sources of new resistance gene specificities against fungal plant pathogens. The durability of such resistance gene introgressions is highly variable, a phenomenon that remains poorly understood mainly because the corresponding avirulence effectors are unknown. Until their breakdown, the resistance genes *Pm8* and *Pm17*, located on independent rye to wheat translocations, conferred resistance against the wheat powdery mildew disease caused by *Blumeria graminis* f.sp. *tritici*. We used genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping in mildew to identify the corresponding *AvrPm8* and *AvrPm17* effectors both encoding small, secreted proteins that are highly expressed during the early stages of

infection. Diversity analysis in powdery mildew collections from major wheat growing areas as well as related powdery mildew lineages revealed that several gain-of-virulence variants of *AvrPm17* and one variant of *AvrPm8* are likely ancient and predate the introgressions of *Pm17* and *Pm8* from rye to wheat. We concluded that standing genetic variation can underlie rapid resistance breakdown of introgressed resistance genes. Our studies demonstrate the relevance of pathogen-based genetic approaches to understand resistance gene durability. We, therefore, argue that the effort to identify durable resistance genes cannot be dissociated from studies of pathogen avirulence genes.

HYDROXYCINNAMIC ACID AMIDE ACCUMULATION AND PR-PROTEIN ENCODING GENE EXPRESSION ARE MAJOR RESPONSES OF WHEAT DURING THE EARLY STAGES OF POWDERY MILDEW INFECTION

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Text

Blumeria graminis f.sp. *tritici* (*Bgt*) is an obligate biotrophic fungal pathogen responsible for powdery mildew in bread wheat (*Triticum aestivum* L.). During the first steps of this pathogenic interaction, basal defense mechanisms take place in wheat leaves. We used RT-qPCR and metabolomic approaches to analyze these early stages of the interaction between *Bgt* and the moderately susceptible wheat cultivar Pakito. The expression of genes encoding pathogenesis-related (PR) proteins (PR1, PR4, PR5 and PR8), known to target the pathogen, increased during the first 48 hours post-inoculation. Moreover, RT-qPCR and metabolomic analyses pointed out the importance of the phenylpropanoid pathway in quantitative resistance against *Bgt*. Among metabolites of this pathway, hydroxycinnamic acid amides containing agmatine and putrescine as amine components accumulated from the second to the fourth day after inoculation. This suggests the involvement of these metabolites in quantitative resistance via cross-linking processes in cell wall for reinforcement, what is supported by the up-regulation of PAL (phenylalanine ammonia-lyase), PR15 (encoding an oxalate oxidase) and POX (peroxidase) further inoculation. This study increases knowledge on basal resistance in wheat, giving new targets to investigate induced resistance in wheat towards *Bgt*.

POWDERY MILDEW ON BLUEBERRIES: TRACKING THE WORLDWIDE SPREAD OF A FUNGAL PATHOGEN IN SPACE AND TIME

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Text
Herbarium specimens can be an opportune source of genetic material that can shed light on the recent past, and sequencing them can be a valuable tool for reconstructing the spread of pathogens. In response to a novel epidemic, it is not always known what the causative agent is and, in the case of a newly introduced pathogen, there is often a lag between detection and identification, and between identification and ascertaining the pathway of movement.

Erysiphe vaccinii is a North American powdery mildew species that infects *Vaccinium* species and up to the present, has never been reported outside of North America. *E. vaccinii* is an important obligate pathogen since it can cause damage to the blueberry industry, especially in an era of reduced fungicide availability/activity. Recent inquiries, in collaboration with a proprietary breeding, marketing and distribution berry company, has found *E. vaccinii* in all other parts of the world (China, Morocco, Mexico, Peru, Portugal, and South Africa) where it is causing damage to blueberries by reducing yields, and causing an increased need for fungicide applications. Understanding the pathogen diversity and geospatial distribution is also key for successfully deploying host genetic resistance.

In this study we took a multi-locus phylogenetic approach on herbarium and fresh specimens collected throughout the world to ascertain the spread of this pathogen in space and time as well as to determine its resistance to commonly used fungicides

Rice diseases

FUSARIUM INCARNATUM AND EQUISETI SPECIES COMPLEX ASSOCIATED WITH RICE BAKANAE DISEASE

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Text
Symptomatic rice plants were found with adventitious roots, thin stems, yellowish leaves, and tall plant-like bakanae disease during a series of surveys conducted in rice-growing areas of peninsular Malaysia and Bangladesh from 2019 to 2022. The pathogens were identified using morphological characteristics, DNA sequences, and phylogenetic analyses of two genes, namely, tef1-α and rpb2. A total of 50 isolates recovered from diseased stems of rice, adventitious roots, roots, leaves, and grains showed morphological features that resembled Fusarium incarnatum-equiseti species complex (FIESC). The identity of the isolates was further determined up to the species level by comparing DNA sequences and phylogenetic analyses of two genes. The phylogenetic analyses of the combined dataset of tef1-α and rpb2 clarified that all the isolates obtained were species within FIESC. The results of

pathogenicity tests revealed that isolates of Fusarium incarnatum are pathogenic toward rice. Leaves of inoculated plants became yellowish, and plants stunted. Mostly FIESC is typical saprophytes, endophytes, or opportunist pathogens associated with plants. Species delimitation and their pathogenic traits within the FIESC are still lacking. The aims of the present study were to identify the species, determine the phylogenetic linkage among the different species of FIESC commonly occurring in rice and assess their ability to cause diseases in rice.

COMPARED ANALYSIS OF PATHOGENICITY OF RICE BLAST FUNGUS MAGNAPORTHE GRISEA

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Text

To master pathogenic characteristics of *Pyricularia oryzae* is the premise and foundation of screening germplasm, breeding resistance, and deploying rice varieties resistant to blast. In this study, total the 166 isolates of rice blast fungi were collected from Japonica rice planting area in Jilin Province, northeast China, and were inoculated to the 7 Chinese Differentials Varieties (CDVs) and the Monogenic Differential Varieties (MDVs) which harbored 23 resistance genes in greenhouse condition.Those isolates were categorized into 7 groups and 44 races according to the CDVs'phenotypes, the dominant Chinese race group was ZA, and the frequency was 45.18%, the dominant races were ZA17(19.28%), ZG1(9.64%), ZB31(7.83%), and ZA25(6.63%), respectively. No dominant race types were demonstrated according to the MDVs' phenotype data, but the sub-groups data were prominent, and the higher sub-groups and frequency were U73(47.0%), i5(31.9%), i7(31.9%), U53(24.7%), k177(21.5%), and ta330(21.7%), respectively. Based on the avirulent gene(s) frequency of Avr*Pi12* (74.70%), Avr*Pi20* (72.29%), Avr*Pi19* (68.67%), Avr*Piz-t* (68.07%) and Avr*Piz* (60.45%), it presented that broad spectrum genes were *Pi12*, *Pi20*, *Pi19*, *Piz-t*, and *Piz*, respectively. The information of dominant avirulent genes type of blast fungi not only presented the pathogenicity, but also reflects the varieties broad spectrum resistance gene types, so it would be benefit for breeding resistant to rice blast.

VARIABILITY AND EVOLUTION INSIGHTS IN XANTHOMONAS ORYZAE PV. ORYZAE TO DETERMINE THE SUSTAINABILITY OF GENETIC RESISTANCE

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Text

Study of diversity and dominance pattern within existing pathogen population helps to identify, select, and deploy effective and sustainable resistance genetics in target

geographies for better and long-lasting product performance. Xanthomonas oryzae pv. oryzae (Xoo) is a major rice pathogen, and its genome harbors extensive inter-strain and inter-lineage variations. We studied pathotype variation in rice bacterial leaf blight pathogen-Xoo strains from India, and performed population based whole genome sequencing and analysis of 100 diverse Xoo strains to understand relationship between pathotypes and genomic lineages. Pathotype analysis of 1024 Xoo strains revealed 11 distinct groups, dominated by 4 major ones while others have restricted distribution. Genomic analysis revealed a major clonal lineage with predominant population and four minor but highly diverse lineages among Indian Xoo strains. This study allowed us to gain insights into the origin of lineages, pathotypes and highly pathogenic strains from India, and further, we were also able to identify core effector genes in Xoo strains. Specific mutations in the effector genes are clearly associated with an ability to cause disease on corresponding resistance genes containing cultivars. Such mega-genomic studies and resources are highly valuable in surveillance of the plant pathogen and to track their diversity and movement, thereby effective deployment of durable resistance genetics.

CHARACTERIZATION OF VIRULENCE FACTORS POTENTIALLY INVOLVED IN HOST-SPECIFICITY OF MAGNAPORTHE ORYZAE ISOLATES

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Text

The blast fungus *Magnaporthe oryzae* is a threat to global food security causing high yield losses in rice and wheat production. Molecular analyses identified multiple divergent lineages and sub-divisions within the pathogen population, which are preferentially associated with particular host genus.[1] Understanding the molecular basis for host preferences is crucial to improve disease management and prevent future spread to new hosts. Comparative genomic analysis has been used to enquire for differences within the genomes of different isolates and this led to the identification of potential host-specific genes. However, experimental proof for these virulence factors and their molecular function is mainly lacking. To close this gap, we established a toolbox for in-depth characterization of such candidate genes. Actually, we investigate two gene candidates: a Nudix-hydrolase present in most *Magnaporthe*-isolates except those infecting wheat, and a trehalase solely present in wheat-isolates. Confocal laser-scanning microscopy confirmed that both genes are secreted and qPCR pointed to a precise regulation during the infection process. To determine a contribution to host specificity of *M. oryzae*, we utilized CRISPR/Cas9 mediated marker-free genome editing and generated mutants with gene deletion, complementation, *in locus* mRFP-tagging and overexpression. Impact of these genetic modifications on fungal virulence was observed for both genes.

[1] DOI:10.1128/mBio.01219-17

RESPONSE OF CUBAN CULTIVARS TO DIFFERENT ISOLATES OF PYRICULARIA ORYZAE

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Text

Blast rice caused by Pyricularia oryzae Cavara, is the main fungal disease of rice (Oryza sativa L.), which has been widely studied due to its vast distribution and destructiveness throughout the world. In Cuba, blast rice affects during all planting seasons and the high variability of cultivars to the breeds of this phytopathogenic fungus confirms that in our country there are cultivars resistant to certain breeds. At present, the western zone, specifically the province of Pinar del Río is the one with the highest incidence of the disease. That is why the improvement strategy to minimize escape includes the selection of lines and new cultivars are traditionally carried out in this province, where its populations and inoculum pressure remain high as well as pathogenic diversity. In this work, the behavior of 14 rice cultivars that are currently used in Cuba with a variable behavior before the attack of this disease compared to resistant controls was evaluated in beds of infection. The isolation and morphological characterization of isolates of Pyricularia oryzae was performed. The in vitro effect of a chitosan on mycelial growth of the fungus was observed. The results showed that three cultivars behaved as highly susceptible. The isolates were characterized morphologically.

EVALUATION OF THE INCIDENCE OF PIRICULARIA ORYZAE ON RICE PLANTS TREATED WITH CHITIN DERIVATIVES AT A HOT SPOT SITE IN CUBA.

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Text

Pyricularia oryzae is one of the most important diseases in rice crop in the world and Cuba. In this work, seeds of rice (Oryza sativa L.) from J-104, a Cuban susceptible cultivar to Pyricularia oryze, were treated with partially acetylated chitosan at 100 and 250 mg L-1 and hydrolyzed chitosan at 100 mg L-1 and then planted in the field at "Caribe" rice farm, a high hot spot site for Pyricularia oryzae infestation. Plant cultivation was done in favorable conditions for disease development. Blast incidence on rice leaves in the vegetative stage at 35 days after planting and blast incidence in the panicle neck at 85 days were evaluated according to the standard scale proposed by the International Rice Research Institute. No chitosan derivative was effective to protect plants from treated seeds at vegetative stage, however, hydrolyzed chitosan at 100 mg L-1 made plants tolerant to P. oryzae at reproductive stage, which was associated with the size of chitosan. Several symptoms of infection by P. oryzae were shown in both stages, so different strains occurred in this hot spot site.So, this site can be a good place for conducting assessments of rice cultivar resistance to P. oryzae and evaluating in vivo elicitors against this fungus in the future.

POST-TRANSLATIONAL MODIFICATION OF OSWRKY31 ARE INTEGRAL TO OSMKK10-2-MEDIATED PATHOGEN DEFENSE OVER GROWTH IN RICE

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Text

The mitogen-activated protein kinase (MPK) cascade plays vital roles in plant innate immunity, growth, and development. Here, we report that the activation of OsMKK10-2 enhances resistance against the rice blast pathogen *Magnaporthe oryzae* and suppresses root growth through the increase of jasmonic and salicylic acid accumulation and decrease of indole-3-acetic acid levels. The transcription factor gene *OsWRKY31* is a key component in OsMKK10-2-OsMPK3-OsWRKY31 cascade involved in plant disease resistance and root growth in rice, as knockout of *OsWRKY31* transcription factor gene compromises most of the responses activated by OsMKK10-2. Phosphomimetic OsWRKY31 has an elevated DNA-binding activity and confers enhanced resistance to *M. oryzae*. In addition, OsWRKY31 stability is regulated by phosphorylation and ubiquitination for fine-tuning the defense and growth balance. Taken together, our findings indicate that OsWRKY31 can physically interact with OsMPK3 and its upstream OsMKK10-2, forming a ternary complex, and OsWRKY31 modified by phosphorylation and ubiquitination functions in OsMKK10-2 mediated signaling pathway for defense priority.

THE GENETIC VARIATION OF FUNGAL EFFECTOR AVR-PIK AND COMPARATIVE PATHOGENICITY OF RICE BLAST FUNGUS POPULATIONS IN THAILAND.

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Text

The blast fungal effector *AVR-Pik* in Thailand exhibits high variability, with 4 of 6 alleles identified (*AVR-PikA*, *D*, *E*, and a novel allele *F*). Isolates containing 2 alleles, *D/F*, are also detected. Allele *F* is unable to recognize by any *PiK* resistance allele, while the *D/F* combination can only infected rice harboring *Piks*. In this study, isolates carrying *AVR-Pik*, those carrying *AVR-PikA*, *D*, *F*, or more than 1 allele (THL794, UBN207129, RBR55004), showed similar pathotype as previous results. The isolates UBN207129 and RBR55004 carry *AVR-Pik* alleles that are recognized by all *Pik* alleles. Therefore, PCR product of *AVR-Pik* from each isolate containing more than 1 allele was cloned and 10 white colonies/isolate were sequenced and analyzed. Sequence analysis showed that UBN207129 and THL794 encoded AVR-PikD, while RBR55004 encoded 9 AVR-PikD and 1 AVR-PikA, which is the first report of this combination in Thailand. Analysis of pathotype in 41 isolates from different regions showed that 9 isolates had a similar pathotype to isolates containing *AVR-PikD*, *F*, and *D/F*, while 32 isolates showed different pathotypes from previously reported. Future research will focus on the role of *AVR-PikD* in isolates containing *D/A* combination in relation to *Piks* resistance gene.

RELIABLE INOCULATION PROTOCOL FOR STUDYING PANTOEA ANANATIS IN RICE

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Text

Bacterial leaf blight-like symptoms were observed on several rice genotypes (*Oryza sativa*) in research field plots of global rice germplasm grown in Arkansas, USA in August of 2021 and 2022. We demonstrated that the disease, which was characterized by spreading lesions on leaves, panicle sterility and reduced yield, was caused by *Pantoea ananantis*, a pathogen not previously described on rice in the USA. The bacterial pathogen isolated from symptomatic plants forms uniform, distinct, yellow-colored bacterial colonies on nutrient agar. Amplicons of the 16S rRNA and *gyrB* genes of the rice *P. ananatis* isolate are 100% identity with the corresponding regions of the *P. ananatis* PA13 reference genome. Pathogenicity of *P. ananatis* to rice was confirmed by scissor-clip inoculations of leaves from seedlings (cultivar Kitaake), and re-isolation of *P. ananatis* from the symptomatic inoculated leaves. While leaf blight disease associated with *P. ananatis* on rice has been reported in other regions of the world, studying the disease interaction in rice plants has proved difficult because methods for inoculating and consistently surveying symptoms are lacking. Here, we report a reliable assay for inoculating plants with *P. ananatis* and characterizing symptom development throughout disease progression on several commonly grown rice cultivars.

SPATIOTEMPORAL MONITORING OF MULTIPLE RICE DISEASES IN WESTERN BURKINA FASO: HIGH FREQUENCY OF CO-OCCURRENCES AND RAPID TURNOVER OF VIRAL POPULATIONS

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Text

Understanding the spatiotemporal dynamics of diseases and identifying risk factors is of paramount importance in guiding the deployment of effective control measures.
We conducted the present study between 2016 and 2019 in western Burkina Faso in three geographical areas, each of which included one irrigated perimeter, and a nearby lowland. Symptom monitoring annually at the same developmental stage showed that bacterial leaf streak (BLS) disease and rice blast were preferentially found in irrigated areas, compared to rainfed lowlands. High levels of co-occurrences were found between the four rice diseases under study, particularly in irrigated areas.
The rice yellow mottle disease, due to the RYMV, is not affected by the rice production system, but instead by the specific site. Partial sequencing of a set of 132 samples revealed a very high genetic diversity, with the co-occurrence of four distinct lineages at small geographical scale. One of these lineages results from a recombination event between two others. Temporal dynamics suggest an evolution of the relative frequencies of each genetic lineage within the studied period. Aggressiveness estimates were obtained from

experimental infections, and differences between isolates likely contribute to explain observed RYMV population evolution.
The implications of these results, including the potential consequences of co-occurrences and co-infections for the evolution of pathogen populations, will be discussed.

EVALUATION OF THE BEHAVIOR OF 49 LINES OF RICE (ORYZA SATIVA L) INTROGRESSED WITH KNOWN RESISTANCE GENES AGAINST RICE BLAST IN IRRIGATED AND RAINFED RICE GROWING SYSTEMS IN BURKINA FASO

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Text
Rice blast caused by Magnaporthe oryzae is one of the major rice diseases in Burkina Faso with losses up to 77% under favorable disease conditions. For the management of this disease, the use of resistant cultivars remains the most economical, and most protective method for the environment. This study focuses on the evaluation of the resistance of 49 lines of rice resulting from crosses between popular cultivars of different countries of Sub-sahara against blast. The experimental design used is a 7 x 7 Alpha lattice with 3 repetitions. The study was conducted in two rainfed sites (Farako-Bâ and Karfiguela) and two irrigated sites (Bagré and Tengrela) in Burkina Faso. The results showed that the rice genotypes developed the disease differently depending on their developmental stages andRice growing systems. In rainfed rice cultivation, 32 genotypes were resistant to leaf blast and 03 (AR-67, IR 130412 and CSR 36) were resistant to leaf and panicle blast. In irrigated conditions, 44 genotypes were resistant to leaf blast and 06 (TZLR-74, IR 133136-B, NERICA 4, NERICA 10, NERICA 11 and CSR 36) were resistant to leaf and panicle blast. The genotype (CSR 36) was disease resistant in both ecologies. The results of this study will make it possible to choose the best rice cultivars, tolerant or resistant to blast, and to identify the effective resistance genes in their genomes.

THE SUSTAINABILITY OF THE RESISTANCE OF ORYZA SATIVA VAR CHHOMRONG DHAN TO PYRICULARIA ORYZAE AND ITS IMPACTS ON RAINFED RICE CULTIVATION IN THE VAKINANKARATRA REGION.

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Text
In Madagascar, rice is the staple food of the population. With the increase in population, rice cultivation in lowlands has become insufficient. Therefore, rainfed upland rice farming has

gained importance.
Since the 1990s, different varieties of rainfed upland rice have been made available to farmers. Unfortunately, their resistance to blast disease was overcome by Pyricularia oryzae populations after a few years of cultivation. But since 2006, a Nepalese variety called Chhomrong Dhan (ChD), which was first tested at the FOFIFA Antsirabe research station in the mid-1990s, has been massively deployed. Thanks to its resistance to blast disease, it was quickly adopted by farmers and covers 85% of the land dedicated to rainfed upland rice in 2017.
Studies conducted by Raveloson et al. from 2014-2017 suggest that ChD displays resistance to P. oryzae, that the strains attacking ChD panicles come from neighboring varieties by spill-over at late stage, but no pathogen population has yet adapted to ChD.
The resistance of ChD reduces the pressure of the disease on rainfed upland rice cultivation in the Vakinankaratra region. Rice blast incidence survey on ChD and neighboring varieties will be continued. The genetic diversity of P. oryzae will be characterized to monitor the evolution of its population structure over the years, on ChD and neighboring varieties. Finally, the genetics of resistance of ChD will be characterized by crossing with susceptible varieties and genetic mapping.

GENE PYRAMIDING FOR ENHANCED BLAST RESISTANCE INTO SPANISH RICE CULTIVARS

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Text
Blast disease, caused by the fungus Magnaporthe oryzae, greatly reduces yield and grain quality and can ruin field crops. Blast disease is a complex trait and preventive treatment with fungicides is the most common method to control the disease, but this is coming to an end due to the restrictions in the use of phytosanitary products and the increasing social demand for a healthier agriculture. Integrated management of crop diseases relies on varietal resistance, agronomic practices, and application of pesticides. In this sense, varietal resistance has a crucial role. Race specific resistance is mediated by R-genes. Breeding for resistance is carried out for a long time and there are a few examples of durable resistance to blast, combining several specific resistance genes and some level of partial resistance. Results from previous research projects allowed us to identify effective R-genes in Spain and served as the basis for a breeding program to introduce R-genes into local varieties. We have generated two highly yielding varieties, displaying long and medium grain size each, by pyramiding the R-genes Pi-ta, Pi-b and Pi-km. Field trials under favourable infection conditions during several years in two different regions of the country, showed that these new varieties are resistant to the blast races present in Spain. These new varieties will contribute to reduce the impact of disease by minimizing the use of fungicides and to the sustainability of the crop.

DISTINCT ALLELIC AND GENOTYPIC STRUCTURES FEATURED BY XIAN/GENG TYPE RESISTANCE GENES RESPONSIBLE FOR RICE BLAST BETWEEN THE SOUTHERN AND NORTHEASTERN REGIONS OF CHINA

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Text

Rice blast, caused by *Magnaporthe oryzae*, it is a major hurdle in the productivity of rice (*Oryza sativa*). Asian rice includes two major subspecies: *Xian*/*indica* and *Geng*/*japonica*, resistance genes (*R*) have been recognized in both subspecies, which can provide a foundation to breed improved cultivars with better invulnerability against the pathogen.Having employed a set of updated comprehensive functional nucleotide polymorphic (FNP) marker systems for deeper allele mining at nine loci, at least 37 candidate functional alleles were identified in both Southern and Northeastern populations in China. As for the functional/non-functional haplotypes at the nine *R* gene loci, *Pid2*, *Pid3*, *Pit*, *Pib*, and *Pi63 w*ere strictly diverged into *Xian* type cvs in the Southern population, and *Pid4*, *Pi54*, *Pi36*, and *Pi37* into *Geng* type cvs in the Northeastern population. Similarly, the candidate functional alleles at the former five loci were almost predominant in the *Xian*, and those at the latter four loci in *Geng* populations. The distinct allelic and genotypic structures between the Southern and Northeastern rice populations in China, which were featured by such *Xian* and *Geng* type *R* genes, will be discussed.

MOLECULAR IDENTIFICATION OF XANTHOMONAS ORYZAE PV. ORYZAE AND BACTERIAL LEAF BLIGHT RESISTANCE GENES XA5, AND XA21 IN BENIN

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Text

In Benin, Xanthomonas oryzae pv. Oryzae (Xoo) causing bacterial leaf blight (BLB) was first described in 2013 on wild rice. So far, no study has been done on Beninese Xoo strains to better characterize these strains. We do not know whether the pathogen has already passed into the cultivated rice varieties and what the disease index is.This study aims to identify Beninese Xoo strains and screen rice varieties grown in Benin for the major BLB resistance genes Xa5, and Xa21. Diseased rice leaves collected from different rice fields in the three phytogeographic areas of Benin were analyzed by PCR for Xoo-specific sequence identification. Furthermore, collected rice accessions were screened by PCR to identify resistance genes Xa5 and Xa21. The results reveal that Xoo was identified in Banikouara and Malanville. Sphingomonas Sp has been identified in several rice fields in Benin. Rice fields infected with Xoo are also coinfected with Sphingomonas Sp. Of the 61 rice accessions screened for the Xa5 resistance gene, 10% are resistant, 53 87% are susceptible and 3% lack the xa5 resistance gene. For Xa21, 60.65% were found to be resistant of which 28 were homozygous (Xa21/Xa21) and 9 were heterozygous (Xa21/xa21). Three accessions possess both xa5 and Xa21 genes. These results indicate that Xoo has moved from the wild rice variety to the cultivated variety in northern Benin. Consequently, a varietal improvement program must be put in place to prevent a BLB pandemic in Benin.

GENOMEWIDE ANALYSIS OF TRANSCRIPTIONAL NETWORKS UNDERLYING RICE RESPONSES TO THE DISEASE BACTERIAL PANICLE BLIGHT AND HIGH NIGHT TEMPERATURES

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Text

Bacterial Panicle Blight (BPB) caused by Burkholderia glumae has impacted rice production worldwide. Outbreaks of BPB have been associated with high night temperatures, suggesting that BPB incidence can be exacerbated by the increases in global temperatures. To understand the effect of high night temperature on BPB, this work screened 20 accessions from the USDA mini-core collection to evaluate BPB disease symptoms in rice panicles in response to B. glumae inoculation under low and high night temperatures. The screening revealed a wide spectrum of disease symptoms in the panicle among accessions. Interestingly, depending on the accession, the severity of symptoms was temperature-dependent or temperature-independent. Within the temperature-independent accessions, we identified susceptible and moderately resistant accessions. To further understand the transcriptional networks controlling rice resistance or susceptibility to BPB, one susceptible accession and one moderately resistant accession were chosen for RNA-sequencing analyses. Comparative transcriptomics analyses between the moderately resistant and the susceptible accession after B. glumae inoculation, uncovered jasmonic acid (JA) biosynthetic and JA-regulated genes, as well as genes involved in cell wall remodeling and disease resistance. These findings highlight the function of these genes and pathways regulating and executing rice responses to BPB in combination with heat stress.

MULTIPLE MUTATIONS IN SDHB AND SDHC SUBUNITS CONFER RESISTANCE TO THE SUCCINATE DEHYDROGENASE INHIBITOR CYCLOBUTRIFLURAM IN FUSARIUM SPP.

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Text

Fusarium fujikuroi is one of the dominant phytopathogenic fungi causing rice bakanae disease worldwide. Cyclobutrifluram is a novel succinate dehydrogenase inhibitor (SDHI) developed by Syngenta, which shows strong inhibitory activities against *Fusarium* spp. The baseline sensitivity of 112 *F. fujikuroi* to cyclobutrifluram was determinated with a mean EC_{50}

value of 0.025 µg/mL. A total of 17 resistant mutants were obtained by fungicide adaptation and displayed equal or slightly weaker fitness than parental isolates, which suggests that the resistance risk of *F. fujikuroi* to cyclobutrifluram is medium. A positive cross-resistance was detected between cyclobutrifluram and fluopyram. The amino acid substitutions H248L/Y of FfSdhB and G80R or A83V of $FfSdhC_2$ conferred cyclobutrifluram resistance in *F. fujikuroi*, which was validated by molecular docking and protoplast transformation. We also found the point mutations H248Y in FpSdhB and A83V or R86K in $FpSdhC_1$ conferred the resistance of *F.pseudograminearum* to cyclobutrifluram. The results indicate that the affinity between cyclobutrifluram and Sdhs obviously decreased after point mutations, therefore multiple mutations in SDHB and SDHC subunits confer resistance to cyclobutrifluram in *Fusarium* spp.

MULTIPLE INFECTION STRATEGIES UNCOVERED BY FUNCTIONAL STUDIES OF EFFECTOR PROTEINS IN USTILAGINOIDEA VIRENS

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Text

Rice false smut is a devastating disease caused by the fungal pathogen *Ustilaginoidea virens*. Effector proteins are known to play a critical role in pathogen virulence, but molecular mechanisms underlying virulence functions of *U. virens* effectors remain largely unknown. Our results identify three *U. virens* core effector proteins SCRE1, SCRE4, and SCRE6 via large-scale screening and knockout analyses. We reveal that SCRE6 is a tyrosine phosphatase, which represents the first identified phosphatase in fungal effector proteins. The effector targets the rice immune negative regulator OsMPK6 and specifically dephosphorylates OsMPK6. The dephosphorylation inhibits OsMPK6 degradation and promotes the accumulation of the negative immune regulator, and thus suppressing plant immunity. Additionally, we show that the SnRK1A-XB24 phosphorelay module positively contributes to plant immunity. SCRE1 inhibits the ATPase activity and phosphorylation of XB24 mediated by SnRK1A, thereby suppressing the rice immune response. We also demonstrate that SCRE4 is an essential virulence effector that transcriptionally suppresses the expression of *OsARF17*, a positive immune modulator in rice, and thus inhibiting plant immunity and promoting pathogen invasion. These studies reveal previously unidentified fungal infection strategies and provide insights into potential targets for improving rice disease resistance.

SOWING DATES INFLUENCE INCIDENCE OF BACTERIAL LEAF STREAK AND RICE YIELD UNDER IRRIGATED PRODUCTION SYSTEMS IN BURKINA FASO

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Text
Bacterial leaf streak caused by Xanthomonas oryzae pv. oryzicola is a disease that is prevalent in the main rice-growing sites of Burkina Faso. It causes foliar incidences of up to 100% on some crop varieties and is variable from season to season. Control methods include the use of resistant varieties, integrated pest management and good cultural practices, including sowing dates. Indeed, data of the influence of sowing dates on the disease incidence are not well known, especially under the rice cultivation conditions in Burkina Faso. In order to identify sowing dates that would reduce BLS incidence on irrigated plains, we set up a trial in the Kou Valley and Di, using a split-plot design with three rice varieties with susceptibility contrasting levels, at three different date of transplantation. The results show that among the three evaluation dates, the sowing in June is the best because it avoids a high incidence of BLS and allow to obtains a better average yield of the three varieties with 8.10 t/ha and 7 t/ha respectively in the both sites. At June sowing date, infection is less severe for the two susceptible varieties FKR62N and TS2, ranging from 0.33 to 1.93 at Kou Valley compared to 8.06 to 10.16 at Di. However, when sown in August, BLS incidence was higher with a yield reduction of at least 3t/ha compared to June sowing. Therefore, the early planting dates can be recommended for effective control of BLS under irrigated rice growing conditions in Burkina Faso.

EMERGENCE OF XANTHOMONAS ORYZAE PV. ORYZAE IN MADAGASCAR: EPIDEMIOLOGY AND IDENTIFICATION OF RESISTANCE SOURCES TO CONTROL BACTERIAL LEAF BLIGHT OF RICE

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Text
In Madagascar rice is the staple food grain. In this country where the consequences of climate change are dramatic, rice production represents a major challenge for food security. Bacterial Leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a devastating disease of rice crops in many countries of Asia and West Africa. In Madagascar, despite a continuous monitoring for rice diseases since the 1980s, BLB had never been reported until recently. In 2019, BLB-like symptoms were observed in two fields in the Central Highlands of Madagascar. Strains were isolated and their identity confirmed as *Xoo*. Since then, regular surveys allowed to observe the dispersion of the disease in different geographical areas of the island and a sharp increase in incidence. 92 malagasy strains collected between 2019 and 2022 were genotyped with the aim to 1) evaluate if this outbreak results from one or several introductions, 2) define its origin and 3) date the introduction. A Multilocus VNTR Analysis (MLVA-14 scheme) suggests a recent unique introduction from Asia and highlights a large clonal expansion from primary founders. Whole genomes of 2 malagasy strains confirm and precise the Asian origin. Strains representative of the diversity were selected for pathogenicity tests, which allowed to identify 4 potentially effective resistance genes. Overall, we show that efficient surveys, genotyping, effector sequencing and phenotyping provide tools to rapidly react to new outbreaks.

ALLELE SPECIFIC RECOGNITION OF THE MAGNAPORTHE ORYZAE EFFECTOR AVR-PITA BY THE UNCONVENTIONAL RICE RESISTANCE PROTEIN PTR

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Text

Blast disease caused by the fungus *Magnaporthe oryzae* is one of the most devastating rice diseases. To secure rice production from losses by blast, disease resistance genes are critical. *Pi-ta* is a widely used blast resistance gene. Published work reports that it codes for a nucleotide-binding and leucine-rich repeat protein (NLR) and recognizes the fungal protease-like effector AVR-Pita. However, this model was challenged by the recent finding that another rice gene named Ptr, which codes for a membrane protein with a cytoplasmic Armadillo repeat domain is required for AVR-Pita recognition. Using *NLR Pi-ta* and *Ptr* RNAi knock-down and CRIPSR-Cas9 mutant rice lines, we found that AVR-Pita recognition relies only on *Ptr* and that the *NLR Pi-ta* has no role. Analysis of the natural diversity of AVR-Pita, showed that different alleles of *Ptr* have different recognition specificities. While the Ptr allele B recognizes a restricted set of AVR-Pita alleles, PtrA detects all natural sequence variants of the effector. We confirmed the escape of certain AVR-Pita alleles from detection by PtrB using mutant and transgenic isolates of the fungus and identified one specific polymorphism that controls the break-down of PtrB-mediated resistance.
Taken together, our work establishs that the *M. oryzae* effector AVR-Pita is detected in an allele-specific manner by the unconventional resistance protein Ptr and that the NLR Pi-ta has no function in Pi-ta resistance and the recognition of AVR-Pita.

Tn-Seq to reveal microbial lifestyles along plant interaction processes

IDENTIFICATION OF BACTERIAL PLANT COLONIZATION GENES AT THE GENOME SCALE

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Text

Bacteria are critical drivers of plant health and performance, and therefore can make key contributions to sustainable agriculture. However, the molecular mechanisms enabling them to effectively colonize plants remain poorly understood. Traditional methods of studying

plant-bacteria interactions have sought to characterize the function of one gene at a time, but the slow pace of this work means that the functions of the vast majority of bacterial genes remain unknown or poorly understood.

Recently developed high-throughput techniques such as transposon sequencing (TnSeq) have been used to identify which are the genetic determinants that are involved in a function of interest. In our study we use RB-TnSeq (random barcode transposon-site sequencing), which combines the advantages of TnSeq and rapid quantification of each transposon mutant using unique barcodes that can be amplified by PCR, in order to identify a comprehensive set of microbial genes that control or influence root colonization.

We found numerous genes that, when mutated, affect the ability of bacteria to competitively colonize the rhizosphere of plants. Interestingly, several genes seem to be important for colonization across different bacterial species, while other genes seem to be specific for a given plant-bacteria interaction. The identification of bacterial genes shaping plant colonization will pave the way for the future development of innovative biostimulants for sustainable agriculture.

TN-SEQ REVEALED DIFFERENT LIFESTYLES OF PLANT PATHOGENS

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Text
Agrobacterium tumefaciens is a biotrophic pathogen that colonizes the galls (plant tumors) it causes, as well as the roots of host and non-host plants. Dickeya and Pectobacterium species are necrotrophic pathogens that macerate stems (blackleg disease) and tubers (soft rot disease) of Solanum tuberosum. They proliferate by exploiting the remains of plant cells. They also colonize roots, even if no symptoms are observed. In this review, we present our results on the use of Tn-Seq to reveal the different lifestyles of these pathogens when they colonize roots (pre-symptomatic lifestyles) or lesions (pathogenic lifestyles).

THE MAKING OF A PATHOGEN: HOW XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS ADAPTS TO PLANT ENVIRONMENTS

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Text
Xanthomonas campestris pv. *campestris* (*Xcc*) is a bacterial pathogen causing black rot disease on wild and cultivated Brassicaceae. *Xcc* starts its disease cycle on the surface of the leaf, after which it gains entry to the plants via the hydathodes. Next, *Xcc* colonizes the underlying epithem, xylem vessels, mesophyll and finally the plant seeds, which allow it to travel through plant generations. Despite the fact that the virulence mechanisms of *Xcc* have been studied since the early eighties, little is known about the genetic determinants that allow *Xcc* to adapt to the various environments it encounters during its disease cycle. In order to identify the genetic determinants of bacterial adaptation to the different plant environments, we performed high-throughput randomly barcoded-transposon insertion site sequencing (RB-TnSeq) screens i*n vitro* and *in planta*. Here, we discuss the results of these screens, which lead to a better understanding of the physiological state of the bacteria in different stages of the disease cycle.

Understanding the ecology and evolution of bacterial wilt disease in the plant microbiomes

HOST RANGE OF A NEW RALSTONIA PSEUDOSOLANACEARUM (PHYLOTYPE I) STRAIN DETECTED IN DUTCH SURFACE WATERS AND BITTERSWEET

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Text
Species of the *Ralstonia solanacearum* species complex (RSSC) cause bacterial wilt and are aggressive, contagious and persistent. As such, all species within the RSSC, including *Ralstonia pseudosolanacearum* (phylotype I), have a quarantine status. Until recently, *R. pseudosolanacearum* (phylotype I) was only sporadically found within the European Union, and the pathogen was absent from the aquatic environment within this region. However, starting from 2020, the bacterium has repeatedly been found in Dutch surface waters, as well as in bittersweet, a known host of *R. solanacearum* (phylotype II). Here, we used one of the original isolates from 2020 to determine the host range of this novel pathogen. We introduced a rifampicin resistance marker via homologous recombination and used the transformed strain to inoculate 24 different plant species from the *Solanaceae*, *Rosaceae*, and other plant families that are known or suspected hosts of the RSSC. Both cultivated crops and weeds were included in the assays. Young plants were stem inoculated, grown under controlled conditions and monitored for 8 weeks. In case of symptomatic or asymptomatic infection, the assays were repeated using soil inoculation. Research was supported by the Dutch Food and Consumer Product Safety Authority, Naktuinbouw, the Nederlandse Algemene Keuringsdienst, Glastuinbouw NL (Stichting Kennis in je Kas),

HZPC, BO Akkerbouw, Stichting Aardbeionderzoek, Deliflor Chrysanten and the Nederlandse Aardappelorganisatie.

EMERGENCE AND EVOLUTION OF A NOVEL LINEAGE OF RALSTONIA SOLANACEARUM WITH EXPANDED HOST RANGE

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Text

Members of the *Ralstonia solanacearum* species complex (RSSC) are historically known to cause brown rot of potato and Moko disease of banana in Central America. A novel lineage detected in the island of Martinique in 1999 exhibited a dramatically different host range and pathogenicity profile. This new lineage, referred to as 4NPB, can infect cucurbits *Anthurium* and *Heliconia* spp. and is more aggressive on solanaceous crops than previous variants. We sequenced 460 RSSC strains sampled across Martinique and French Guiana during the emergence of this novel lineage. Pangenome and phylogenetic analyses were performed to identify genomic changes linked with the emergence of the new lineage. This analysis reveals the 4NPB population likely emerged from a population of Moko-causing strains found in association with a tomato host on the mainland, followed by dispersal to Martinique. While 4NPB and Moko-causing strains are closely related, variation in the accessory genome includes the exchange of Type 3 secreted effectors and multiple genes with predicted catalytic activity. Recombination hotspots found between 4NPB and Moko strains include various toxin-antitoxin systems, which are potentially involved in intra-species competition and signaling. These changes may underly the altered pathogenicity and host-range profile of 4NPB, contributing to the dramatic expansion of this lineage across Martinique.

CONTACT DEPENDENT GROWTH INHIBITION IN RALSTONIA PSEUDOSOLANACEARUM

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Text

Ralstonia pseudosolanacearum is a deadly bacterial plant pathogen known to infect many plant species. Comparative genomics of a South Korean population of *R. pseudosolanacearum* revealed that recombination frequently targets secreted gene products including type III secreted effectors and CdiA proteins, similar to hemagglutinin. CdiA proteins vary in the presence of a C-terminal toxin domain that inhibits the growth of adjacent cells lacking cognate immunity protein CdiI, a phenomenon called contact-dependent growth inhibition (CDI). CDI mediates both antagonistic and cooperative contact-dependent interactions, contributing to the formation of populations sharing identical CDI loci. *R. pseudosolanacearum* carries an expanded set of CDI loci compared to Burkholderia spp.,

where their function is better characterized. My work examines whether these loci mediate contact-dependent interactions and contribute to phenotypes associated with virulence and cooperative behavior in *R. pseudosolanacearum*.

COMPARISON OF RHIZOSPHERIC BACTERIAL COMMUNITIES OF POTATO GENOTYPES WITH DIVERSE DEFENSE RESPONSES AGAINST RALSTONIA SOLANACEARUM.

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Text

Potato (Solanum tuberosum) is one of the most important widespread hosts of Ralstonia solanacearum, the causal agent of bacterial wilt. In Uruguay, germplasm with resistance to R. solanacearum has been identified and advanced clones with different responses to R. solanacearum infection were selected and characterized. Previous results showed that plant resistance was correlated with differential bacterial colonization patterns and induced defense responses after infection. The aim of this work is to study the correlation between plant resistance and rhizosphere microbiome in selected genotypes with different responses to bacterial wilt. Plants were grown in a macrotunnel greenhouse with soil collected from a potato field. Pathogen colonization effects on rhizosphere microbiota were evaluated in healthy and infected plants. Disease progression was recorded and pathogen was quantified in rhizosphere samples. A resistant genotype showed delay in pathogen colonization and high final pathogen concentration in the rhizosphere, comparable to the susceptible genotype. These results suggest that the resistant genotype restricts rhizosphere pathogen colonization, preventing root and stem infection. Bacterial community composition is being analyzed in 76 samples comparing the sequence of V3-V4 region of 16S rARN. It is expected to identify potentially beneficial microbial groups related with resistant plants, contributing to an integrated disease control.