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**BOOK OF ABSTRACTS**



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## **Innovative Smart Technologies for Agricultural Production and Plant Health**

TITO CAFFI, *Università Cattolica del Sacro Cuore*

Pests are defined by FAO and IPPC as “any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products”. They can cause enormous crop loss worldwide with damages that can occur in the field, from sowing to harvesting, and also in the storage. Secure food supply for the future generations requires environmentally safe and sustainable production. Main goal of the modern agriculture is to reduce intensive fertilizer and pesticide use and decrease heavy exploitation of natural resources (water, soil, energy). Precision agriculture (PA) is a collection of agricultural practices that focus on specific areas of the field at a particular moment in time. The main goal of precision agriculture can be summarized in doing “the right thing, at the right time in the right place”). With the recent scientific advancements, technological innovations and legislative tools it is nowadays possible to achieve these strategic goals and to increase sustainability in agriculture. In 2020, the International Year of Plant Health, the use of Smart Technologies can contribute to enhance plant health, reduce hunger and poverty, protect the environment, and boost economic development.

The actual availability of innovative tools and data management techniques, also leading to big data management and analysis requirement, allow to think about an integrated system that provides phytosanitary monitoring for some pilot crops which is effective, rapid, objective, repeatable in the most varied environmental contexts, and therefore suitable to provide appropriate support to the various phytosanitary control needs in a region or an area. It is possible to integrate into modern decision support systems information, protocol, guidance in order to allow trained personnel to carry out surveys and data collection, through information, alerts and guidelines (photographic or video supported) provided by the system.

This approach could lead to a complementary monitoring system and enhancement to the forecast models, an aspect that will allow to optimize and increase the information and alerts provided to the operator, guaranteeing a clearer and more aware picture of the phytosanitary and physiological state of the plant.

**The plant inside as a diverse microhabitat: communities, niches, colonization behaviour of beneficial endophytes and relation to plant growth and health.** S. COMPANT<sup>1</sup>. <sup>1</sup> *AIT Austrian Institute of Technology, Center for Health and Bioresources, Bioresources Unit, Tulln, Austria. E-mail: stephane.compant@ait.ac.at*

Plant host different fungal and bacteria in organs like roots, stem, leaves, fruits and seeds. Most of them derive from the soil and the rhizosphere as stated since the XIXth century, but other sources like the anthosphere, carposphere, phyllosphere, lamosphere and caulosphere can also lead to establishment of endophytes inside plant tissues. Other microbes can further derive from animals or from plants growing near each other. A thorough understanding of the communities, routes and niches colonization of endophytes in the phytobiome viewpoint has led to a better knowledge on how to use specific microbes for stimulating plant growth and increase host health but also to understand better how plants shape their microbiome. Most of the research has been done either on fungi or bacteria but recent studies further show that some bacteria can colonize beneficial fungi internally as endofungal bacteria and increase their beneficial effects on plants, leading further to a strategy of using multipartite interaction to boost plant protection.

**Session topic:** Microbiomes and their role in plant health

## **Plant Biology and Climate Emergency**

DIRK INZE<sup>1</sup> VIB-UGent Center for Plant Systems Biology, Belgium

The world is experiencing an unprecedented climate crisis that requires urgent action at all fronts. Plant biology offers great opportunities both for adapting to climate change as well as for mitigating the accumulation of greenhouse gasses.

It is expected that higher temperatures and longer drought episodes will greatly reduce crop Productivity in many areas including the Mediterranean basin. Understanding how plants rewire their growth and development in response of environmental cues such as drought is the first step towards breeding and/ or engineering stress tolerant crops. I will illustrate the challenges of achieving this ambitious goal by our research on drought responses in maize. The molecular and phenotypic comparison of drought response of maize plants cultivated in growth chambers, greenhouse and field conditions highlighted the importance of applying an experimental 'lab to field to lab' approach. Furthermore, drought responses involve many interacting genes. Genome editing offers great perspectives not only to tackle complex multi-genic traits such as drought but also to engineer drought tolerant crops.

Plants are fantastic organisms that evolved to efficiently use CO<sub>2</sub> for growth and development. The selection of plants with improved CO<sub>2</sub> sequestration capabilities, above ground and below ground, are likely to become a valuable tool to combat climate change. Obviously, such plants should be resilient to environmental stresses and show a low dependency on fertilizers and will discuss various approaches on how to develop such plants for climate emergency.



## **Genome editing: Enabling genome editing for European agriculture: will scientific evidence prevail?**

DIRK INZE<sup>1</sup> VIB-UGent Center for Plant Systems Biology, Belgium

Agriculture and food production must become more sustainable in a world facing a growing population under changing climate conditions and environmental degradation. All possible approaches, including improved plant breeding technologies, are essential to address these challenges. In order to develop new crop varieties, scientists and plant breeders need to have access to the widest possible array of breeding tools. The most recent addition to the toolbox is precision breeding, also known as genome editing with a preference for CRISPR. This technology allows for scientists and breeders to develop desired crop varieties in a faster, relatively simple and much more directed way compared to older breeding technologies.

However, there are legal and procedural uncertainties regarding the status of genome edited crops in Europe. While the CRISPR technology is being adopted at an unprecedented speed, the current regulatory framework remains outdated. Moreover, the European Court of Justice (ECJ) ruling from 2018 (C-528/16) brought even more confusion because of the interpretation that crops obtained by precision breeding are subject to the GMO regulatory provisions. This regulatory burden even applies to crops with the smallest, specific CRISPR-mediated DNA alteration, that can also occur spontaneously in nature.

The ruling leads to inconsistency in the legislation because of the fact that conventional mutagenesis -which results in thousands of random DNA alterations- is exempt from the GMO regulatory provisions. The legislation no longer correctly reflects the current state of scientific knowledge. Besides, subjecting genome-edited crops to the current EU GMO regulation will delay the development of climate-resilient crops, hinder progress in sustainable agriculture, reduce EU's competitiveness and hamper global trade.

The ruling hampers the cultivation of genome edited crops with beneficial traits for our health or the environment such as improved nutritional composition, improved digestibility, lower content of anti-nutritional components, reduced allergenicity or requiring less input.

The scientific community in Europe responded united and published a position paper and an open statement to call upon the European Commission and the European Parliament. With a growing number of signatories, reaching currently to 129 European research institutes and organizations, from 21 different Member States and the UK, the network EU-SAGE was launched. EU-SAGE stands for European Sustainable Agriculture through Genome Editing and aims to provide information about genome editing and to promote the development of European and EU member state policies that enable the use of genome editing for sustainable agriculture and food production. I will provide an update on the ongoing discussions with the European Commission. Hopefully scientific evidence will prevail.

## **Plant and seed priming for improved growth and abiotic stress protection under a changing climate**

VASSILIS FOTOPOULOS, *Cyprus University of Technology*

Increased frequency of extreme environmental events resulting from global climatic changes remarkably influences plant growth and development. Close examination of plant-to-plant communication in nature has revealed the development of unique strategies from plants for responding to abiotic stress, with one of the most interesting being through priming for improved defense responses. The process of priming involves prior exposure to a biotic or abiotic stress factor making a plant more tolerant to future exposure. Priming can also be achieved by applying natural or synthetic compounds which act as signaling transducers, 'activating' the plant's defense system. An up-to-date overview will be presented describing the research carried out at the Cyprus University of Technology using priming agents towards induced acclimation of plants to environmental challenges. In addition, recent findings will be presented on the evaluation of chemical compounds as well as nanoparticles that potentially display growth promoting properties in plants, closely related to our existing expertise and previous observations in priming against stress.

**Cypress Canker: a model pathosystem to study fungal invasions** M. GARBELOTTO<sup>1\*</sup>, G. DELLA ROCCA<sup>2</sup>, C. EYRE<sup>1</sup>, T. OSMUNDSON<sup>1</sup> and R. DANTI<sup>2</sup> <sup>1</sup> *Department of ESPM, 54 Mulford Hall, University of California, Berkeley, CA 94720, USA* <sup>2</sup> *IPSP, National Research Council CNR, Via Madonna del Piano 10, 50127 Sesto Fiorentino, FI, Italy*  
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Our understanding of invasions by plant pathogens lags behind compared to that of other biological invasions. Cypress Canker Disease (CCD) caused by the fungus *Seiridium cardinale* (Sc), has emerged as an excellent pathosystem to further our understanding of fungal invasions. By using a population genetics approach, we have shown that Sc is endemic to the interior of California, where the disease is expressed exclusively on exotic, off-site or artificially-bred cypress species. The early 1920s outbreak in the interior of California was responsible for two introductions: one in Italy and one in New Zealand. Coalescent analyses showed that Italian population of Sc played a major role in the diffusion of the pathogen throughout the Mediterranean. Phenotypic traits in Mediterranean invasive Sc populations indicate that small conidial size, high phenotypic plasticity and high sporulation potential are key traits for a successful invasion, while high virulence and high growth rate are not necessary. Comparative analyses indicate that geographically isolated *S. cardinale* populations are now genetically and phenotypically distinct, and by inoculating a range of California Sc genotypes on Italian cypress, we show that resistance to CCD can be eroded by California genotypes. Will a further Sc introduction occur? In a recent study, we show that within the last 20 years, the Mediterranean and California populations served as sources for new introductions in New Zealand and Morocco, respectively, thus proving additional introductions are a distinct possibility. In conclusion, our data prove that invasive populations cannot be regarded analogous to source populations that generated them.

## **CIHEAM Policy in Plant Health to Enhance Food Security in the Mediterranean Region**

A. M. D'ONGHIA<sup>1</sup>. <sup>1</sup>*Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Via Ceglie, 9 - 70010 Valenzano (BA), Italy.*

Most of pests and diseases affecting Mediterranean crops are seriously compromising food security and, consequently, the sustainability of rural populations in several countries in the Mediterranean region. The importance of Plant Health has been clearly highlighted by UN that has declared the 2020 as the International Year of Plant Health.

CIHEAM, an intergovernmental Organization of 13 Member States, has gained a long experience in plant health which is one of the main pillars of the CIHEAM Strategic Agenda 2025 – adopted in 2016 by the Ministries of Agriculture of CIHEAM Member States. It is approached through: training of researchers, officers, professionals; applied research linking local scientists with the international research communities; participatory governance that means encouraging discussions and interactions among scientific, institutional and private stakeholders; and cooperational developing programmes enhancing country capacity building and awareness raising.

The CIHEAM Institute of Bari, in particular, has more than 30 years of experience in plant health, implementing and financing numerous international research initiatives in the Mediterranean region and neighboring countries (e.g. Iran, Sultanate of Oman, Iraq). It is also engaged in several initiatives funded by the Italian Cooperation addressing plant health in several countries (Mediterranean, Balkan, African, Near eastern and Middle eastern countries). These initiatives have delivered institutional capacity building and technical assistance to hundreds of officials of national and local entities. Furthermore, in line with the statutory mission of CIHEAM Bari (higher education, research and development cooperation), since 1985 the Institute has delivered specialized and post-graduate diplomas on Integrated Pest Management to thousands of trainees coming from over 50 countries.

Particular attention of CIHEAM is posed on early surveillance, detection and control of transboundary pests and diseases which may seriously threat crops, environment and sustainability of rural populations (e.g. *Xylella fastidiosa*). The achieved results have demonstrated that it is possible to ensure the shift towards sustainable production if quarantine measures are strengthened and a comprehensive phytosanitary management is applied. CIHEAM initiatives are therefore focusing on harmonizing national rules with the EU technical/ phytosanitary/ legal standards, in order to safeguard agriculture and promote improvement in domestic and export trading of safer food in conformity with Governments' strategic plans.

Session Topic: European and Mediterranean Plant Pathology

## **Fungal pathogens of wood: are they a threat to Mediterranean fruit crops?**

VLADIMIRO GUARNACCIA<sup>1</sup> *University Of Torino*

Intensification, diversification and globalization regularly happen, causing a broad range of collateral effects on agriculture. The risks of disease dissemination via trade and the movement of goods and people is increasing. Moreover, climate change is also affecting plant health by modifying the interactions between host plants, pathogens and the environment. Fruit tree plantations are increasingly threatened by fungal diseases. It has been also observed how fruit trees share fungal pathogens with woody plants that are not considered to be trees. Both primary and opportunistic pathogens can cause various symptoms such as cankers, twig blight and wood rotting. *Diaporthe* and *Neofusicoccum* spp. are examples of pathogens able to infect a broad range of fruit trees. Moreover, there are numerous latent pathogens such as those in the Diatrypaceae family or in the *Cytospora* and *Neocosmospora* genera, and many others.

Recent findings about fungus/host combinations will be presented, with emphasize on the connection between species identification studies and the development of modern diagnostic tools. Moreover, factors such as cultivar diversity, propagation material health and rootstock selection, need to be investigated in relation with the pathogens, for providing the necessary support to the producers of a such high economic important agriculture sector.

**Current situation of *Xylella fastidiosa* impact in Spain: Main research initiatives ongoing to understand and tackle this pathogen.** B.B. LANDA. *Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Córdoba, Spain. E-mail: blanca.landa@ias.csic.es*

*X. fastidiosa* (Xf) was first reported in 2016 in Majorca, in the Balearic Islands, and in 2017 in Alicante province, Mainland Spain. Currently, three Xf subspecies and four STs have been detected in the Balearic Islands (subsp. *fastidiosa* ST1, subsp. *multiplex* ST7 and ST81, and subsp. *pauca* ST80). On the other hand, only Xf subsp. *multiplex* ST6 have been detected in the Valencian Community where the Demarcated Area covers an extension of >136,200 hectares, of which over 1,100 hectares, 12,500 orchards and 90,000 trees have been already eradicated as of November 2021. This talk will present the main research initiatives going on in Spain aimed to understand Xf epidemics and mitigate its impact not only in Spain, but also in Europe. These main research initiatives have contributed to fill knowledge gaps on Xf in Europe, gathering fundamental information on several aspects: i) Characterizing the genetic population structures of *X. fastidiosa* in the different EU outbreaks, including Spain, and linking the genetics of the bacterium with its pathways of entry, ii) testing and developing new diagnostic tools based on molecular and proximal- and remote-sensing approaches; iii) searching for new control tools targeting the bacterium in the plant or searching for host resistance, and iv) understanding the epidemiology and modeling disease development and developing risk analysis. All these results have contributed to provide elements to inform Spanish and EU policy related to Xf management at different spatial scale (from regional to EU level) and socio-economic contexts.

This research was financially supported by the PTI-SolXyl on *X. fastidiosa* from CSIC, Projects 727987 XF-ACTORS (H2020- UE), PID2020-114917RB-I00 (AEI-MICINN Spain and FEDER-EU), and E-RTA2017-00004-C06-02 (AEI-INIA), and by the Spanish Olive Oil Interprofessional

## **From Myco-key to myco-twin: mycotoxin management along food/feed chain**

A.F. LOGRIECO<sup>1</sup>. <sup>1</sup>*Institute Of Sciences Of Food Production (Isipa)*

Fungal disease is one of the most important contributors to the occurrence and severity of mycotoxin contamination of crop plants. Phenotypic and metabolic plasticity has enabled mycotoxigenic fungi (MF) to colonize a broad range of agriculturally important crops and to adapt to a range of environmental conditions. New mycotoxin-commodity combinations provide evidence for the ability of fungi to adapt to changing conditions and the emergence of genotypes that confer enhanced aggressiveness toward plants and/or altered mycotoxin production profiles. Among diseases caused by MF, the most important are the result of attacks carried out by species complexes. Examples of these diseases are the Fusarium ear rot of maize, Fusarium head blight (e.g. wheat, barley, and oat), black point of wheat kernels by *Alternaria alternata* species complex and related species; and various rot caused by *Aspergilli*. Mycotoxins in plant products and in processed food and feed have a significant economic impact and pose a serious problem for animal and human health. The management of good agricultural practices in the pre-harvest is a key issue for minimizing the risk of mycotoxin accumulation in the crops before the harvest. Such practices can involve crop rotation, tillage, proper fertilization and fungicide or biological control distribution, variety selection, timely planting and harvests and the control of the insects which often facilitate the toxigenic fungal species infection. Moreover, it is extremely important to prevent post-harvest contamination and develop practical and effective post-harvest procedures for mycotoxin reduction in the food supply chains and to provide alternative and safe use options for contaminated batches. An update review will be given on integrated management of pre-and post harvest practices aiming at the minimizing the risk of mycotoxin contamination along chain and main effective solutions developed by EU MycoKey (<http://www.mycokkey.eu/>) and MycoTwin (<https://www.mycotwin.eu/project>) projects.

**Integrated pest management smart technologies to precisely detect and control plant diseases.** D.I. TSITSIGIANNIS. *Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens. E-mail: dimtsi@aua.gr*

The ever-increasing demands of international markets for safe food have led to the development of integrated plant protection strategies for a more efficient and sustainable agriculture and to more robust certification and control systems for agricultural products. Novel integrated management systems (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 advanced methods of artificial intelligent diagnosis using spectral imaging techniques or mass spectrometry sensors for accurate detection and assessment of disease severity; (c) evaluate novel biocontrol and chemical plant protection products to control effectively the diseases; (d) develop innovative prototype sprayers actuating different nozzle types and adopting variable rate control based on canopy characteristics, the pathogen dispersal and disease development. We also develop and validate Decision Support Systems (DSS) based on computer-based knowledge systems that enable disease prediction and monitoring by combining epidemiological data, biological and chemical control strategies and precision farming tools. These systems determine the critical stages of the various plant protection spray interventions taking into account (a) the environmental conditions (temperature, rainfall, relative air humidity and leaf wetness obtained from local meteorological stations), (b) the developmental stage of the host, (c) the cultivation practices, (d) the microbiological load and other parameters. The ultimate goal of the 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*)



**The sanitary crisis caused by *Xylella fastidiosa*, a plant pathogenic bacterium recently discovered in Europe with a focus on the situation in France.** M-A JACQUES<sup>1</sup>, E DUPAS<sup>1</sup>, and S CESBRON<sup>1</sup>. <sup>1</sup>Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France.

Emergences of plant pathogenic bacteria have a resounding effect when they are responsible for diseases with major socio-economic consequences or are threatening valuable plant productions and the environment. This is the case for diseases caused by *Xylella fastidiosa* recently discovered in Europe. Our ability to anticipate, prevent and mitigate this type of emerging infectious diseases depends on a better understanding of current pathogen distribution over space and time, invasion routes, conditions favoring their emergence and population reservoirs. It is within this framework that we are participating in the development and test of several detection/identification methods (multiplex qPCR assays, nested MLST, metagenomics) and reconstructed the invasive scenario of *X. fastidiosa* subsp. *multiplex* ST6 and ST7 in France. Our studies rely on genome sequence analyses and tipdating to date the divergence of French *X. fastidiosa* subsp. *multiplex* strains from their American relatives. We developed and used a MultiLocus Variable Number of Tandem repeat Analysis (MLVA) on infected plant samples to take advantage of both a large sample collection and the capacity of these markers to monitor recent evolutionary events. The divergence time between French strains and their American relatives suggests not so recent introductions that remained unnoticed, probably because of the unspecific symptoms affecting diverse ornamental and native species in a diversified landscape. Bayesian methodologies were used to infer the number of introductions and the most probable scenario of population evolution and spread in these two French regions, Corsica and PACA.

This research was financially supported by the European Union's Horizon 2020 research and innovation program under grant agreement 727987 XF-ACTORS (*Xylella fastidiosa* Active Containment Through a Multidisciplinary-Oriented Research Strategy), and INRAE SPE division and ANSES.

**Euphresco: a platform to link research and policy in the Mediterranean area and beyond** BALDISSERA GIOVANI, IBRAHIM AL-JBOORY, MEKKI CHOUIBANI, NICO HORN, LAURA MUGNAI, ANNA MARIA D'ONGHIA

Mediterranean agriculture, forests and other environments are seriously threatened by numerous quarantine and emerging pests. The negative impacts caused by these pests are expected to increase due to the acceleration of global trade and to climate change that respectively favour the movement of these organisms over long distances and facilitate their adaptation to and establishment in new environments.

In the face of these challenges, the Mediterranean region is particularly vulnerable due to the shortcomings in national quarantine systems, limited expertise and phytosanitary infrastructures, and the lack of funds for research activities in support of statutory plant health.

The strengthening of research in the field of plant health is one of the main challenges that countries in the Mediterranean region have to address. The diversity of priorities both in terms of pests and in terms of infrastructures and skills has reduced the impact of national efforts, but plant health challenges require rethinking of the organization of research activities in all countries and their coordination in order to increase efficiency and impact.

Coordination at Mediterranean level will reduce the fragmentation of actions; it will promote convergence of national programmes; it will build critical mass.

In this context, several Mediterranean organizations and initiatives have joined forces to improve international collaboration in and coordination of research efforts on plant health and plant protection of Mediterranean countries. The various activities undertaken so far and the results of discussions with Mediterranean country representatives will be presented.

**Applications of remote sensing and information technology in the surveillance of quarantine diseases of fruit tree crops.** A. M. D'ONGHIA<sup>1</sup>. <sup>1</sup>*Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Via Ceglie, 9 - 70010 Valenzano (BA), Italy.*

Early and accurate detection systems for quarantine diseases of fruit tree species are essential for efficient large-scale surveillance, rapid implementation of control measures and evaluation of their effects. The new frontiers of technology can also offer several smart solutions in the field of plant health, facilitating the task of operators in implementing more efficient, accurate, timely and cost-effective real-time and large-scale surveillance programs. It is therefore necessary to have a considerable amount of data (e.g. climate, geographical, satellite, diagnostic) accurately captured at a distance and on the ground, using applications, sensors, forecasting models, specific diagnostic methods, etc. To this aim, Remote sensing (RS) can help to identify infections on a large scale, even when symptoms are not yet visible. There are many platforms and sensors designed for the acquisition of remote sensing data, ranging from satellite, aircraft and, more recently, UAS. The availability of high resolution time series of images such as Sentinel-1 and Sentinel-2, introduced with the European Copernicus programme, has greatly facilitated the scale-step in the use of Earth Observation (EO) data for agricultural and phytosanitary applications. In addition to RS, Information Technology (IT) is another important tool for plant protection programs allowing accurate storage, retrieval, transmission and manipulation of monitoring data. Various IT tools can be used for this purpose such as diagnostic devices, Apps (accurate on-site data acquisition), DSS, etc. An overview on RS and IT systems is provided, with particular reference to those already available and applied in the official pathogen monitoring programmes (*Citrus tristeza virus*, *Xylella fastidiosa* in Apulia region, Italy), e.g.: (i)an automatic procedure for tree counting, using GeoEye-1 multispectral image; (ii)prediction maps using WorldView-2 satellite image in GIS environment for early detection of CTV-suspected trees; (iii)photointerpretation of high-resolution aerial images for the identification of OQDS trees in the demarcated area of *X. fastidiosa*; (iv)several applications for accurate field data acquisition by NPPOs (e.g. Apps for *Xylella fastidiosa*, XylApp, XylAppEU, XylAppNENA) and citizens (XylAppcitizens); (v) integrated DSS made of database, forecasting models and users interfaces.

**Gender in plant health and pathology: Exploring the research agenda**  
MARGREET VAN DER BURG<sup>1</sup> Wageningen University

This presentation will take the audience on a short journey to explore gender into the plant pathology research agenda. With some historical and contemporary examples it will be illustrated how women differently from men are connected to plant health and pathology and its research. It ends with showing windows of opportunity what research including gender can help dealing with the current challenges the field faces.

## **Molecular host-microbe interactions in plants: from microbes to host innate immunity**

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In their long history of interspecific interaction, plants and invading microorganisms – beneficial or pathogenic – are constantly embroiled in complex co-evolutionary dynamics. Several plant associated microorganisms have acquired an arsenal of sophisticated colonization mechanisms to manipulate the physiological processes of their hosts. These mechanisms also include specialised proteins, known as “effectors” that are utilized by microbes to promote colonization.

However, their molecular function(s) and their targets in the host-cell, are largely unknown, for the vast majority of these effectors. Today, this still is one of the major questions in the Molecular Plant-Microbe Interactions (MPMI) research field. However, the effectors' activities/functions can be used as tools to identify important components of plant innate immunity and physiology that could potentially lead to innovative strategies for crop improvement.

Plants, on the other hand, have evolved a well-organized and complex innate immunity system, to recognize invasion. The recognition occurs through the perception of microbial structural patterns, as well as, the secreted effector proteins, by specialized transmembrane or intracellular receptors of the host-cell. The microbial effectors are mainly recognised, in resistant hosts, by members of the NLR (Nucleotide-binding Leucine-rich Repeats receptors) receptors' family. The elucidation of plant NLRs' molecular function, will significantly help the future development of novel and/or synthetic immune receptors with new recognition capacities, in crops.

**Fungicide resistance in *Botrytis cinerea* populations from protected crops in the Mediterranean basin: Current status and implications with its management fungicide sensitivity and genetic diversity of *Botrytis cinerea* populations from conventional and organic tomato and strawberry fields in Cyprus and Greece. G. S. KARAOGLANIDIS<sup>1</sup>**

<sup>2</sup>*Plant Pathology Laboratory, Faculty of Agriculture, Forestry and Natural Environment, Aristotelian University of Thessaloniki, POB 269, 54124, Thessaloniki, Greece*

*Botrytis cinerea* is one of the most destructive pathogens infecting numerous crops. Among them are included several important crops cultivated in the Mediterranean basin such as greenhouse grown vegetables or strawberries. Resistance of the pathogen to fungicides used for its control is widespread throughout the Mediterranean basin in crops heavily treated such as those cultivated in greenhouses and it is associated with reduction in product's performance and control failures.

In this report the current status of resistance to major botryticide groups such as SDHIs, QoIs, hydroxylanilides or anilinopyrimidines in *Botrytis spp.* populations will be reviewed and detailed information will be given on the methodologies of resistance detection, variability of mutations conferring resistance to fungicides, their effect on the fungal sensitivity to the different active ingredients and the fitness of resistant strains. Furthermore, implications of cross-resistance patterns complexity and fitness cost for the resistant mutants will be discussed in relation to resistance management in greenhouse grown vegetables taking into account the recent introduction of new active ingredients into the agricultural practice, such as new molecules belonging into SDHIs.

Additionally, future prospects and challenges will be presented with emphasis on the role of alternative control methods such as the integration of Biological Control Agents (BCAs) with conventional fungicides in reducing the fungicide selection pressure on fungal population and, thus, minimizing the risk for resistance development.

**Phytoplasmal and Viral diseases of fruit crops in the East Mediterranean countries.** ELIA CHOUEIRI. *Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, P.O. Box 287, Zahlé, Lebanon*

Fruit crops including stone and pome fruits and grapes are important crops adapted to the eastern Mediterranean region. Quality and quantity losses in these crops due to viral and phytoplasmal infections are reported from these countries. Almond witches'-broom associated with the presence of '*Candidatus Phytoplasma phoenicium*' is responsible for the death of more than 100,000 almond and peach trees in Lebanon. In Egypt, serious economic loss due to infection with European stone fruit yellows (ESFY) on apricots and peaches are reported. However, in Turkey, ESFY disease was mostly detected in symptomatic apricot and plum. In Jordan, aster yellows phytoplasma (16SrI) affecting peach trees was reported in addition to the recent incidence of '*Candidatus Phytoplasma solani*' infecting plum. On pome fruits, pear decline induced by *Ca P. pyri* has been observed on pear cultivars in Lebanon and in pear and quince orchards in Turkey. Some apple varieties were infected with apple proliferation disease in Turkey. *Ca. Phytoplasma solani*, the causal agent of bois noir in grapevine has been recorded in Lebanon, Syria, Turkey and Jordan associated with typical grapevine yellows, whereas *Ca. Phytoplasma omanense*' was detected in a grapevine sample, cultivar Syrah in Lebanon. Many viruses were recorded on fruit trees such as Prunus necrotic ringspot virus, Prune dwarf virus, Apple mosaic virus in several countries, whereas American plum line pattern virus, plum pox virus were detected only in some countries. On grapes, grapevine leafroll associated viruses, vitiviruses and nepoviruses (GLRaV-1, GLRaV-2, GLRaV-3, GVA, GVB, GFLV) were the most common viruses.

**Epidemiology and management of legume and cereal viruses in Arab and Mediterranean region.** SAFAA G. KUMARI<sup>1</sup>, ASMA NAJAR<sup>2</sup>, NADER ASAAD<sup>3</sup> and ABDUL RAHMAN MOUKAHEL<sup>1</sup>.

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In the Arab and Mediterranean region, cool-season food legumes (chickpea, faba bean, lentil and pea) are infected naturally by many viruses, and the number of viruses involved continues to increase. However, at any specific location only a few of these viruses are of economic concern. Yield losses resulting from virus attack vary widely, from little, as in the case of *Broad bean mottle virus* in Tunisia and Morocco, to complete crop failure when conditions permit widespread virus infection at the vulnerable early plant growth stage, as with *Faba bean necrotic yellows virus* in Egypt and Syria. Cereal crops (wheat, barley and oat) in Arab and Mediterranean region are also affected by viruses, mainly *Barley yellow dwarf virus-PAV* and it is reported to occur in epidemic levels in only few countries of this region including Algeria, Morocco and Tunisia. Epidemic spread of most viral diseases was always associated with high vector populations and activity. Although virus disease management can be achieved through the combined effect of several approaches, development of resistant genotypes is undoubtedly one of the most promising control components. Experience gathered over the last few decades clearly showed that no single method of virus disease control suffices to reduce yield losses in legume crops. Some progress was made on the disease management of some legume and cereal viruses using a combination of healthy seed, host resistance, cultural practices (such as adjustment of planting date, plant density, roguing of infected plants early in the season) and chemical vector control.



## **First report of Glomerella leaf spot (GLS) in South Tyrolean (Italy) apple orchards**

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Glomerella leaf spot (GLS) on apple, caused by various *Colletotrichum* species, is an emerging disease worldwide. GLS was restricted to apple growing areas with humid and subtropical climate. Thus, was not reported from European apple growing areas. Extreme weather conditions, including heavy rainfall and warm temperatures at the end of August 2020, led to an unknown symptomatology in South Tyrolean (Italy) apple orchards: leaves showed necrotic lesions, proceeding to extended chlorosis of the leaf within a short period of time. Affected leaves dropped prematurely and resulted in complete defoliation approximately a month after. A few days after the appearance of the first leaf lesions, brownish spots, often surrounded by a purple halo, started to develop on fruit, finally affecting up to 100 % of the crop. Fungal isolates were recovered from symptomatic leaves and fruit spots and morphological analysis identified these isolates belonging to the genus *Colletotrichum*. A multi-locus sequence analysis based on the ITS (internal transcribed spacer) region and on fragments of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT) and beta-tubulin (TUB2) genes assigned all isolates to *Colletotrichum fructicola*. Further, pathogenicity assays on apples were performed, and Koch's postulates were confirmed. Climatic conditions like increasing air temperatures, frequency and intensity of precipitations may lead to a further spread of this pathogen. Subsequently result in notable yield losses in commercially managed orchards. Thus, targeted plant protection measurements and containment strategies should be implemented to control and prevent the spread of this disease.

**Fusarium head blight and crown rot diseases of wheat in Algeria and other southern Mediterranean countries: distribution, identification and pathogenicity of associated species.** H. BOUREGHDA. *Laboratory of Phytopathology and Molecular Biology, department of botany, The National Higher School of Agronomy (ENSA), El Harrach, Algiers, Algeria.*  
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Fusarium head blight (FHB) and crown rot (CR) of wheat are worldwide serious diseases which may affect yield and also kernel contamination by mycotoxins. FHB occurs when prolonged wet weather coincides with anthesis. CR is a chronic problem where dry climatic conditions are present and when continuous wheat cropping is adopted. In the southern Mediterranean countries, climatic conditions are conducive for both diseases that can coexist. In Algeria, CR is more widespread because wheat is grown much more in arid and semi-arid regions; whereas FHB is restricted to humid and sub-humid stages where also CR is present. In Tunisia, FHB was also reported only in the two bioclimatic sub-humid and semi-arid upper stages. Based on published data, in Algeria, *Fusarium culmorum* was reported as the dominant species associated to both diseases with *F. pseudograminearum* as the second causal agent; while in Tunisia, the same data were recorded for CR, but for FHB, the dominant species was *Microdochium nivale* followed by *F. culmorum*. In Morocco and Egypt, *culmorum* and *Bipolaris sorokiniana* were reported as major species associated to CR. In addition *Rhizoctonia oryzae* was also associated to CR and *F. graminearum* to FHB in Egypt. Pathogenicity assessments have shown that in Algeria, *F. culmorum* was the most aggressive on the wheat seedlings and on the head, while *F. pseudograminearum* was the most aggressive on the crown. In Tunisia, *F. culmorum* and *F. pseudograminearum* were the most aggressive on the crown, and in Egypt *F. culmorum* was also the most aggressive.

**The consequences of co-infection by *Cucumber green mottle mosaic virus* and *Pythium* species under different environmental conditions.** O. FRENKEL<sup>1</sup>, A. M. PHILOSOPH<sup>1,2</sup>, Y. ELAD<sup>1</sup>, A. KOREN<sup>3</sup>, N. MOR<sup>4</sup>, and A. DOMBROVSKY<sup>1</sup>. <sup>1</sup>*Department of Plant Pathology and Weed Research, ARO, Volcani Center, Rishon LeZion POB 15159, Israel;* <sup>2</sup>*Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, POB 12, Rehovot, 76100, Israel;* <sup>3</sup>*Hishtil Nurseries, Moshav Nehalim, 4995000, Israel;* <sup>4</sup>*Extension Service, Ministry of Agriculture and Rural Development Rishon LeZion, IL 7505101, Israel;* E-mail: [omerf@volcani.agri.gov.il](mailto:omerf@volcani.agri.gov.il)

Plant pathology studies mainly focused on one host/one pathogen paradigm, but pathosystems can also involve co-infection by several pathogen species. Co-infection may show symptoms dissimilar to infections by each pathogen alone and may even increase the damage to the host. The case study describes the synergistic effect of co-infection by *Cucumber green mottle mosaic virus* (CGMMV) and *Pythium* spp. In the last decade, the phenomenon of late-wilting has increased in cucumber greenhouses during CGMMV outbreaks. As wilting appears in defined patches accompanied by root rot, we hypothesized that the phenomenon is caused by co-infection of soilborne pathogen/s and CGMMV. A field 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* and CGMMV leads to a significant synergistic wilting effect and reduced growth parameters. Furthermore, this synergistic effect was detected under a wide range of (optimal and suboptimal) temperatures, and *P. spinosum* that mostly prevails in mild temperatures, caused high mortality incidence at extended temperature range and even at 32°C. This study demonstrates the complexity of pathosystems involving co-infections by two pathogens. They encourage a broader perspective of the complexity of agricultural diseases to apply the most proper disease management.

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**Physiological and morphological changes in tomato plants infected with tomato leaf curl virus.** M. AHMAD ZESHAN. *Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha. Pakistan. 40100. E-mail: [muhammad.ahmad@uos.edu.pk](mailto:muhammad.ahmad@uos.edu.pk)*

The main objective of the study was to evaluate the effects of TLCVD on growth and yield parameters of tomato plants including their height, no. of fruits, intermodal distance, weight of healthy and infected leaves, no. of leaves and no. of branches. The presence of virus was confirmed by the biological assays such as whitefly mediated inoculation and leaf patch grafting. Growth and yield parameters of both healthy and diseased plants were compared to assess the losses. Leaf area was measured by using leaf area meter while vernier caliper was used for the fruit size. Leaves and fruits were weighed by using electric balance. Plant height was taken by measuring tape. Membrane stability index and electrolyte leakage was calculated by the method of Sairam (1994) and Sullivan and Ross, 1979, respectively. All the aforementioned growth and yield parameters were significantly reduced in infected plants as compared to healthy plants. Plant height, no. of fruits/plant, no. of branches, intermodal distance, no. of leaves/plant etc. decreased in diseased plants. Average electrolyte leakage of healthy leaves was 23% while 94% EL leakage recorded in infected leaves. The membrane stability index of healthy leaves was 61% that reduced to 44% in infected leaves. Average leaf surface area of infected leaves reduced to 5.23 cm<sup>2</sup> as compared to 6.33 cm<sup>2</sup> of healthy plants. TLCV infection interrupted the plant physiology considerably due to which all plant parts and physiological factors that contribute towards yield and quality of produce were greatly affected resulting in heavy yield losses.

**Detailed survey of fungal communities of four vineyards with different cultivars located in the Palava region (Czech Republic).** A. BERRAF-TEBBAL<sup>1</sup>, D. TEKIELSKA<sup>1</sup>, J. PECENKA<sup>1</sup>, M. SPETIK<sup>1</sup>, A. E. MAHAMED<sup>2</sup>, K. STUSKOVA<sup>1</sup>, J. WOHLMUTH<sup>1</sup>, D. CERNOHORSKA<sup>3</sup>, E. PENAZOVA<sup>1</sup>, J. CECHOVA<sup>1</sup>, M. BARANEK<sup>1</sup>, R. POKLUDA<sup>1</sup>, D. GRAMAJE<sup>4</sup>, A. EICHMEIER<sup>1</sup> <sup>1</sup>*Faculty of Horticulture, Mendeleum-Institute of Genetics, Mendel University in Brno, Valticka 334, 69144 Lednice, Czech Republic* <sup>2</sup>*Laboratoire de Biologie des Systèmes Microbiens (LBSM), Département des Sciences Naturelles, Ecole Normale Supérieure de Kouba, Alger BP 92, Vieux-Kouba, Alger, Algeria* <sup>3</sup>*Vinařství Plenér s.r.o., třída 1. máje 3414/11c, 690 02 Břeclav, Czech Republic* <sup>4</sup>*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas— Universidad de La Rioja-Gobierno de La Rioja, Ctra. de Burgos Km. 6, 26007 Logroño, Spain. E-mail: ales.eichmeier@mendelu.cz*

Grapevine cultivars are differentially vulnerable to infections caused by fungal trunk disease (TD) pathogens. The objective of this study was to examine four vineyards with different cultivars located in the Palava region, the most famous winegrowing region in the Czech Republic, on the total mycoflora, including TD pathogens. For this purpose, ten plants of cvs. Blaufränkisch, Palava, Pinot Noir and Welschriesling showing symptoms of TD were collected in each vineyard in July 2019. Fungal communities were examined using both traditional isolation on artificial media and high-throughput amplicon sequencing (HTAS) of the internal transcribed spacer (ITS2) region. HTAS increased the resolution of the fungal community analysis and revealed a highly diverse and complex mycoflora of grapevine wood as compared to the classical approach. According to the symptoms severity evaluated in the last two years, Pinot Noir was identified as the cultivar most sensitive to TD pathogens in our study. This observation correlated with the results of the fungal detection. The most prevalent fungal species detected by classical approach belonged to the genera *Diaporthe* and *Phaeoacremonium* and this was compared with the HTAS results. The study provides important and practically useful insights into fungal communities among four cultivars and represents the first approach to study fungal communities on grapevine plants by using MiniSeq, 2-channel sequencer combined with classical isolation. This study also provides the most comprehensive survey of TD pathogens in Czech vineyards and tolerance against TD pathogens in the Palava region.

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**Modelling the airborne inoculum of *Polystigma amygdalinum*, causal agent of the red leaf blotch of almond in Catalonia, NE Spain.** G. PONS-SOLÉ<sup>1</sup>, E. LÁZARO<sup>2</sup>, A. VICENT<sup>2</sup> and J. LUQUE<sup>1</sup>. <sup>1</sup>*Sustainable Plant Protection Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Ctra. de Cabrils km 2, 08348 Cabrils, Spain.* <sup>2</sup>*Institut Valencià d'Investigacions Agràries (IVIA), Ctra. CV-315 km 10.7, 46113 Moncada, Spain.* E-mail: [gemma.pons@irta.cat](mailto:gemma.pons@irta.cat)

The red leaf blotch (RLB) of almond, caused by the ascomycete *Polystigma amygdalinum*, is the main foliar disease affecting almond orchards in the Mediterranean Basin. The pathogen overwinters in leaf litter and ascospores are released from perithecia in spring to infect new almond leaves. RLB is characterized by a long incubation period and fungicide sprays should be scheduled during the period of ascospore release. We monitored the airborne *P. amygdalinum* ascospores from 2019 to 2021 (February to September each year), by placing a 7-day volumetric spore trap (Hirst type) in two RLB-affected almond orchards in Catalonia (NE Spain). Exposed tapes were collected weekly and analysed through real-time qPCR to quantify *P. amygdalinum* trapped ascospores on a daily basis. Hierarchical Bayesian beta regression models were developed to fit the dynamics of accumulated ascospore catches to several environmental variables for both locations. The best model included accumulated degree-days (ADDs) and ADDs considering both rainfall and vapor pressure deficit (ADDwet) as fixed factors, and year as random factor. For this model, the linear regression of the median posterior predictive distribution against observed values accounted for 78% of the total variance, with a mean absolute error of 0.1415 and a root mean square error of 0.1799. A Decision Support System using this model is currently under development to schedule fungicide sprays and optimize the control of almond RLB.

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**Uncovering the mechanisms involved in *Pinus pinaster* resistance to pine wilt disease by analysis of coding and non-coding transcriptomes.** I. MODESTO<sup>1,2,3</sup>, L. STERCK<sup>3,4</sup>, V. ARBONA<sup>5</sup>, A. GÓMEZ- CADENAS<sup>5</sup>, I. CARRASQUINHO<sup>6,7</sup>, Y. Van de Peer<sup>3,4,8</sup> and C.M. MIGUEL<sup>2,9</sup>. <sup>1</sup>ITQB NOVA, Universidade Nova de Lisboa, Oeiras, Portugal. <sup>2</sup>iBET, Oeiras, Portugal. <sup>3</sup>Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium. <sup>4</sup>VIB-UGent Center for Plant Systems Biology, Ghent, Belgium. <sup>5</sup>Departament de Ciències Agràries i del Medi Natural. Universitat Jaume I, Castelló de la Plana, Spain. <sup>6</sup>INIAV, Oeiras, Portugal. <sup>7</sup>LEAF – Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. <sup>8</sup>Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa. <sup>9</sup>Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal. E-mail: [cmmiguel@fc.ul.pt](mailto:cmmiguel@fc.ul.pt)

Pine wilt disease (PWD), caused by the parasitic nematode *Bursaphelenchus xylophilus*, or pinewood nematode (PWN), is a serious threat to conifer forests in Europe and Asia. *Pinus pinaster* is one of the most affected species due to its high susceptibility. Heritable resistance has been reported in *P. pinaster* trees, opening the possibility for selecting and breeding for this trait. To uncover the mechanisms underlying resistance, we have analysed the transcriptional responses after inoculation with PWN in plants showing resistant and susceptible phenotypes. Our analysis revealed a strong reprogramming of gene expression, particularly in resistant plants. Differential gene expression analysis pointing to roles for lignin synthesis and jasmonic acid defence pathway in resistance phenotypes were confirmed by biochemical analyses of cell wall composition and hormone pathways. Additionally, secondary metabolism, oxidative stress response and resistance genes also seem relevant to overcome PWD. Approximately 100 miRNAs were responsive to PWN inoculation, some of which with predicted targets associated to roles in jasmonate-response pathway, ROS detoxification and terpenoid biosynthesis. Furthermore, several *P. pinaster* genes putatively targeted by PWN miRNAs were identified, which was supported by degradome analysis. Targets for *P. pinaster* miRNAs were also predicted in PWN, suggesting a role for trans-kingdom miRNA transfer and gene silencing both in PWN parasitism as in *P. pinaster* resistance to PWD. Our results provide novel insights into transcriptional and post-transcriptional regulatory mechanisms which may be useful for the development of new strategies to protect *P. pinaster* forests from PWD.

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**Spread and current situation of *Fusarium oxysporum* f. sp. *ubense* tropical race 4 affecting banana in Israel and the Middle East.** M. MAYMON<sup>1</sup>, N. SELA<sup>1</sup>, U. SHPATZ<sup>1,2</sup>, N. GALPAZ<sup>3</sup> AND S. FREEMAN<sup>1</sup>. <sup>1</sup>*Department of Plant Pathology and Weed Research, ARO, The Volcani Institute, Rishon LeZion, 7505101 Israel.* <sup>2</sup>*R & D, Kiryat Shmona 11016, Israel.* *E-mail: freeman@volcani.agri.gov.il*

*Fusarium oxysporum* f.sp. *ubense* tropical race 4 (TR4) is considered one of the most devastating soilborne fungal pathogens of banana worldwide, causing mortality to Cavendish group bananas. The pathogen was first detected in South East Asia, spread to the greater Mekong subregion, Australia and disseminated to India, Pakistan, Oman, Turkey and Mozambique (Africa), and recently was identified in the South American continent in Colombia and Peru. TR4 was discovered in the Middle East (Jordan and Lebanon in 2014, and Israel in 2016). In Israel, typical TR4 symptoms of leaf-yellowing and wilting symptoms, accompanied by internal vascular discolorations of rhizomes and pseudostems were observed in mature 'Grand Naine' Cavendish cultivar plants from Shfeya, Carmel coastal plain and various locations along the eastern shores of Lake Galilee. TR4 representative isolates were tested for pathogenicity and identification from symptomatic plants was reconfirmed by PCR. Sequenced genomes of 5 representative TR4 isolates (two from Israel, one from Jordan, the Philippines, and Indonesia each) and 11 additional worldwide isolates were compared by single nucleotide polymorphisms (SNPs) analysis, to determine the origin of the Israeli isolates. SNP detection and phylogeographical analyses indicated that the Middle Eastern isolates are closely related, implying that the origin of the pathogen in Israel is from Jordan, while those from Colombia were related to a representative isolate from Indonesia. Host range, susceptibility/tolerance of germplasm to TR4, and epidemiological studies and survival of the pathogen are currently being investigated.

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***In vitro* effects of cell density on development and metabolism of *Phaeomoniella chlamydospora* a pathogen of Esca disease of grapevine** Z. KARÁCSONY<sup>1</sup>, K.Z. VÁCZY<sup>1</sup>.

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The ascomycetous fungus *Phaeomoniella chlamydospora* is one of the most important pathogens of Esca disease of *Vitis vinifera*. Esca belongs to the group of grapevine trunk diseases (GTDs), which are chronic fungal infections of vascular tissues leading to symptoms on both the perennial (wood necrosis) and annual parts (stunted shoots, foliar chlorosis and necrosis) of the host. The process of the pathogenesis of GTDs is still an unsolved question. The uneven expression of external symptoms on infected plants and the lack of obvious correlation between the pathogens' abundance in the host and the occurrence of symptoms suggest that external factors and unique events greatly affect disease development. One such event can be the realization of a critical cell density of pathogens in the host, which shifts the otherwise harmless fungi to pathogenic behavior. Microorganisms perceive cell density by the "Quorum sensing" mechanism, which affects the virulence of several bacterial and fungal pathogens. Our results pointed out that cell density has an important role in the development and metabolism *P. chlamydospora*. Experiments on solid-state cultures suggest that higher cell density promotes the germination of conidia and later the formation of polysaccharide-rich mycelial patches, which is an indicator of biofilm formation. Higher cell densities promoted the pigmentation of colonies and also the synthesis of antibacterial compound(s) suggesting a shift towards secondary metabolism. These phenomena may also occur in infected plants possibly leading to disease development.

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The effect of volatile organic compounds emitted by the biocontrol agent *Paenibacillus alvei* K165 grown on Luria Broth medium against *Verticillium dahliae*. E.G. POULAKI<sup>1</sup>, F.L' HARIDON<sup>2</sup>, R. CARRON<sup>2</sup>, L. WEISSKOPF<sup>2</sup> and S.E. TJAMOS<sup>1</sup>. <sup>1</sup>Agricultural University of Athens, Crop Science Department, Lab of Phytopathology. <sup>2</sup>Department of Biology, University of Fribourg, Fribourg, Switzerland. E-mail: sotiris@aua.gr

In the present study, we investigated the effect of the volatile organic compounds (VOCs) emitted by the biocontrol agent *Paenibacillus alvei* K165 grown on Luria Broth (LB) medium against *Verticillium dahliae*, under *in vitro* and *in planta* conditions. The GC/MS analysis revealed that K165 grown on Luria Broth medium, produces a number of volatile compounds, such as 2,3 butanediol and tropone. It was found that the pure compounds of 2,3 butanediol and tropone inhibited the growth of *V. dahliae in vitro* by 10 and 20 %, respectively. The *in planta* effect of the K165 emitted VOCs against *V. dahliae* was examined in a novel dual compartment device, where the plants are placed in soil in the upper part of the device and the bacterial cells are applied in the lower part, ensuring the spatial separation of plant roots from K165 cells. For the experiments, K165 was inoculated on LB medium in the lower part of the device. The endophytic presence of *V. dahliae* was examined in the plants of the different treatments at 3 and 7 dpi, by Real Time PCR analysis. It was shown that the K165 treated plants were less colonized by *V. dahliae* compared to controls, at both sampling time points. In agreement with the RTPCR results, disease scoring revealed that the percentage of disease symptoms, mainly in the form of wilting, were significantly less in the plants exposed to the K165 VOCs compared to controls.

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**Bacillus velezensis K165 mediated resistance against *Verticillium dahliae*, *Botrytis cinerea* and *Hyaloperonospora arabidopsidis* and the role of histone acetyltransferases in biocontrol.** D. GKIZI<sup>1</sup>, M. MALAI<sup>2</sup>, K. DOUKA<sup>2</sup>, E. POULAKI<sup>2</sup>, V. NTOUKAKIS<sup>3,5</sup> and S.E. TJAMOS<sup>2</sup>. <sup>1</sup>*School of Food Sciences University of West Attica, Ag. Spyridonos str, Egaleo, 12243, Athens, Greece,* <sup>2</sup>*Agricultural University of Athens, School of Crop Science, Laboratory of Plant Pathology, Iera Odos 75, 11855, Athens, Greece,* <sup>3</sup>*School of Life Sciences University of Warwick, Coventry, CV4 7AL, UK,* <sup>4</sup>*Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry CV47AL, UK,* E-mail: danai\_gkizi@aua.gr

The use of microbes for the biological control of plant diseases during the last decades has been well studied along with the molecular aspect of plant- biocontrol agent (BCA) interactions. Nevertheless, the epigenetic effect of the BCAs on plants is a relatively new research field to be explored. Recently, we showed that treatment of *Arabidopsis thaliana* with the BCA *Bacillus velezensis* (previously *Paenibacillus alvei*) K165 confers inherited resistance to the soilborne pathogen *Verticillium dahliae* in K165-treated plants and their offspring. The observed plant protection was attributed to histone acetylation of genes participating in lignin biosynthesis and immune responses; resulting in lignin accumulation and induction of the jasmonate/ethylene pathway. In the present work, we studied the role of K165 in the *Arabidopsis-Botrytis cinerea/Hyaloperonospora arabidopsidis/Pseudomonas syringae* pv tomato (Pst) interactions in K165-treated wild type plants and their offspring. It was revealed that K165 protected the plants against *B. cinerea* and *H. arabidopsidis*; while, the offspring of the K165-treated plants were as susceptible as the controls. Furthermore, the performance of pathogenicity experiments in *Arabidopsis* mutants showed the role of histone acetyltransferases (HAT) of GNAT-MYST (HAG) and CBP (HAC) families in the K165 mediated disease resistance.

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**Biological Control of *Aspergillus carbonarius* and *Botrytis cinerea* in Grapevine Berries and Transcriptomic Changes of Genes Encoding Pathogenesis-Related (PR) Proteins.** D.GKIZI<sup>1</sup>, E. POULAKI<sup>2</sup>, S. E. TJAMOS<sup>2</sup>

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*Botrytis cinerea* and *Aspergillus carbonarius* are among the most important widespread fungal pathogens causing bunch rot in grapevine berries, resulting in significant economic losses. Additionally, *A. carbonarius*, although a secondary invader, is the main source of ochratoxin A in grapes. In this work, the plant protective activity of the biological control agents, *Bacillus velezensis* (Previously *Paenibacillus alvei*) K165, *Blastobotrys* sp. FP12 and *Arthrobacter* sp. FP15 was examined against *B. cinerea* and *A. carbonarius* on grapes. In vitro preliminary dual culture experiments showed a significant reduction of both fungi growth by K165, while FP15 restricted only the growth of *A. carbonarius* and FP12 was ineffective. Next, ex vivo experiments on grape berries, revealed that K165, FP12 and FP15 reduced *A. carbonarius* rot severity by 81%, 57% and 37%, respectively, compared to the control. *B. cinerea* growth in berries was significantly reduced only by K165 (by 75%). Gene expression analysis of pathogenesis-related proteins PR2, PR3, PR4 and PR5, using real-time PCR, indicated the activation of multiple defense responses involved in the biocontrol activity of the examined biocontrol agents.

Gkizi, D.; Poulaki, E.G.; Tjamos, S.E. Towards Biological Control of *Aspergillus carbonarius* and *Botrytis cinerea* in Grapevine Berries and Transcriptomic Changes of Genes Encoding Pathogenesis-Related (PR) Proteins. *Plants* 2021, 10, 970. <https://doi.org/10.3390/plants10050970>  
plants10020207

**Evaluation of the biocontrol capabilities of *Clonostachys rosea* against grapevine trunk diseases.** A. GEIGER<sup>1,2</sup>, Z. KARÁCSONY<sup>1</sup>, J. GEML<sup>3</sup>, K. Z. VÁCZY<sup>1</sup> <sup>1</sup>*Eszterházy Károly Catholic University, Food and Wine Research Center, Leányka utca 6, H-3300, Eger, Hungary.* <sup>2</sup>*Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Páter Károly u. 1. H-2100, Gödöllő, Hungary,* <sup>3</sup>*ELKH-EKKE Lendület Environmental Microbiome Research Group, Eszterhazy Károly Catholic University, Leányka u. 6. H-3300, Eger, Hungary.*

The biocontrol agent *Clonostachys rosea* is a soil-born ascomycete. This fungus is known for its antagonistic abilities against numerous plant pathogenic fungi, nematodes and insects. However, in the point of grapevine trunk diseases (GTDs), it has not been investigated in details yet. The aim of this study was to characterize the biocontrol capabilities of *C. rosea* against the pathogens of GTDs. Confrontation tests were carried out with five *C. rosea* isolates and six GTD-related pathogens. The highest growth inhibitions were observed against the pathogens *Phaeomoniella chlamydospora* and *Eutypa lata* (50% and 30%). Mycoparasitism was observed against *Botryosphaeria* and *Phomopsis* species. Elicitor and biomass production and the sporulation of the isolates were also compared. Based on the obtained results, isolate 19b1 was selected for *in planta* experiments. Cabernet sauvignon cuttings were grown under greenhouse conditions, and inoculated with the pathogens *P. chlamydospora*, *E. lata* and *Botryosphaeria dothidea*. Plants were further grown in untreated soil or a soil amended with  $10^4$  spores/g of 19b1 isolate. After three months of incubation, the development of symptoms was examined. Lesion lengths caused by *P. chlamydospora* and *E. lata* were significantly decreased in the presence of *C. rosea* 19b1, while the virulence of *B. dothidea* was unaffected. The biocontrol agent was frequently re-isolated from the base of the cuttings, and its colony forming units were increased from  $10^4$  to  $10^5$  in one gram of treated soil. All the above results suggest, that *C. rosea* can be used as a biocontrol agent against GTDs.

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**Mineral oils against powdery mildew: how paraffin oil induces resistance in grapevine against *Erysiphe necator* and how is applicable in disease management.** X. PÁLFI<sup>1</sup>, M. LOVAS<sup>1</sup>, Z. KARÁCSONY<sup>1</sup>, J. KÁTAI<sup>3</sup>, K.Z. VÁCZY<sup>1</sup> and ZS. ZSÓFI<sup>2</sup>. <sup>1</sup>Food and Wine Research Institute, Eszterházy Károly Catholic University, H3300 Eger, Hungary. <sup>2</sup>Institute for Viticulture and Enology, Centre for Research and Development, Eszterházy Károly Catholic University, H3300 Eger, Hungary. <sup>3</sup>Faculty of Agricultural and Food Sciences and Environmental Management Institute of Agrochemie and Soil Sciences, University of Debrecen, H4032, Debrecen, Hungary. E-mail: palfi.xenia@uni-eszterhazy.hu

Petroleum-derived spray oils (PDSOs) have been used widely and for a long time in pest management of several crops. They are mostly used as washing sprays and adjuvants, but they also have an antifungal effect. Good results were observed in field experiments against powdery mildew (GPM) of grapevine (*Vitis vinifera* L.). Although, the mode of action of this antifungal property is poorly understood. The possible direct fungicidal activity against GPM and the stress-inducing capability of 2 v/v% paraffin oil (PFO) on grapevine were examined using cv. Kékfrankos cuttings and *E. necator* fungus. In our experiments, no direct fungicide activity on *E. necator* was detected, however PFO induced significant physiological changes in the grapevine. Several stress-related processes were observed: increased H<sub>2</sub>O<sub>2</sub> and salicylic acid production, secondary thickening of cell wall through lignin deposition and accumulation of phenolic compounds. Changes in some enzyme activities related to oxidative stress or metabolism of phenolics were also measured and were in accordance with the physiological changes. Our results suggest that PFO could induce systemic acquired resistance in the grapevine through the elicited stress responses which leads to reduced susceptibility to GPM. The potential benefit of PFO to plant immunity was also experienced in our field spraying experiment in 2015-2016, where combining PFO with conventionally used fungicides showed increased efficacy against GPM. In addition to its elicitor role, PFO can support the adherence and absorption of spray agents, thus application of PDSOs in disease management can be rewarding.

This research was supported by the Project GINOP-2.3.2-15-2016-00061.

**Development of cost-effective methods for detection of the DMI fungicide resistance marker A495T of the grapevine powdery mildew fungus *Erysiphe necator*. M.Z. NÉMETH<sup>1</sup>, A. PINTYE<sup>1</sup>, O. MOLNÁR<sup>1</sup>, F. MATOLCSI<sup>1,2</sup>, Á.N. HORVÁTH<sup>1</sup>, ZS. SPITZMÜLLER<sup>3</sup>, K.Z. VÁCZY<sup>3</sup> and G.M. KOVÁCS<sup>1,2</sup>.**

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Demethylase inhibitor (DMI) fungicides applied to control *Erysiphe necator*, causal agent of grapevine powdery mildew, are integral part of disease management in grapevine cultivation. Intensive application of DMIs resulted in fungicide resistance in some *E. necator* populations. Resistance is most commonly caused by a point mutation, A495T, in the *CYP51* gene resulting in an amino acid change in the CYP51 protein, rendering the fungus resistant to DMIs. Monitoring fungicide resistance is essential for effective disease control. Our aim was to develop cost-effective methods for detection of A495T point mutation. We optimized a simple protocol for DNA extraction from *E. necator*. Our method is based on crushing single chasmothecia in extraction medium, and the resulting extract could be directly used in downstream applications. These extracts were used for direct amplification of the gene region containing A495T by PCR. A quantitative real-time PCR (qPCR) assay was also adapted to detect A495T. To further simplify diagnostics, utility of loop mediated isothermal amplification (LAMP) for detection of A495T was tested. Primers were designed for detection of A495T. The developed qPCR and LAMP protocols combined with the quick DNA extraction are suitable for cost-effective genotyping of *E. necator* for the A495T mutation.

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**The compositional turnover of grapevine-associated plant pathogenic fungal communities are greater among intraindividual microhabitats and terroirs than among healthy and Esca-diseased plants** A. GEIGER<sup>1,2</sup>, Z. KARÁCSONY<sup>1</sup>, R. GOLEN<sup>1</sup>, K. Z. VÁCZY<sup>1</sup>, J. GEML<sup>1,3</sup>. <sup>1</sup>*Food and Wine Research Centre, Eszterházy Károly Catholic University, Leányka u. 6. H-3300, Eger, Hungary.* <sup>2</sup> *Doctoral School of environmental Sciences, Hungarian University of Agriculture and Life Sciences, Páter Károly u. 1. , H-2100, Gödöllő, Hungary.* <sup>3</sup>*ELKH-EKKE Lendület Environmental Microbiome Research Group, Eszterhazy Károly Catholic University, Leányka u. 6. H-3300, Eger, Hungary. E-mail: [geml.jozsef@uni-eszterhazy.hu](mailto:geml.jozsef@uni-eszterhazy.hu)*

Grapevine is vulnerable to numerous diseases, one of them is grapevine trunk diseases (GTD), which is a threat in the vine industry causing serious losses due to the premature decline of the vine and yield loss. In the past decades, several studies dealt with the causing agents of GTD, however key questions related to the emergence and severity of the disease remained unanswered, including possible differences in plant pathogenic fungal communities of asymptomatic and symptomatic grapevines. Fungal DNA metabarcoding data were generated from bark, soil and perennial wood samples of symptomatic and asymptomatic grapevines from three terroirs. Larger compositional differences in plant pathogenic fungi were found among different plantparts within grapevine plants than among individuals. GTD related fungi were dominant in the perennial woody tissue while non GTD pathogens in soil. Asymptomatic plants do not differ from symptomatic ones. Our result suggest that fungi generally associated with Esca disease belong to the core grapevine microbiome and likely are commensal endophytes and/or latent saprotrophs, some of which can act as opportunistic pathogens on stressed plants. In addition we found significant compositional differences among sampling sites, particularly in soil, which suggest a certain influence of local edaphic and mesoclimatic factors on plant pathogenic fungal communities. Furthermore, we observed differences among terroirs in plant pathogenic fungal communities in grapevine woody parts indicate that environmental factors likely are important for the development of Esca disease and further studies are needed to investigate the abiotic conditions on fungal compositional dynamics in Esca-affected plants.

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**Botryosphaeriaceae species as fungal pathogens associated with olive trunk diseases in southern Italy.** A. CARLUCCI, F. LOPS, M.L. RAIMONDO *Department of Agriculture, Food, Natural resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy.*

Botryosphaeriaceae species have a cosmopolitan distribution and a wide range of plant hosts including olive trees. Olive cultivation in southern Italy represents a very important and productive crop with high number of hectares cultivated and high yields. Different species of this family have been associated with trunk diseases causing symptoms such as cankers, dieback and olive decline. Several wood samples collected over 15 years from olives showing symptoms yielded a collection of fungal isolates, many of which resulted to belong to Botryosphaeriaceae family. A phylogenetic study, carried out on representative number of strains combining sequences from internal transcribed spacer region and translation elongation factor 1- $\alpha$  gene, allowed to identify five *Neofusicoccum* species, such as *N. cryptoaustrale*, *N. luteum*, *N. mediterraneum*, *N. parvum* and *N. vitisifusiforme*, and one *Botryosphaeria* species such as *B. dothidea*. Morphological description by conidia size, colour and shape confirmed the molecular identification. This study highlighted that the olive symptomatic wood analyzed is mainly attacked by *Neofusicoccum* species. In particular, *N. cryptoaustrale* has been isolated and described for first time, to our knowledge, from olive wood and in Italy.

Structure analysis of the ribosomal intergenic spacer (IGS) region as a putative marker for *Phaeoacremonium* phylogeny. M.L. RAIMONDO, F. LOPS, A. CARLUCCI. *Department of Agriculture, Food, Natural resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy*

Most species of *Phaeoacremonium* are associated with wood diseases of various plants, such as *Vitis vinifera*, *Olea europaea* and *Prunus* species. The increasing recognition of novel *Phaeoacremonium* species (to date 63), and their recent taxonomic reassignment through phylogeny based on the  $\beta$ -tubulin and actin genes, have highlighted the presence of paraphyly, intraspecific variation, and incongruence of some *Phaeoacremonium* species. For these reasons, the entire IGS region of a collection of 57 *Phaeoacremonium* strains was amplified, sequenced and subjected to phylogenetic analysis. A detailed analysis of the structure of IGS region was done on *Phaeoacremonium italicum* strains as study model, and compared with those of the closest related species, *P. alvesii* and *P. rubrigenum*. This structure analysis showed five categories of repeat elements that were organised into distinct patterns. The comparison of the trees (IGS, and  $\beta$ - tubulin and actin) indicated that the intergenic spacer rDNA region was able to distinguished intraspecific and interspecific variations. Preliminary studies on phylogenetic informativeness suggested that IGS could be an useful marker able to resolve shallow divergence in the *Phaeoacremonium* species. Further molecular studies are required to determine whether intergenic spacer sequences can improve precision in defining *Phaeoacremonium* phylogeny, and prevent misidentification and the introduction of vague species boundaries for the genus.

**Characterization of endophytic *Alternaria* species isolated from grapevine (*Vitis vinifera*) shoots.** A. MOLNÁR<sup>1</sup>, D.G. KNAPP<sup>2</sup>, G. TÓTH<sup>2,3</sup>, I. BOLDIZSÁR<sup>2,4</sup>, K.Z. VÁCZY<sup>1</sup>, G.M. KOVÁCS<sup>2</sup>. <sup>1</sup>*Food and Wine Research Centre, Eszterházy Károly Catholic University, Leányka utca 6, Eger 3300, Hungary.* <sup>2</sup>*Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest 1117, Hungary.* <sup>3</sup>*Department of Pharmaceutical Chemistry, Semmelweis University, Hőgyes Endre u. 9, Budapest, 1092, Hungary.* <sup>4</sup>*Department of Pharmacognosy, Semmelweis University, Üllői út 26, Budapest, 1085, Hungary. E-mail: molnar.anna@uni-eszterhazy.hu.*

Grapevine is associated with highly diversified endophytic fungal communities affecting the health status and productivity of the host plant. According to our previous findings, a significant number of fungal species belonging to the genus *Alternaria* are among the most common endophytes inhabiting the different grapevine varieties. Recently, *Alternaria* species have been the main focus of numerous studies primarily in connection with their challenging taxonomy and ability to produce a high variety of secondary metabolites, however data on well-identified species in Hungarian vineyards and their secondary metabolites are limited. In the present study we addressed the molecular identification of endophytic *Alternaria* species colonizing the above-ground tissues of grapevine. We also aimed to determine the metabolite profile of the *Alternaria* isolates. Based on the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, we identified the *Alternaria* species belonging to *Alternaria* sect. *Alternaria* in a predominant number among the fungal isolates from asymptomatic leaves and clusters. Since species-level discrimination within the *Alternaria* sect. *Alternaria* is not adequate by using solely the ITS region, our isolates were subjected to combined analysis of five additional genomic loci (RPB2, ALTA1, endoPG, OPA10-2 and KOG1058). The metabolite profiling was carried out using ultra-high performance liquid chromatography (UHPLC)-high-resolution mass spectrometry (HRMS). We can infer that the endophytic fungal isolates gained from healthy shoots of grapevine belong to two distinct lineages considered as *A. alternata* and *A. arborescens* species complex, and numerous compounds characteristic to the genus have been found in the different *Alternaria* lineages.

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## **Towards Nutrition-Sensitive Agriculture: an evaluation of biocontrol effects, nutritional value, and ecological impact of bacterial inoculants**

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The integration of Nutrition Sensitive Agriculture with plant protection is a promising domain which gave new insights to a balanced agriculture production by filling the gap of nutritional and ecological aspects alongside food safety. Based on this novel concept, application of microbial-based products can be an alternative to synthetic products without compromising health-related properties, sustainable production and maintaining biodiversity in the agroecosystem. Therefore, in this study, we evaluated the performance of romaine lettuce plants (*Lactuca sativa*) in pots under optimized greenhouse conditions with and without the treatment of potential plant-beneficial bacterial strains *Paenibacillus pasadenensis* 'R16', *Pseudomonas syringae* '260-02' and *Bacillus amyloliquefaciens* 'CC2' against soil-borne fungal pathogens *Rhizoctonia solani* and *Pythium ultimum*. The considered factors included the biocontrol efficacy against pathogens, microbial biodiversity in the bulk soil, rhizosphere and root endosphere, nutraceutical and plant growth promotion parameters. Strain R16 significantly reduced the symptom severity against both pathogens, while the other two strains, as well as a Trichoderma-based commercial product, showed less efficient biocontrol ability. Among all treatments, a radical variation was observed in the microbial composition of rhizosphere and root endosphere but not in the bulk soil, showing no substantial ecological side effects. Most nutraceutical parameters like photosynthetic efficiency, carotenoid content, phenolic compound content remained uninfluenced by the treatments. Additionally, chlorophyll content was higher in R16-treated leaves challenged with *Rhizoctonia solani*, demonstrating a positive physiological effect on carbon fixation. Furthermore, none of the treatments had negative effects on plant growth, showing the suitability of these strains.

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**Multilocus-sequencing–based genetic composition and DMI fungicide resistance in *Erysiphe necator* populations in Hungary.** M.Z. NÉMETH<sup>1</sup>, A. PINTYE<sup>1</sup>, O. MOLNÁR<sup>1</sup>, E. MATOLCSI<sup>1,2</sup>, Á.N. HORVÁTH<sup>1</sup>, V. BÓKONY<sup>1</sup>, ZS. SPITZMÜLLER<sup>3</sup>, K.Z. VÁCZY<sup>3</sup>, L. KISS<sup>4</sup> and G.M. KOVÁCS<sup>1,2</sup>. <sup>1</sup>*Plant Protection Institute, Centre for Agricultural Research, ELKH, Herman Ottó út 15., 1022 Budapest, Hungary.* <sup>2</sup>*Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary.* <sup>3</sup>*Food and Wine Research Centre, Eszterházy Károly Catholic University, Leányka utca 6., 3300 Eger, Hungary.* <sup>4</sup>*Centre for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, 487-535 West Street, 4350 Toowoomba, Queensland, Australia. E-mail: matolcsi.fruzsina@atk.hu (presenter), nemeth.mark@atk.hu (corresponding author)*

Grapevine powdery mildew (GPM) caused by *Erysiphe necator* is usually controlled by sterol demethylation inhibitor (DMI) fungicides, but long-term use of these agents has resulted in resistance in GPM. A common marker of resistance is the A495T nucleotide substitution in the *CYP51* gene. The GPM populations in Europe can be divided into two groups, which may differ in their seasonality and fungicide resistance. We sampled GPM in Hungary to describe genetic composition of these populations and to investigate the prevalence of the A495T-marker, and the relationship between its occurrence, fungicide treatment, sampling sites and years and *E. necator* genetic groups. Sampling was conducted in six wine regions for three years. Fragments of four genes were sequenced. Occurrence of A495T was determined by sequencing or real-time PCR, and the probability of A495T occurrence was analyzed with a generalized linear model.

Fourteen haplotypes were obtained, of which eight were previously unknown. These included haplotypes which represented SNPs characteristic to both genetic groups, and might represent recombinants. A495T was detected in all wine regions, in ~16% of the samples. We found significant differences in the occurrence of A495T among several wine region and cultivar combinations and between study years. Occurrence of A495T was not significantly different between treated and untreated sites, neither between seasons nor between genetic groups. Our results indicate that in Hungary, *E. necator* populations consist of diverse haplotypes, including recombinants. These frequently harbor A495T mutation, occurrence of which is mainly influenced by wine region and cultivar, and the sampling year.

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**Rapid risk appraisal for potential entry, establishment and spread of *Xylella fastidiosa* in NENA countries.** M. DIGIARO<sup>1</sup>, K. DJELOUAH<sup>1</sup>, M. FREM<sup>2</sup>, H. EL BILALI<sup>1</sup>, G. CARDONE<sup>1</sup> and T. YASEEN<sup>3</sup>. <sup>1</sup>*International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM-Bari), Via Ceglie 9, 70010 Valenzano (Bari), Italy.* <sup>2</sup>*External consultant of CIHEAM-Bari.* <sup>3</sup>*Food and Agriculture Organization of the United Nations (FAO)/ Regional Office for the Near East and North Africa Region (RNE) 11 Al Eslah El Zerai St., Dokki, Cairo, Egypt. E-mail: [Thaer.Yaseen@fao.org](mailto:Thaer.Yaseen@fao.org) E-mail: [digiaro@iamb.it](mailto:digiaro@iamb.it)*

The potential risks of introduction, establishment and spread of *Xylella fastidiosa* (Xf) on the main host crops olive, vine, citrus, stone fruit and ornamental plants in NENA countries were assessed, by examining the cases of Algeria, Egypt, Jordan, Lebanon, Libya, Morocco, Palestine, Syria and Tunisia. The study was based on data retrieved directly from official public sources and questions to experts. Questions were aimed at assessing the risk of entry of Xf (volume of potential host commodities imported from officially infected countries, number of entry points, existing technical readiness and legislation for prevention) or the risks of its establishment and spread (existing surveillance programs and certification programs of plant propagation material, presence of vectors, favorable climatic conditions, abundance of main crops and alternative hosts). For each parameter, score ranged from 1 (low risk) to 6 (high risk). Results showed a level of Xf-entry risk from medium to high. Morocco resulted the most vulnerable country, followed by Palestine, Lebanon, Syria and Egypt. The risk of Xf-establishment and spread was instead higher for Syria and Lebanon, followed by Palestine, Morocco and Jordan. Combining these results, Morocco, Lebanon, Palestine and Syria were the countries most exposed to Xf entry and establishment risks with values, in a scale from 2 to 12, ranging between 6.95 and 7.75, above the value of 6.0 set as a high-risk threshold. Other countries fall into an intermediate and low risk class. The prompt and effective implementation of legislative, financial, technical and scientific measures becomes necessary in each country

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**Role of early-season control on Botrytis bunch rot epidemics in vineyards.** G. FEDELE<sup>1</sup>, E. GONZÁLEZ-DOMÍNGUEZ<sup>2</sup> and V. ROSSI<sup>1</sup>. <sup>1</sup>*Department of Sustainable Crop Production (DIPROVES), Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, 29122, Piacenza, Italy.* <sup>2</sup>*Horta srl, via Egidio Gorra, 55, 29122, Piacenza, Italy.* E-mail: vittorio.rossi@unicatt.it

Botrytis bunch rot (BBR) is one of the main diseases affecting grapevines, which require adequate control, often based on routine application of fungicides at the end of flowering (A), pre- bunch closure (B), veraison (C), and before harvest (D). This simple, calendar-based scheduling of fungicides is at odds with the complexity of *Botrytis cinerea* life cycle and the existence of different infection pathways. Recently, a mechanistic model showed that early-season development of *B. cinerea* plays a key role in determining the severity of BBR on ripe bunches, because of latent infection of young berries and saprophytic colonisation of bunch floral debris (or bunch trash). A meta-analysis of 116 studies confirmed that BBR control in A is more effective than in B, the latter being useful under high disease pressure only, when the full ABCD strategy is needed for effective BBR control; the combination of ACD provides better and additive control of the early-season infection pathways and the multiple infection events during berry ripening. Following an integrated BBR management approach, the early-season use of alternative products (e.g., biological control agents and botanicals) based on model predictions provides effective BBR management in vineyards.

**Development and evaluation of a weather-driven, mechanistic model for predicting blossom blight caused by *Monilinia laxa* and *M. fructicola* on stone fruits.** V. ALTIERI<sup>1</sup>, I. SALOTTI<sup>1</sup> and V. ROSSI<sup>1</sup>. <sup>1</sup>*Department of Sustainable Crop Production (DIPROVES), Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, 29122, Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it*

*Monilinia laxa*, *M. fructicola*, and *M. fructigena* are three closely related fungi that affect blossoms, twigs and fruit, and can cause substantial economic damage to stone fruits; blossom blight is mainly caused by *M. laxa* and *M. fructicola*. Disease control generally relies on fungicide sprays; however, spray programs often provide inconsistent disease control because of poor spray timing or may result in unjustified sprays when the conditions are not suitable for infection. To improve decision making in disease control, we developed a mechanistic, weather-driven model based on the available knowledge on processes leading to blossom infection by *M. laxa* and *M. fructicola*. By using systematic literature review and systems analysis, the available knowledge was retrieved, analyzed, and synthesized in a relational diagram, which considers the following compartments: i) production of conidia on mummified fruits; ii) release and deposition of the conidia produced on mummies; iii) infection caused by conidia on stone fruit blossoms; iv) incubation and disease onset. The model is driven by weather-dependent mathematical equations developed based on literature information. Model validation is ongoing by comparing model predictions vs. independent (i.e., not used in model development), observed data. Different cropping systems and stone fruit hosts are being considered to evaluate the model's accuracy and robustness in a wide range of conditions.



**A mechanistic weather-driven model for *Ascochyta rabiei* infection and disease development in chickpea.** I. SALOTTI<sup>1</sup> and V. ROSSI<sup>1</sup>. <sup>1</sup>*Department of Sustainable Crop Production (DIPROVES), Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, 29122, Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it*

*Ascochyta* blight caused by *Ascochyta rabiei* is one of the most important diseases of chickpea (*Cicer arietinum* L.). The available knowledge on *A. rabiei* was retrieved from literature, analysed and mobilised to develop a mechanistic, weather-driven model for the prediction of *ascochyta* blight epidemics. By using the systems analysis, the life cycle of the pathogen was used to draw the model structure; published, quantitative information was used to develop algorithms driving model variables. The model was validated using data from literature and a field trial performed in Southern Italy in 2019. The ability of the model to predict primary infections was evaluated using data from Washington (USA) in 2004 and 2005, Israel in 1996 and 1998, and in Spain from 1988 to 1992. The model showed good accuracy and specificity. The probability of correctly predicting infections was 0.838 and the probability that there was no infection when not predicted was 0.776. The model failed to predict some real infections, which however originated very low disease (only 8.1% of the total disease). Model capability to predict the disease progress during the growing season was evaluated by using data collected in Australia from 1996 to 1998 and in Southern Italy in 2019. Significant linear regression ( $R^2=0.904$ ,  $P<0.001$ ) between predicted and observed data was obtained, indicating that the model is accurate and robust in predicting infections and dynamics of *ascochyta* blight epidemics. The model could then be used for supporting *ascochyta* blight control.

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**Changes in the xylem microbiota associated to infection by *Xylella fastidiosa* in Brazilian olive groves.** M. ANGUITA-MAESO<sup>1</sup>, J.A. NAVAS-CORTÉS<sup>1</sup>, H.D. COLETTA-FILHO<sup>2</sup>, B.B. LANDA<sup>1</sup>. <sup>1</sup>*Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Avenida Menéndez Pidal s/n, 14080, Córdoba, Spain.* <sup>2</sup>*Centro de Citricultura Sylvio Moreira, Instituto Agronômico-IAC, Rod. Anhanguera, km 158 - Cascalho, 13490-000, Cordeirópolis - SP, Brasil. E-mail: manguita@ias.csic.es*

Nowadays, the health of olive groves is under threat due to an increase on diseases caused by vascular pathogens such as the bacterium *Xylella fastidiosa* and the soil-borne fungus *Verticillium dahliae*. Both pathogens may compromise the olive production worldwide due to their capacity to adversely affect the plant growth by colonizing and blocking the xylem vessels causing desiccation of leaves and branches and ultimately the death of the tree. Knowledge of the xylem-inhabiting microbiota is essential to understand the resistance response observed in some olive cultivars under high inoculum pressure, and to select potential biocontrol microorganisms. For that purpose, we investigated the xylem-inhabiting bacterial communities present in stem and root tissues of olive trees of cv. Grappolo growing at five field locations (two in the state of Sao Paulo and three in Minas Gerais State) in Brazil, and compared the changes occurring on trees infected and non-infected by *Xylella fastidiosa*. Sequencing data resulted in a total of 925 bacteria ASVs, distributed in 15 phyla and 421 genera. Proteobacteria was the most abundant bacterial phylum (91.41%) followed by Firmicutes (4.19%) whereas *Pseudomonas* and *Methylobacterium* (31.16% and 24.21%, respectively) were the dominant genera. Microbial diversity was firstly determined by field location followed by ecological niche and in a lesser extent by *X. fastidiosa* infection. Furthermore, network analysis identified 32 keystone species with positive and negative associations with *X. fastidiosa*. Interestingly, 1174-901-12 and *Pseudomonas* showed the greatest number of negative ASVs associated to *X. fastidiosa*, whereas *Acidiphilium* and *Corynebacterium* co-occurred with the pathogen. This work contributes to improve our understanding on the plant microbiome in relation to plant health status in order to promote and maintain sustainable olive agroecosystems.

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Endophytic *Trichoderma* spp. from Hungarian grapevines with biocontrol potential

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*Trichoderma* species are opportunistic colonizers of various habitats, and while most species live on the basidiocarps of basidiomycetes, they can as well be isolated from soil and plants. Reports on endophytic strains from grapevine are rare, and have scarcely ever characterized or identified on the species level. Here we report on ten *Trichoderma* strains found during screening for grapevine trunk disease (GTDs) pathogens in a local vineyard in the Tokaj Wine Region in Hungary. They were identified as *Trichoderma gamsii*, *T. orientale*, *T. simmonsii*, *T. afroharzianum*, *T. atrobrunneum* and *T. harzianum sensu stricto* on the basis of their ITS1 and ITS2 and *tef1* sequences. The growth potential of the strains was assessed at a range of temperatures. Potential human pathogens—based on temperature preferences and taxonomic characteristics—were discarded. Two strains - identified as *T. simmonsii* and *T. afroharzianum* - were selected for further studies based on the criteria of good growth at 5 °C, as well as at ambient temperature range, but with limited growth at 37 °C. They both exhibited outstanding biocontrol activity against the tested GTD pathogens isolated from grapevine (*Diplodia seriata*, *Eutypa lata*, *Neofusicoccum parvum*). Their Biocontrol Indices were also good towards Oomycota and Ascomycota plant pathogens isolated from other hosts.

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## **Fungal-bacterial interactions in grapevine wood: consequences on plant health**

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Viticulture worldwide is confronted with heavy economic losses caused by Grapevine Trunk Diseases (GTDs). Fungi have been described as the main cause of GTDs, however bacteria diversity is very high in the grapevine wood. The function of these wood-inhabiting bacteria is relatively unknown, only a few information being available about their interactions with grapevine or the wood-inhabiting fungi.

Using different bacterial strains isolated from wood of Bordeaux grapevines and three major GTDs-pathogenic fungi, we observed that bacterial-fungal interactions ranged from synergism to antagonism, depending on the bacterial and fungal species involved. Screening of bacterial strains against *Neofusicoccum parvum*, *Phaeoemoniella chlamydospore* (*Pch*) and *Fomitiporia mediterranea*, showed some strains inhibited the growth of these fungal pathogens and reduced the wood necrosis length caused by *N. parvum* and *Pch* in young grapevines. On the contrary, synergistic relationships were observed for other bacterial strains and *N. parvum* or *mediterranea*. Regarding the functions of bacteria, it was shown that (i) some strains can independently destructure grapevine-wood components such as cellulose and hemicellulose, and (ii) fungal ability to degrade wood structures is strongly influenced by bacteria inhabiting wood. Specifically, a new cellulolytic and xylanolytic bacterial species we called, *Paenibacillus xylinteritus*, displayed a synergistic interaction with *F. mediterranea* and promoted wood degradation compared to that caused by the fungus alone.

Altogether these results raise the question of the functional diversity of the bacterial communities colonizing grapevine wood and their direct, or indirect, involvement in GTDs.

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**Fungal pathogens associated with grapevine trunk diseases in Cyprus.** G. MAKRIS, M. CHRISTODOULOU, S. SOLONOS, L. I. KANETIS and MICHALIS CHRISTOFOROU. *Department of Agricultural Sciences, Biotechnology, and Food Science, Cyprus University of Technology, Limassol, 3036, Cyprus. E-mail: [loukas.kanetis@cut.ac.cy](mailto:loukas.kanetis@cut.ac.cy)*

Grapevine trunk diseases (GTDs) pose a major concern to the grape industries worldwide. However, in Cyprus, there is no comprehensive study about the population structure and the etiology of this important disease complex. During 2017, 182 fields located in the main grape-producing areas of the country (Limassol and Paphos), were surveyed. Wood pieces from 3-5 selected vines per field, exhibiting typical GTDs symptoms, were excised for fungal isolations. Samples were collected out of 24 grapevine cultivars, although 62% were from the two most prevalent, indigenous wine-cultivars Mavro and Xinisteri. More than 600 samples were processed and approximately 750 fungal strains were isolated. Molecular identification based on the ITS (internal transcribed spacer) sequences, revealed the involvement of over 60 taxonomic groups at the species or genus level. GTD-related pathogenic species of the genera *Phaeomoniella*, *Phaeoacremonium*, *Neofusicoccum*, *Botryosphaeria*, *Diplodia*, *Eutypa*, *Eutypella*, *Diaporthe*, *Cryptovalsa*, *Cytospora*, *Neofabraeae*, *Seimatosporium*, *Kalmusia*, and *Paraconiothyrium* were identified. Further phylogenetic analysis of the aforementioned groups was performed, using specific to the genus markers, accordingly. *P. chlamydospora* (14.5%), *Phaeoacremonium minimum* (4.4%) *Diplodia seriata* (4%), *Botryosphaeria dothidea* (3.5%), and *Eutypa lata* (3.2%) were the predominant GTD-related species. Furthermore, isolates of the genera *Alternaria*, *Cladosporium*, *Fusarium*, *Neosetophoma*, *Ulocladium*, *Paecilomyces*, and *Penicillium*, commonly associated with the grapevine microbiome, were detected. Pathogenicity tests were performed by wood inoculation of young potted grapevine plants. Symptoms were recorded and re-isolations were confirmed, based on morphological characteristics, thus fulfilling Koch's postulates. To the best of our knowledge, this is the first systematic record of GTDs in Cyprus.

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**The necrosis and ethylene inducing gene *VdNEP* as a molecular marker for differentiation between *Verticillium dahliae* pathotypes.** A. TRIANTAFYLLOPOULOU<sup>1</sup>, A.K. TZIMA<sup>1</sup>, A. TZANAKI<sup>1</sup>, O.I. BALOMENOU<sup>1</sup>, I. TSOUTSOS<sup>1</sup>, R.M. JIMÉNEZ-DÍAZ<sup>2</sup>, and E.J. PAPLOMATAS<sup>1</sup>. <sup>1</sup>*Laboratory of Phytopathology, Agricultural University of Athens, Iera Odos 75, Athens, Greece.* <sup>2</sup>*Universidad de Córdoba, College of Agriculture and Forestry (ETSIAM), Departamento de Agronomía, Campus de Excelencia Internacional Agroalimentario, Edificio C-4 Celestino Mutis, Campus Rabanales, Córdoba, 14071, Spain.*

*Verticillium dahliae*, is a cosmopolitan soilborne fungal pathogen with a wide host range leading to major agricultural losses. Isolates of the pathogen are categorized in the defoliating (D) and non-defoliating (ND) pathotypes, with the former being highly virulent causing distinct defoliation in cotton, okra and olive. To differentiate isolates, a new molecular marker based on the necrosis and ethylene inducing *VdNEP* gene has been developed. Southern blots probed with *VdNEP*, showed fragment polymorphisms between isolates of the two pathotypes. In order to isolate regions flanking the gene, inverse PCR was performed on genomic DNA of D and ND reference strains. Sequencing detected differences in the 3' untranslated region of *VdNEP* between the two pathotypes. Based on these findings, specific primers were designed for PCR detection. The primers were evaluated via screening of a collection of olive and cotton *V. dahliae* isolates from Greece, as well as a limited number of isolates from different parts of the world. Their efficiency was compared with already available markers. For the designated as D isolates, pathogenicity experiments were conducted in cotton plants so as to verify the molecular findings. As a whole, the results indicate that the new primers present a more robust and reliable tool for the differentiation of pathotypes, leading to subsequent higher chances for successful management of the pathogen.

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**Induction of grapevine defence mechanisms by the oomycete *Pythium oligandrum* against, *Neofusicoccum parvum*, a pathogenic fungus involved in Esca.** A. YACOUB<sup>(1,2)</sup>, R. HAIDAR<sup>(1,2)</sup> J. GERBORE<sup>3</sup>, M.C. DUFOUR<sup>(1,2)</sup> and P. REY<sup>(1,2)</sup>. <sup>1</sup>*Université de Pau et des Pays de l'Adour/E2S UPPA/ CNRS, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux - UMR 5254, IBEAS Avenue de l'Université, Pau, 64013, France.* <sup>2</sup>*Bordeaux Sciences Agro, UMR1065 SAVE, Université de Bordeaux, ISVV, F-33140 Villenave d'Ornon, France.* <sup>3</sup>*Université de Bordeaux, ISVV, Unité de recherche Œnologie EA 4577, USC 1366 INRA, Bordeaux INP, F-33140 Villenave d'Ornon, France.* <sup>3</sup>*BIOVITIS, 15400 Saint Etienne de Chomeil, France.* Email: [amira.yacoub@univ-pau.fr](mailto:amira.yacoub@univ-pau.fr)

Grapevine trunk diseases (GTDs) are on the rise in vineyards all over the world. Many pathogens are involved in these diseases, e.g. *Phaeomoniella chlamydospora*, and *Neofusicoccum Parvum*. Nowadays, there is no chemical treatments available to control these diseases. Accordingly, biocontrol of pathogens implicated in GTDs was developed using microorganisms. Among them, the oomycete *Pythium oligandrum*, whose strains naturally colonized grapevine roots in vineyards from several countries worldwide. In this study, the ability of *P. oligandrum* to induce grapevine resistance against *N. parvum* was evaluated. Two greenhouse assays showed that the necrosis of Cabernet Sauvignon cuttings caused by *N. parvum* was reduced by 65% when *P. oligandrum* colonized the root system of young vines. The expression levels of a set of 96 genes ("NeoViGen96"chip) involved in various grapevine defense pathways were studied by real-time PCR, at the trunk level. This analysis aims at determining plant responses after inoculation by *P. oligandrum* and/or *N. parvum*, at three different sampling time points. Overall, our results showed that the sampling time point present an important effect on studied gene expression levels, whatever the treatment applied. Moreover, at each sampling time point, specific grapevine responses to the different treatments (control, *P. oligandrum*, *N. parvum*, *P. oligandrum* + *N. parvum* treatments) were also differentiated. When *P. oligandrum* colonizes grapevine root systems, infection with the pathogen is associated with a more intense up-regulation of certain genes such as PR protein and signaling pathway genes. A priming effect of plant defense system is certainly induced in presence of *P. oligandrum*.

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***Fusarium tricinctum* species complex members emerging pathogens in several crops: the case of apple** M.T. SENATORE<sup>1</sup>, R. SOLDESTI<sup>1</sup>, M. CALI<sup>1</sup>, E. CAPPELLETTI<sup>1</sup>, M. SULYOK<sup>2</sup>, A. PRODI<sup>1</sup> <sup>1</sup>*Department of Agricultural and Food Sciences, Alma Mater Studiorum University of Bologna, Viale Fanin, 44, 40127 Bologna, Italy. E-mail: antonio.prodi@unibo.it* <sup>2</sup>*Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Konrad Lorenz Strasse, 20, A-3430, Tulln.*

Members of *Fusarium tricinctum* species complex (FTSC) are polyphagous and widespread pathogenic species even if considered of secondary importance on several crops. However, their presence has grown in the last year in crops like wheat, barley, ryegrass, redcurrant, box and apple causing yield losses causing quality reduction and mycotoxins contamination. In detail, in the last years, FTSC members have been reported as emerging pathogens on apple fruits and wood in apple orchards. This study analyzes from a morphological, and genetic point of view FTSC fungi isolated from Italian apple wood compared with isolates from different crops; furthermore, it assesses the ability of the FTSC isolates to produce emerging mycotoxins such as Enniatins and Beauvericin (ENNs and BEA) through molecular detection of *Esy1n* gene (confirmed in all isolates examined) and mycotoxin production. A phylogenetic analysis based on TEF-1 $\alpha$  gene was conducted assorting the analyzed isolates in four different phylospecies: FTSC 2 (*F. acuminatum*), FTSC 3 (*F. tricinctum*), FTSC 4 (*F. avenaceum*) and FTSC 14. In addition, one isolate did not cluster with any of the reference sequences representing a potential novel species in our population. The pathogenicity and mycotoxin productions of *F. acuminatum* and *F. avenaceum* strains was evaluated by artificial fruit inoculation on two different apple cultivars: Golden Delicious and Fuji. If the main mycotoxin problem related to apple production has always been represented by patulin, to date, with *Fusarium* representing a threat for wet apple core, the problem is extended to emerging mycotoxins such as ENNs and BEA.



**Evaluation of epiphytic grape yeasts for the control of *Aspergillus carbonarius* and ochratoxins in grapes.** M.K. ILIADI<sup>1</sup>, E.G. POULAKI<sup>1</sup>, C.K. KAVROUMATZI<sup>1</sup>, M.F. VARVERI<sup>1</sup>, D.N. PERIVOLARIS<sup>1</sup>, S.E. TJAMOS<sup>1</sup>, E.J. PAPLOMATAS<sup>1</sup> and D.I. TSITSIGIANNIS<sup>1\*</sup>. <sup>1</sup>*Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece.* \*Email: dimtsi@aua.gr

Yeasts are considered ideal biological control agents, as they are able to survive in a wide range of environmental conditions, grow rapidly with simple nutritional requirements, colonize plant surfaces relatively easy even under prolonged dry conditions and do not secrete toxic substances for humans. Yeasts have been widely used against plant pathogens at pre- or post-harvest level. The purpose of this study was to discover effective endemic yeasts as biocontrol agents against the black rot of grapes. *Aspergillus carbonarius*, the causative agent of this disease, adversely affects the organoleptic characteristics of the wine and produces carcinogenic ochratoxins. In this study, we tested various grapevine yeasts from the collection of Laboratory of Phytopathology (AUA) as well as new isolates from different grapevine varieties and regions in Greece. Several yeast strains were tested *in vitro*, for their ability to inhibit the production of conidia in the ochratoxigenic strain Ac-29 of *A. carbonarius* on a solid Yeast Malt Agar medium. Antagonism and ochratoxin inhibition bioassays were also carried out on detached grape berries. Yeasts with high antagonistic properties against *A. carbonarius* were selected for further field trials on Greek grape white varieties (Savatiano and Asyrtiko). The results of the comparative study of the different yeasts against *A. carbonarius* and ochratoxins will be presented. The discovery of endemic yeast strains that are acclimated and adapted to the local environment and local flora and fauna and can effectively inhibit the ochratoxin production may lead to the creation of novel biocontrol products.

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**New and emerging fungal diseases of super-high-density olive trees in California.** F.P. TROUILLAS, M.T NOURI, R. TRAVADON and D.P. LAWRENCE. *Department of Plant Pathology, University of California, Davis, 95616 Davis, CA, USA. E-mail: flotrouillas@ucanr.edu*

California produces 95 percent of the olives grown in the United States with a bearing acreage of approximately 14,700 hectares. During the winter of 2016, *Neofabraea* leaf and twig lesions was first detected in super-high-density (SHD) oil olive orchards in California. Affected trees showed leaf and shoot lesions, and cankers in branches, which developed at wounds caused by mechanical harvester. *Phlyctema vagabunda* and *Neofabraea kienholzii*, were found to be consistently associated with the disease and Koch's postulates were completed. The cultivar 'Arbosana' was highly susceptible to the disease, whereas cultivars 'Arbequina' and 'Koroneiki' appeared to be tolerant. Field trials indicated that several fungicides can reduce disease incidence and management strategy guidelines were implemented to limit further spread of the disease. Pleurostoma decline of olive trees caused by the fungal pathogen *Pleurostoma richardsiae* was also recently detected in SHD olive orchards. Symptoms of *Pleurostoma* decline in olive trees included leaf yellowing and browning, leaf drop as well as wilting and dieback of twigs and branches, and brown to dark discoloration of the wood, while severely affected trees died. Field observations suggested that infections by *P. richardsiae* initiate at wounds in trunks and branches caused by field equipment. Following a state-wide survey of olive orchards in California, olive anthracnose was not detected in SHD olive orchards. However, the disease was observed in an orchard of Gordal-Sevillana olives located at the Kearney Agricultural Research and Extension Center. Fungal isolates obtained from the olive fruits in this orchard were identified as *Colletotrichum fioriniae*.

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**Etiology and management of trunk and scaffold canker diseases of almond in California.**  
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Trunk and scaffold canker diseases (TSCD) caused by fungal pathogens are destructive diseases of almond trees. Common symptoms of TSCD include sunken bark lesions, wood discoloration and gummosis in trunks and branches. Recent surveys conducted by our laboratory showed the occurrence of four major canker diseases in California: Botryosphaeriaceae cankers, *Ceratocystis* canker as well as *Cytospora* and *Eutypa* cankers. Nonetheless, up to 21 fungal pathogens were found associated with TSCD of almond. Pruning wounds made for scaffold selections as well as maintenance pruning were common entry sites for canker pathogens. Therefore, we investigated various strategies to prevent infection of pruning wounds and promote tree health. Fungicide trials were carried out to determine best fungicidal products for the protection of pruning wounds. Experiments indicated the superior efficacy of thiophanate-methyl against several canker pathogens. Additionally, the biocontrol agent *Trichoderma atroviride* SC1 provided excellent pruning wound protection thus allowing sustainable control solutions against canker diseases. Additional studies were conducted to investigate the seasonal susceptibility of pruning wound and duration of pruning wound susceptibility according to the time (month) of pruning. Results showed that the duration of pruning wounds susceptibility was lowest when pruning is done in January. Overall pruning wound susceptibility declined substantially after 1 to 2 weeks following pruning. In conclusion, this work indicates that one application of a pruning wound protectant such as thiophanate-methyl (conventional fungicide) or *T. atroviride* SC1 (biological control agent) following late pruning in January can significantly reduce risks of infection of pruning wounds by canker pathogens.

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**Etiology and management of Phytophthora crown and root rot of pistachio in California.**  
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Pistachio is one of the most widely cultivated nut crops in California with approximately 115,000 hectares of bearing pistachio trees. In recent years, several orchards were identified with declining trees leading to substantial tree losses. Symptoms included trees with poor vigor, yellowing and wilting of leaves, crown rot and profuse gumming on the lower portion of trunks. Thirty-seven *Phytophthora*-like isolates were obtained from crown rot tissues in the rootstock of grafted pistachio trees and characterized by means of multi-locus phylogeny comprising ITS rDNA, beta-tubulin and mt cox1 sequence data. The analysis identified *Phytophthora niederhauserii*, *P. mediterranea* and *P. taxon walnut* associated with declining pistachio trees. Pathogenicity studies in potted UCBI rootstocks confirmed that all three *Phytophthora* species can cause crown and root rot of pistachio, thus fulfilling Koch's postulates. The widespread occurrence of *Phytophthora* crown rot in recently planted pistachio orchards and the susceptibility of UCBI rootstocks suggest this disease constitute an emerging new threat to the pistachio industry of California. Recently, we have conducted experiments to determine the relative tolerance of UCBI (*P. atlantica* × *P. integerrima*), PGI (*P. integerrima*) and Platinum (*P. integerrima* × *P. atlantica*) commercial rootstocks to *Phytophthora* and identify tolerant/resistant rootstocks that can be used to sustainably managed this soil-borne disease of pistachio. Experiments conducted using mycelial plugs for stem inoculation and inoculated rice grain or zoospores for soil/root inoculations of the various commercial rootstocks suggested that Platinum is the most tolerant rootstock to crown rot diseases when compared to PGI and clonal UCBI rootstocks.

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**Modeling potential climatic suitability of olive vascular diseases in southern Spain.** L.F. ARIAS-GIRALDO<sup>1</sup>, B.B. LANDA<sup>1</sup> and J.A. NAVAS-CORTÉS<sup>1</sup>. <sup>1</sup>*Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain. E-mail: lfarias@ias.csic.es*

Andalusia at Southern Spain, is the main olive oil producing region in the world with 1.6 million hectares. Verticillium wilt (VW) caused by the soil-borne fungus *Verticillium dahliae* (*Vd*) and the olive quick decline syndrome, caused by the bacterium *Xylella fastidiosa* (*Xf*) are currently the main threats for this crop. *Vd* is broadly extended at Southern Spain causing severe yield losses in some areas. On the contrary, *Xf* is not known to be present in this region but represents a threat since the detection of the bacterium in Spain. The objective of the study was to perform risk analysis that may help to prevent the spread of *Vd* and to avoid the establishment of *Xf* in Andalusia. For that, species distribution modeling was used to determine the relationships between sample location for those pathogens with associated environmental variables, and to estimate the ecological requirements for a particular species.

Our results identified Bio5 and Bio15 as the main climatic factors that determine the potential distribution of *Vd* in Andalusia, with the areas along the Guadalquivir river showing the highest suitability, which agrees with the current known *Vd* distribution in the region. The model also identifies extensive areas with moderate to high suitability in regions where olive plantations are currently expanding. Suitable areas for *Xf* are mainly characterized by warm temperatures during the summer-dry period, mild winter, and a well-defined rainy season, with the eastern part of Andalusia being more at risk.

The developed models would allow better management of VW by identifying the level of risk for both, current and new plantations, as well as prioritizing surveillance programs for *Xf* based on the level of risk to this pathogen.

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**Evaluation of biological control agents for the protection of almond pruning wounds against fungal canker pathogens.** R. TRAVADON, D.P. LAWRENCE, S. LI and F.P. TROUILLAS. *Department of Plant Pathology, University of California, Davis, 95616 Davis, CA, USA. E-mail: rtravadon@ucdavis.edu*

Fungal canker diseases are ubiquitous in perennial crops grown for fruit and nut production in regions with a Mediterranean climate. In almond, these diseases decrease yield and severely limit the productive lifespan of orchards. The fungal pathogens infect almond trees essentially through pruning wounds, as pruning is a common cultural practice to establish a tree architecture and invigorate fruitwood. Biological control agents (BCAs) have recently gained tremendous interests for pruning wound protection given the increasing restrictions for pesticide use. The objectives of this study were to evaluate the biocontrol potential of commercial and experimental BCAs against common fungal canker pathogens of almond. Initial screening of nine BCAs in dual cultures with seven pathogens allowed the selection of the four most promising BCAs for further testing of their antagonistic activities in planta. To this end, four BCAs were evaluated using detached almond branches in the laboratory against a subset of four pathogens: *Cytospora plurivora*, *Eutypa lata*, *Neofusicoccum parvum* and *Neoscytalidium dimidiatum*. This second level of screening revealed that some BCAs were able to reduce almond pruning wound infections by canker pathogens. The four selected BCAs were further evaluated in the field in two almond orchards planted with two distinct almond cultivars, Sonora and Nonpareil. Based on two years of field data from two locations, two BCAs provided levels of disease control similar to those reached with the conventional use of chemicals (i.e. thiophanate-methyl), and are thus promising candidates for use in integrated pest management strategies for almond production.

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**Modelling temperature response of *Xylella fastidiosa* strains and xylem vessel temperature on woody plants** M. ROMÁN-ÉCIJA<sup>1</sup>, B.B. LANDA<sup>1</sup>, L. TESTI and J.A<sup>1</sup>. NAVAS-CORTÉS<sup>1</sup>. <sup>1</sup>*Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Córdoba, Spain. E-mail: mromanecija@ias.csic.es*

Temperature is a key factor affecting *Xylella fastidiosa* (*Xf*) biology and epidemiology. Knowledge of the response of *Xf* to temperature is needed to assess the potential establishment of *Xf* strains occurring in the European outbreaks and develop regionalised risk models. Furthermore, as xylem-inhabiting microorganism, is relevant to determine at which extent air temperature, used to develop these models, is related to that into xylem vessels of host plants. The objectives were to determine the effects of temperature on *in vitro* cell culture of *Xf* strains and to describe the xylem vessels temperature dynamics in an olive orchard. Thus, growth, biofilm formation and survival of 37 *Xf* strains representative of five subspecies and 13 STs from a wide geographic origin and host plants were evaluated in a range from 4 to 40°C. Moreover, several thermocouples were installed within branches and trunks at a 1 and 4 cm depth, to measure air, soil and xylem temperature at 10-min intervals for 31 months.

Our results indicate that *Xf* strains showed a differential response to temperature. When grouped by subspecies, the widest optimal growth range was estimated for *Xf* subsp. *fastidiosa* and *multiplex* on 19 to 33°C and 20 to 31°C, while *Xf* subsp. *pauca* strains had a lower optimal range from 19 to 27°C. Similarly, extreme temperatures differentially affected cell survival. Thus, temperatures between 4 to 10°C did not affect cell survival; conversely, incubation temperatures of 36 and 40°C resulted lethal. Moreover, the relationship between air and xylem or soil temperature estimated by regression models indicated a buffer effect of trunk tissues and of soil, especially for maximum temperatures occurring during summer.

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**The effect of extreme weather conditions on the incidence and spreading of grapevine trunk diseases.** A. CSÓTÓ<sup>1</sup>, P. BALLING<sup>2</sup>, N. RAKONCZÁS<sup>1</sup>, CS. KOVÁCS<sup>3</sup>, A. NAGY<sup>1</sup> and E. SÁNDOR<sup>1</sup>. <sup>1</sup>*University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Debrecen, Hungary.* <sup>2</sup>*Research Institute for Viticulture and Oenology, Tokaj, Hungary.* <sup>3</sup>*National Agricultural Research and Innovation Centre Fruitculture Research Institute Development Institute of Újfehértó, Hungary.* E-mail: [csoto.andras@agr.unideb.hu](mailto:csoto.andras@agr.unideb.hu)

Grapevine trunk diseases (hereinafter: GTD) are among the most significant problems of the viticulture sector nowadays. The complex of diseases may cause decay and death of arms and even the whole trunk with a wide range of symptoms. The loss of vines year by year accelerates the economic aging, thus vineyards become unproductive prematurely. There is no effective preventive or curative treatment or agronomical practice against these diseases yet. This happens because of the diversity of GTD fungal pathogens, (ii) their protected niche within the plants woody tissues difficult to reach with chemicals, and (iii) the effect of abiotic factors on the disease incidence and symptoms development. Our goals were to determine the environmental parameters that may have significant influence for the development and spreading of the GTDs. The patterns of different diseases incidence within the plantation, and the effects of climatic and other environmental factors need long time data collection. More than three-year surveys were carried out in Tarczal and in Pallag regions (Hungary) detecting the incidence of the diseases and isolating fungi from the woody tissues in the cordon. Our results suggest that uneven rainfall, late spring frosts, high groundwater levels, and erosion have all increased the disease incidence. Species of *Botryosphaeriaceae* were the most common GTD pathogens in the examined plantations based on isolation and morphological as well as molecular identification.



**Valorization of *Gelidium sesquipedale* residue in the control of Ascochyta blight of chickpea** ERRATI HAJARE<sup>1,2,3</sup>, LEBBAR SALIM<sup>3</sup>, DARI KHADIJA<sup>1</sup>, HILALI LAHOUCINE<sup>1</sup>, KRIMI BENCHEQROUN SANAE<sup>2</sup> <sup>1</sup>University Hassan 1<sup>st</sup>, Faculty of Science and Techniques, Laboratory of Agro Alimentary & Health, P.O. Box 577, Settat, Morocco. <sup>2</sup>National Institute of Agriculture Research (INRA, CRRA-Settat), P.O. Box 589, Settat, Morocco. <sup>3</sup>Setexam Company for the Study and Exploitation of Algae and Maritime Products, Kenitra - 14000 Morocco. Email: [sanae.krimibencheqroun@inra.ma](mailto:sanae.krimibencheqroun@inra.ma)

The use of biological products as an alternative to chemical fungicide become important considering adverse health and environmental impact. The red algae *Gelidium sesquipedale* is mainly used for the extraction of Agar-Agar in the industry. However, a large quantity of by-product residues is also produced and is unused. The objective of this study is to valorize the bioactive compounds of *G. sesquipedale* residues and to evaluate their antifungal activity in controlling Ascochyta blight of chickpea. The algae residue extraction was performed using different solvents: water, dichloromethane, and dichloromethane: ethanol, 1:1 (v/v). The antifungal activity against the pathogen was first tested *in vitro* on PDA medium amended with different extracts using 7 concentrations from 0 to 8 mg/ml. A phytotoxicity effect of these products was evaluated on chickpea seeds. Further experiments were carried out *in vivo*, in the greenhouse to evaluate the efficacy of aqueous extract of residue to control the disease. Two technics of treatment application were tested; seeds and foliar applications. A systemic chemical fungicide (Azoxystrobine 250g/l) was used for comparison. Results indicated that aqueous extract of residue was the most effective in inhibiting the mycelium growth of pathogen by 80% using 8mg/ml. No phytotoxic effect of aqueous extract was observed on germination seeds of chickpea at all tested concentrations. Moreover, the application of aqueous extract of residue as foliar treatment was effective in controlling the disease and reducing disease severity by 73%, similar to chemical fungicide. However, no significant effect was observed, using seed treatment. Therefore, the aqueous extract of *G. sesquipedale* residues, used as foliar treatment, could be more investigated to develop a biological antifungal product.

**Determination of antibiotic residues in the endemic spurge honey (*Euphorbia Resinifera* o. Berg) from Morocco using biochip multi array technology and LC-MS/MS.**

RANIA BENJAMAA<sup>1</sup>, ABDEL KHALID ESSAMADI\*<sup>1</sup>, ABDELKARIM MOUJANNI<sup>2</sup>, BOUBKER NASSER<sup>1</sup>. <sup>1</sup>*Hassan First University of Settat, Faculty of Sciences and Technologies, Laboratory of Biochemistry, Neurosciences, Natural Resources and Environment*, <sup>2</sup>*National Office of Food Safety (ONSSA), Avenue Hadj Ahmed Cherkaoui, Agdal - Rabat – Maroc. E-mail: essamadi@uhp.ac.ma*

The presence of antibiotic residues in honey presents a risk to the health of consumers, because they could be a source of allergic reactions and can lead to obtaining bacterial resistant strains to antibiotics after consumption of honey. Screening methods are the first step in controlling antibiotic residues in food [3]. They can detect the presence of an antibiotic or group of antibiotics at the level of interest, and usually provide qualitative results. Then, in a second step the residues of the positively tested samples are quantified mostly by quantitative confirmation methods such as using an analytical method based on high performance liquid chromatography associated with a mass detector (HPLC-MS/MS). In this study, a total of 37 *Euphorbia resinifera* honey samples were analyzed, using a screening test: Evidence Investigator<sup>TM</sup>, an immuno-enzymatic method for detection of 27 antibiotic residues; followed by a liquid chromatography-tandem mass spectrometry (LC- MS/MS) for confirmation of suspect samples. In all samples, there were no antibiotic residues detected except for one showing the detection of rimethoprim at 6.48 µg kg<sup>-1</sup>.

**Potential insect vectors of *Xylella fastidiosa* in Morocco: case of spittlebug.** N. HADDAD<sup>1,2</sup>, I. MRABTI<sup>1,2</sup>, M. AFECHTAL<sup>1</sup>, K. EL HANDI<sup>3</sup>, R. BENKIRANE<sup>2</sup> and M.C. SMAILI<sup>1</sup>. <sup>1</sup>*National Institute for Agricultural Research (INRA), Regional Center for Agricultural Research of Kenitra, Box: 257, Kenitra, Morocco;* <sup>2</sup>*Faculty of Sciences, Laboratory of Plant, Animal, and Agro- Industry Productions, University Ibn Toufail, Kenitra, Morocco,* <sup>3</sup>*Laboratoire de Biotechnologie Végétale et Valorisation des Bio-Ressources, Faculté des Sciences, Université Moulay Ismail, Meknès B.P. 11201, Morocco. E-mail: [najat.haddad@uit.ac.ma](mailto:najat.haddad@uit.ac.ma)*

The bacterium *Xylella fastidiosa* represents a potential risk to the Moroccan flora. A thorough understanding of the distribution and ecology of potential vectors in wine-growing systems in Morocco is critical to the development of successful control. A sweeping net was used to collect insects (2604 specimens), to assess its occurrence and to establish an inventory of its potential insect during 2019 and 2020. Five insect potential vectors were recorded and mainly located in the northern part of Morocco, namely; *Philaenus tessellatus*, *P. maghresignus*, *Philaenus* sp., *Neophilaenus campestris* and *N. lineatus*. *Philaenus tessellatus* was the most important spittlebug recorded with uneven occurrence throughout the country. *Philaenus spumarius* has not been found in the present surveys which could indicate that it has been replaced by *P. tessellatus*. Therefore, the latter should be considered as the main potential insect vector of *X. fastidiosa* in Morocco.

**Evaluation of the susceptibility of improved and landrace durum wheat genotypes to *Zymoseptoria tritici* under nitrogen supply.** M. HASSINE<sup>1</sup> and S. AYADI<sup>1,1</sup>.<sup>1</sup>*University of Carthage, National Agronomic Institute of Tunisia, LR14AGR01, Laboratory of Genetics and Cereal Breeding, Avenue Charles Nicolle 43, 1082 Tunis, Tunisia. Email:marwa.hassine1@gmail.com*

Nitrogen fertilization is one of limiting factors on durum wheat production. This nutrient is the most important source used in agriculture practices and represents significant environmental and production costs. In the meantime, the fungal pathogen *Zymoseptoria tritici* was responsible on significant yield losses on durum wheat (*triticum turgidum* ssp *durum*) in Tunisia. In this context, the present study was conducted with the aims to evaluate the effect of different nitrogen rates on the development of Septoria leaf Blotch (STB) and yield components. Different levels of nitrogen (0, 50, 75, 150, 200 and 250 kgN/ha) were applied under field conditions in order to evaluate the degree of susceptibility of two improved (Karim and Salim) and two landrace genotypes (Chili and Mahmoudi). Disease severity has increased from 9% without nitrogen supply to 40% under the high rate (250 kgN / ha). Improved genotypes were more sensitive to STB (Karim 35% and Salim 51%) than landrace genotypes (Mahmoudi 17 % and Chili 12%). A significant decrease in yield components as thousand kernel weight (TKW) was observed with the increasing of disease severity and nitrogen rate from 48.58 g to 41.66 g, under 0N and 250 kgN/ ha, respectively. This result could provide novel insights on the implication and the efficiency of the use of different sources of nitrogen fertilizer on field performance of durum wheat and disease control.

This research was financially supported by National Institute of Field Crops (INGC) Boussalem, Tunisia.

**Rhizosphere-enriched microbes as a pool to design synthetic communities with beneficial effects on plant fitness and health.** MARIA-DIMITRA TSOLAKIDOU<sup>1</sup>, IOANNIS A. STRINGLIS<sup>2</sup>, NATALIA FANEGA-SLEZIAK<sup>1</sup>, STELLA PAPAGEORGIOU<sup>1</sup>, ANTRIA TSALAKOU<sup>1</sup>, IAKOVOS S. PANTELIDES<sup>1</sup>. <sup>1</sup>*Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Lemesos, Cyprus.* <sup>2</sup>*Plant-Microbe Interactions, Department of Biology, Science4Life, Utrecht University, Utrecht, the Netherlands.*  
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Suppressive composts represent an environmental friendly approach to combat soil-borne plant pathogens and improve plant growth. In a previous study, the microbial nature of the suppressiveness of a compost was demonstrated. The compost-derived microbial communities enriched in the rhizosphere of plants were identified and characterized for *in vitro* antifungal activity against soilborne fungal pathogens and for their potential to change plant growth parameters. In the present study, we designed two simplified microbial synthetic communities (SynComs) with different composition using representative bacteria from the abovementioned rhizospheric community, to provide evidence of their beneficial effects on plant fitness and health. SynCom1, consisting of bacterial strains reflecting their relative abundance in the rhizospheric community, displayed a negative effect on *Arabidopsis in vitro*, but promoted tomato growth in pot experiments. SynCom2, consisting only of *Bacillus* strains, did not affect *Arabidopsis* growth but had a positive effect on tomato growth and suppressed disease symptoms caused by *Fusarium oxysporum* f. sp. *lycopersici*. Our results demonstrate that the composition of complex microbial communities is a driver of different plant phenotypes, and these effects are observed in a host-dependent manner. Identification and characterization of their traits, could facilitate the design of novel microbial synthetic communities that could be used as inoculants with defined and controllable properties, conferring consistent beneficial effects towards plants.

**Enriched epigenetic marks at Pm-0 locus genes prime courgette and induce SAR responses against the causing agent of powdery mildew. T. MARGARITOPOULOU<sup>1</sup>, D. KIZIS<sup>1</sup>, D. KOTOPOULIS<sup>1</sup>, I. E. PAPADAKIS<sup>2</sup>, C. ANAGNOSTOPOULOS<sup>3</sup>, E. BAIRA<sup>3</sup>, A. TERMENTZI<sup>3</sup>, A.-E. VICHOU<sup>1</sup>, C. LEIFERT<sup>4,5</sup>, E. MARKELLOU<sup>1</sup>. <sup>1</sup>Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, Athens, 14561, Greece <sup>2</sup>Faculty of Crop Science, Agricultural University of Athens, Athens, 11855, Greece. <sup>3</sup>Scientific Directorate of Pesticides' Assessment & Phytopharmacy, Benaki Phytopathological Institute, 14561, Greece. <sup>4</sup>SCU Plant Science, Southern Cross University, Military Rd., Lismore, NSW, Australia. <sup>5</sup>Department of Nutrition, IMB, University of Oslo, 0372 Oslo, Norway.**

Powdery mildew disease, caused by the obligate biotrophic fungal pathogen *Podosphaera xanthii*, is the most reported and destructive disease on cultivated Cucurbita species all over the world. Recently, the appearance of highly aggressive *P. xanthii* isolates has led to powdery mildew outbreaks even in resistant crops making disease management a very difficult task. To challenge this, breeders rely on genetic characteristics for powdery mildew control. Analysis of commercially available intermediate resistance courgette varieties using cytological, molecular, and biochemical approaches showed that the plants were under a primed state and an induced Systemic Acquired Resistance status. Plants exhibited enhanced callose production, upregulation of Salicylic Acid (SA) defense signaling pathway genes and accumulation of SA and defense metabolites. Additionally, the intermediate resistant varieties showed an altered epigenetic landscape in histone marks that affect transcriptional activation. We demonstrated that courgette plants had enriched H3K4me3 marks on SALICYLIC ACID-BINDING PROTEIN 2 (SABP2) and YODA (YDA) genes of the Pm-0 interval introgression, a genomic region that confers resistance to Cucurbits against *P. xanthii*. The open chromatin state of SABP2 and YDA genes was consistent with genes' differential expression, induced SA pathway, altered stomata characteristics and activated SAR responses. Our data suggest that the Pm-0 SABP2 and YDA genes modulate resistance against *P. xanthii* by setting plants in a primed state, and that epigenetic background of courgette varieties on genes has an important regulatory role on defense and induced SAR responses, that could be further explored for production of varieties with enhanced resistance to the pathogen.

**Evaluation of biological and synthetic plant protection products for the management of *Alternaria* leaf blight in carrots.** C.K KAVROUMATZI<sup>1</sup>, M.K. ILIADI<sup>1</sup>, D. AKRIVOPOULOU<sup>1</sup>, E.G. POULAKI<sup>1</sup> and D.I. TSITSIGIANNIS<sup>1\*</sup>. <sup>1</sup>*Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece.* \*Email: dimtsi@aua.gr

*Alternaria dauci* is a necrotrophic fungus responsible for *Alternaria* leaf blight, the worldwide major foliar disease in carrot production. The disease commonly occurs when carrots are cultivated during moderate temperatures and the leaves are exposed to prolonged periods of wetness due to rainfall, dew, or sprinkler irrigation. Severe epidemics have been reported to reduce yields by 40-60%. Under high disease pressure, no single control measure is sufficient to manage the disease adequately. The disease management relies on the combination of the application of synthetic plant protection products (PPPs) with the use of partial resistant varieties and monitored by disease prediction models. In this study, the efficacy of several commercial biopesticides, plant resistance inducers and synthetic PPPs against *Alternaria* leaf blight in carrots were evaluated under greenhouse conditions in two pathogenicity experiments. For the pathogenicity trials, an intermediate disease resistant variety and a susceptible one were used, that were inoculated with a spore suspension of *A. dauci* two days after the application of PPPs. All tested PPPs were able to control successfully *Alternaria* leaf blight in greenhouse. Among the synthetic PPPs, *Luna Sensation*®, *Signum*® and *Dagonis*® showed consistently great decrease in disease severity for both cultivars. *LBG-01F34*®, *Trianum*® and *Sonata*® were some of the most efficient bio-PPPs on both hybrids. The results of the comparative study of the different PPPs will be presented.

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**Physiological, cellular, and molecular responses of *Cucurbita pepo* genotypes infected by *Podosphaera xanthii* and treated with *Reynoutria sachalinensis* plant extract.** T. MARGARITOPOULOU<sup>1</sup>, D. KIZIS<sup>1</sup>, I. THEOLOGIDIS<sup>2</sup>, A. TERMENTZI<sup>2</sup>, E. BAIRA<sup>2</sup>, M. MAKRIDAKIS<sup>3</sup>, J. ZOIDAKIS<sup>3</sup>, N. VAKIRLIS<sup>2</sup>, E. TOUFEXI<sup>1</sup>, G. BALAYIANNIS<sup>4</sup>, C. ANAGNOSTOPOULOS<sup>5</sup>, A.-E. VICHOU<sup>1</sup> L. REMPELOS<sup>6</sup>, C. LEIFERT<sup>7</sup>, E. MARKELLOU<sup>1</sup>. <sup>1</sup>Laboratory of Mycology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 8 Stefanou Delta Street, 14561, Kifissia, Athens, Greece. <sup>2</sup>Laboratory of Toxicological Control of Pesticides, Scientific Directorate of Pesticides' Control & Phytopharmacy, Benaki Phytopathological Institute. <sup>3</sup>Proteomics Laboratory, Foundation of Biomedical Research of the Academy of Athens, 4 Soranou Ephessiou Street, 11527, Athens, Greece. <sup>4</sup>Laboratory of Chemical Control of Pesticides, Scientific Directorate of Pesticides' Control & Phytopharmacy, Benaki Phytopathological Institute. <sup>5</sup>Laboratory of Pesticides Residues, Scientific Directorate of Pesticides' Control & Phytopharmacy, Benaki Phytopathological Institute. <sup>6</sup>School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK. <sup>7</sup>Centre for Organics Research, Southern Cross University, Military Rd., Lismore, NSW, Australia. E-mail: e.markellou@bpi.gr & d.kizis@bpi.gr

Powdery mildew (PM) caused by *Podosphaera xanthii* is one of the most important courgette diseases with high yield losses and is currently controlled mainly by fungicides, biocontrol agents, botanicals, and sulphur applications in conventional and organic production. Giant knotweed (*Reynoutria sachalinensis*, RS) extract is a known elicitor of plant defenses, but its mode of action remains elusive. The aim of this study was to investigate the mechanisms of foliar RS applications and how these affect PM severity and crop performance when used alone or in combination with genetic resistance. Using an array of physiological, cellular, and molecular approaches we showed that RS can have a lasting effect on PM progress on courgette leaves by enhancing plant defense responses at different levels. RS foliar treatments significantly reduced PM severity on both an intermediate resistance (IR) and a susceptible (S) genotype. RS induced the formation of callose papillae, hydrogen peroxide accumulation, and the expression of the Salicylic Acid (SA)-regulated *NPR1*, *PR1*, *PR2* and *PAL* genes which correlated with callose deposits and ROS production. In addition, increased SA production was detected along with increased p-coumaric and caffeic acid concentrations. These findings clearly indicate that RS elicits plant defenses notably through the SA pathway. To gain insight at the molecular level, we performed transcriptomic (Illumina sequencing), proteomic (nanoHPLC-HRMS/MS) and metabolomic (UHPLC-HRMS/MS) analyses on the S genotype inoculated with *P. xanthii* and treated with *R. sachalinensis* prior to inoculation. Functional classifications of the DEGs revealed enriched GO-terms including various hormone-mediated responses, oxidation-reduction processes, lipid biosynthesis and metabolism, and defense responses. Additionally, significant number of glycerophospholipids, lipids that act as chemical signals facilitating signaling during defense response, were detected.

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**Comparative proteome analysis provides new insights into the complex responses of *Citrus aurantium* grafted with *C. sinensis* and infected with *Citrus tristeza virus*.** M. TRINDADE<sup>1§</sup>, S. A. DANDLEN<sup>2§</sup>, L. ANJOS<sup>1</sup>, A. DUARTE<sup>2</sup>, D. M. POWER<sup>1</sup>, N. T. MARQUES<sup>3</sup>.  
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Sour orange (*Citrus aurantium* L., CA) has a lower susceptibility to *Citrus tristeza virus* (CTV). When sweet orange is grafted onto CA and is infected with a quick decline CTV isolate, a quick decline syndrome may develop. This syndrome is related to obliteration and necrosis of the sieve tubes rootstock close to the bud union, which culminates in dieback symptoms. To investigate the molecular response of CA to CTV, the CA stem tissue below the bud union was analyzed by SWATH-MS in two-year-old plants, infected or uninfected with the quick decline isolate T36, in the following conditions: i) CA grafted with CA infected or not; ii) CA grafted with sweet orange [*C. sinensis* 'Valencia Late' (VL)] infected or not. A Library of 2689 proteins was confidently generated for CA. Differentially expressed proteins (DEPs, for a  $p < 0.05$  and  $FD = 1.5x$ ) between CA infected and uninfected were 352, from which 31 were modified in common with VL infected and uninfected. Enriched terms (KEGG pathways) of modified proteins common to CA grafted with uninfected or infected CA and CA grafted with uninfected or infected VL, were mainly assigned to the glycolysis and glyoxylate and dicarboxylate metabolism. Specific modified terms in CA grafted with VL, infected and uninfected, were mainly in alpha-linolenic acid metabolism, tyrosine and carbon fixation metabolism. In summary, a VL grafted scion promotes considerable modifications in stem CA proteome. Furthermore, CTV infection affects the CA stem proteome, differently when the grafted scion is CA or VL.

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**Bacterial mixtures - Combining compatible endophytic *Bacillus* strains with strong biological control potential *in vitro* and *ex vivo*.** P.C. TSALGATIDOU<sup>1,2</sup>, E.-E. THOMLOUDI<sup>1</sup>, E. BAIRA<sup>3</sup>, P. KATINAKIS<sup>1</sup> and A. VENIERAKI<sup>4</sup>. <sup>1</sup>Laboratory of General and Agricultural Microbiology, Crop Science Department, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece. <sup>2</sup>Department of Food Science and Technology, University of the Peloponnese, 24100 Kalamata, Greece. <sup>3</sup>Laboratory of Toxicological Control of Pesticides, Scientific Directorate of Pesticides' Control and Phyto-pharmacy, Benaki Phytopathological Institute (BPI), Kifissia, 14561 Athens, Greece. <sup>4</sup>Laboratory of Plant Pathology, Crop Science Department, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece. E-mail: [venieraki@aua.gr](mailto:venieraki@aua.gr)

The excessive use of chemical plant protection products has caused significant adverse effects on the environment. In agriculture, the alternative use of selective beneficial bacterial biological control agents (BCAs) effectively supports plant protection. Due to the characteristic of their composition, medicinal plants from the Mediterranean basin are an ideal source of efficient endophytic bacterial BCA candidates. Bacterial endophytes are beneficial microorganisms found in the internal tissues of their host plant without causing any visible pathogenic symptoms. In this study, endophytic *Bacillus* bacteria isolated from native medicinal plants *Calendula officinalis* and *Hypericum hircinum* showed remarkable antagonism to phytopathogenic fungi, including *Botrytis cinerea* and *Colletotrichum acutatum* under *in vitro* and *ex vivo* conditions. All *Bacillus* strains studied successfully colonized the plant tissues and competed with the phytopathogenic fungi for nutrients and space. We applied an integrative approach coupling genome mining and metabolic profiling to decipher the potential of these endophytes as BCAs against pathogenic fungi. The chemical analysis of their extract indicated that these *Bacillus* endophytes were able to secrete several bioactive secondary metabolites. Also, we investigate their ability for fungal growth suppression either as individual strains or as bacterial mixtures. Formulation of compatible bacterial strains resulted in some successful mixtures with strong BCA ability.

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***Sclerotinia sclerotiorum*: A new Pathogen of Sugar Beet in the USA.** M.F.R. KHAN<sup>1, 2†</sup>, M.Z.R. BHUIYAN<sup>1</sup>. <sup>1</sup>*Department of Plant Pathology, North Dakota State University, Fargo, ND 58108.* <sup>2</sup>*University of Minnesota, St. Paul, MN, USA.* E-mail: [Mohamed.khan@ndsu.edu](mailto:Mohamed.khan@ndsu.edu)

*Sclerotinia sclerotiorum* (Lib.) DeBary is a destructive necrotrophic fungus that adversely impacts a wide range of crops worldwide (Purdy 1979). This fungus may exist as mycelia and survive in nature through developing hard bodied, blackish resting sclerotia. This pathogen was reported for the first time on sugar beet (*Beta vulgaris*) in North Dakota, USA in 2019 (Khan et al 2020) and was confirmed in several beet producing states areas in 2020. *S. sclerotiorum* was observed causing both foliar blight and root rot. Radial growth of mycelium and development of sclerotia was evaluated on PDA media. Co-cultivation of commercial sugar beet seeds with mycelial plug and sclerotia were done to determine the pathogenicity of inocula in PDA plates. Inoculated seeds showed partial emergence and or complete death of the seedlings whereas non-inoculated check had well developed seedlings. The sensitivity of *S. sclerotiorum* was determined on PDA amended with 0, 0.01, 0.1, 1, and 10 ppm of Proline® (prothioconazole, DMI) Priaxor® (pyraclostrobin, QoI + fluxapyroxad, SDHI) and triphenyltin hydroxide fungicides. Results indicated that Priaxor® was the most effective at reducing mycelial radial growth. Commercial seed varieties from three different seed companies were evaluated for their sensitivity to *S. sclerotiorum*. All varieties evaluated were susceptible to the pathogen suggesting no inherent resistance to this pathogen. The presence of a new emerging pathogen is of concern to the sugar beet industry that is a major economic driver in sugar beet producing states. Field research will be implemented to develop strategies to manage *S. sclerotiorum*.

**SDHI fungicide has potential to reduce storage rot in sugar beet caused by *Botrytis cinerea* in USA.** M.F.R. KHAN<sup>1, 2†</sup>, M.Z.R. BHUIYAN<sup>1</sup>. <sup>1</sup>*Department of Plant Pathology, North Dakota State University, Fargo, ND 58108.* <sup>2</sup>*University of Minnesota, St. Paul, MN, USA.* E-mail: [Mohamed.khan@ndsu.edu](mailto:Mohamed.khan@ndsu.edu)

#### Abstract

*Botrytis cinerea* Pers. is a necrotrophic fungus that affects more than 200 plant species (Brian et al 2007) worldwide. This fungus causes significant economic losses to the sugar beet industry in regions where beet roots are stored for up to nine months. The pathogen is typically present as a characteristic gray mold on the roots that results in increased respiration rates that significantly reduces recoverable sucrose. About 60 to 80% sucrose loss occurs due to respiration during storage (Fugate and Lafta 2009). There are many fungicides that are extensively used for sugar beet production but relatively few chemicals have been evaluated to determine their effect in controlling storage rot of sugar beet. Penthiopyrad, a new SDHI fungicide labeled for sugar beet was evaluated for reducing the activity of *B. cinerea* on sugar beet root in storage conditions. Harvested roots were washed, dried and sprayed or dipped in different concentrations of the fungicides. Harvested sugar beet roots were washed, dried, and sprayed and dipped in different concentrations (10, 20, 40 and 80 ml/L), respectively, followed by inoculation with mycelial plug of *B. cinerea*. The treated beets were kept at 4°C and their respiration rates were evaluated at 0, 30, 60, 90 and 120 days post inoculation. Results indicated that penthiopyrad at 20 ml/L or higher rates significantly reduced the respiration rate (CO<sub>2</sub>) in treated beets and has the potential to be used for reducing storage loss caused by *B. cinerea* on sugar beet in long term storage.

**Strategic management of fungicide resistant *C. beticola* in sugar beet using a holistic management strategy in climate change era.** M.F.R. KHAN<sup>1,†</sup>. <sup>1</sup>*Department of Plant Pathology, North Dakota State University, Fargo, ND 58108; and University of Minnesota, St. Paul, MN, USA. E-mail: [Mohamed.khan@ndsu.edu](mailto:Mohamed.khan@ndsu.edu)*

*Cercospora beticola* causes Cercospora leaf spot (CLS), one of the most damaging foliar diseases of sugar beet grown in warm and humid regions. Since the 1970s, growers have become more dependent on fungicides for effective control of CLS. Growers have used fentin acetate and triphenyltin hydroxide, benzimidazoles, demethylation inhibitors (DMIs), and quinone outside inhibitors (Qols) with varying levels of success at controlling *C. beticola*. Over time, the pathogen, which has the ability to produce large numbers of conidia and multiple generations during a growing season, developed resistance to most of the fungicides used for its control. In 2016, growers in the USA lost over \$200 million because of Qol resistance and a CLS epidemic. Currently, there are few fungicides that provide effective control of *C. beticola* when used alone and very few effective alternating partners. Recently developed varieties (CR+) with improved resistance to *C. beticola* had better resistance to *C. beticola* compared to the best currently approved varieties. Fungicide applications were done on a calendar basis, and on an only when needed based on the presence of symptoms and favorable environmental conditions. Results indicated that CR+ varieties with and without fungicide applications resulted in similar or higher recoverable sucrose compared to similarly treated older varieties. Strategies including incorporating infected debris, crop rotation, planting away from previously infected field, using CR+ varieties, and judicious use of fungicide mixtures will be necessary for sustainable and economic production of sugar beet.

***Trichoderma atroviride* SC1: The biocontrol solution for grapevine pathogens and other important crops.** MARIA KAIIFA, ANDREA NESLER, JONAS GOOSSENS, ANN VERMAETE. *Bi-PA nv (Biological Products for Agriculture), Technologielaan, 7. 1840 Londerzeel, Belgium. E-mail: maria.kaifa@bi-pa.com*

Bi-PA nv is working on the development and registration of biological plant protection products. One of the success-stories of the company is the product Vintec®, a bio-fungicide based on active spores of *Trichoderma atroviride strain* SC1. Vintec® was initially developed and registered as a major tool to protect vines against trunk diseases (Esca, *Eutypa lata*). The product is applied preventively on pruning wounds. The mode of action is mainly based on the colonization of pruning wounds, through creation of a barrier against pathogens and competition for space and nutrient.

After the first registration of Vintec® Bi-PA nv has been continuously working on further developing the product. Vintec® has obtained label extensions for the use against *Botrytis cinerea* on grapes and tomatoes as well as on the main fungal stone fruit pathogens (*Coryneum beijerinckii*, *Monilinia laxa* and *Taphrina deformans*).

Protection of tomato plants against *Botrytis cinerea* was confirmed by efficacy trials in protected conditions in NL, IT and ES. Best efficacy results were observed applying Vintec® at 5 to 10 g/100 L. Efficacy (% Abbott) was better or comparable to the reference products when applied in a preventive application.

In the case of the protection of stone fruits against major pathogens, Vintec® performed very well in efficacy trials performed in open field conditions in IT, ES, GR and PT. Optimal dose is 200 g/ha showing higher or comparable efficacy (% Abbott) to the reference products.

**Fungal diversity in necrotic wood of *Prunus* trees in Germany.** U. DAMM<sup>1</sup> and S. BIEN<sup>2</sup>.

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Wood diseases of fruit trees have far-reaching ecological and economic consequences. The low knowledge about the diversity and taxonomy of the associated fungi impedes quick and targeted control measures in case of a sudden disease outbreak. Therefore, a survey was conducted in orchards of *Prunus avium*, *P. cerasus* and *P. domestica* in three important fruit production areas in Germany. More than 1000 isolates were obtained from symptomatic wood and a diversity of at least 172 species was revealed based on ITS and LSU sequence data with the majority belonging to the *Ascomycota*. *Aposphaeria corallinolutea* (*Dothideomycetes*) and *Pallidophorina paarla* (*Leotiomycetes*) were most frequently detected, from all three host species and in all sampling regions. Based on the number of nucleotide differences to reliable reference sequences, species identifications were divided into categories. According to this scheme, 83 species were identified with high and 20 species with low certainty. The first group already includes 14 taxa that were analysed in- depth within this project, of which 8 species (*Arboricolonus simplex*, *Collophorina badensis*, *C. germanica*, *C. neorubra*, *Cadophora prunicola*, *Ca. ramosa*, *Minutiella pruni-avium*, *M. simplex* and *Proliferodiscus ingens*) and two genera (*Arboricolonus* and *Pallidophorina*) were newly described. Further 69 taxa could not be assigned to any species and were therefore regarded as potential new species. Reduced conidiogenous cells and a yeast-like phase were frequently observed e.g. in *P. paarla*, *Collophorina* and *Minutiella* species and regarded as adaptation to the life inside wood.

This research contributes to the German Barcode of Life project, funded by the Federal Ministry of Education and Research of Germany.



**Pathogenic viability of wheat rust diseases in the southern and eastern Mediterranean region: current status, challenges, and regional collaboration. KUMARSE NAZARI, ICARDA, Izmir, Turkey**

The wheat yellow, stem and leaf rust diseases pose continues threat to wheat production in Central West Asia and North Africa (CWANA) and Mediterranean region. The rust pathogens are capable of rapidly developing new virulence to resistance genes following the Boom and Bust disease cycle. Considering the transboundary nature of the rust pathogens, regional monitoring of rust pathogens and information exchange are the first and foremost requirements in disease resistance mitigation strategy. In response to a call for regional rust research collaboration, the Turkey-ICARDA regional cereal rust research center (RCRRC) was established in Izmir in 2013 and since then the center has coordinated the regional rust surveillance and Trap Nurseries and since 2018 regional race analysis of Yr and Sr was carried out using a Cereal Rust Biosafety Laboratory at RCRRC. A regional precision wheat rust phenotyping platform was also established within the RCRRC which has provided precise field phenotyping data for more than 60,000 wheat accession from the international and national breeding programs. The RCRRC has received more than more than 1000 Yr and Sr samples from Middle east, Mediterranean region and East Africa since 2018. Using differential varieties and molecular marker approaches, it was evident that most of the Yr and Sr rust samples from these regions, have common virulence structures with the European races. This shows similarity of the rust races and freely movements of the rust pathogens. In current presentation, regional race analysis and Trap Nurseries, regional collaboration for precision phenotyping capacity will be presented and discussed.

**A New Disease Complex Threatening Fig (*Ficus carica* L.) in Southern Italy.** W. HABIB<sup>1</sup>, V. CAVALIERI<sup>1,2</sup>, M. CARLUCCI<sup>3</sup>, C. DONGIOVANNI<sup>1</sup>, F. NIGRO<sup>1,3</sup>. <sup>1</sup>*Centro di Ricerca, Sperimentazione e Formazione in Agricoltura – Basile Caramia (CRSFA), Via Cisternino 281, Locorotondo, 70010 Bari, Italy.* <sup>2</sup>*Istituto per la Protezione Sostenibile delle Piante – CNR, Via Amendola 122/D, 70126, Bari, Italy.* <sup>3</sup>*Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari - Aldo Moro, Via Amendola 165A, 70126 Bari, Italy.*

Symptoms of severe decline of fig (*Ficus carica*) have been recently detected in Salento area of the Apulia region (southeastern Italy). Symptoms begin with leaf chlorosis and evolve into necrosis, twig defoliation and death of lateral branches. Cankers are often observed, associated with extensive wood discoloration under the dead bark and together with bark beetles' galleries. In September 2021, wood sections and adult insect specimens were collected from symptomatic trees in several orchards. Bark beetles were morphologically and molecularly identified as *Cryphalus dilutus* (Curculionidae, Scolytinae). Fungal isolation was performed on PDA amended with 0.5 g/l streptomycin sulfate from 200 wood fragments and 52 *C. dilutus* adults. Three groups of fungal pathogens were recovered from both plant tissues and insects, namely Botryosphaeriaceae (38.5%), *Fusarium solani* species complex FSSC (36.9%), and Ophiostomatoid fungi (6.7%). Mixed infections with at least two pathogens were common (67%). A set of 47 single spore isolates were therefore identified using multilocus phylogenetic analyses based on the sequences of three informative genes. The preliminary results showed that almost 50% of the FSSC isolates belong to the newly described species *Neocosmospora perseae* causing trunk cankers on avocado in Sicily. Also, at least three species of Botryosphaeriaceae (*Neofusicoccum parvum*, *Lasiodiplodia theobromae*, *Botryosphaeria dothidea*) and two genera of Ophiostomatoid fungi (*Graphium* and *Ceratocystis*) were identified. Phylogenetic analyses and pathogenicity tests are ongoing in order to determine the role of each fungal species in the aetiology of the observed decline syndrome, and verify the potential role of the *C. dilutus* as a vector.

**Pathogenesis-related proteins of *Arabidopsis thaliana* in response to combination of abiotic (salinity) and biotic (fungus gnats and dodder) stresses.** L. ZAGORCHEV, D. TEOFANOVA, K. GEORGIEVA, A. ATANASOVA. *Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski", 1164, 8 Dragan Tsankov Blvd., Sofia, Bulgaria*  
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The aim of the present study was to follow the response of the model plant *Arabidopsis thaliana* L. to a combination of abiotic and biotic stresses: 1) salinity; 2) herbivores – fungus gnats (Sciaridae) and 3) parasitic plants – *Cuscuta campestris* Yunck. (dodders), as well as the indirect effect on the dodder. Two major pathogenesis-related (PR) proteins classes – chitinases and proteases, were profiled under different stress conditions – single, double and triple stress in directly and indirectly affected organs. Enzymes were studied by zymography analyses after separation on polyacrylamide gel electrophoreses and data were analyzed by ANOVA and principle component analysis. All stress factors affected both the number of isoforms and the relative activity of both enzymes with the effect of herbivores being the most pronounced. The combination of stresses lead to mostly antagonistic effect in roots and cumulative effect in leaves. Salt stress caused similar effect to parasitism, differential effect in comparison to herbivores and did not interact with biotic stresses, while herbivores and parasitism affected the PR-proteins differentially, but interacted strongly. Most interestingly, both salinity and herbivores, applied on the host plant, affected significantly the parasitic plant as well.

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**Genome-wide characterization of WD40 protein family in *Monilinia fructigena*.** A. ZAMBOUNIS<sup>1</sup> and A. XANTHOPOULOU<sup>1,1</sup>.<sup>1</sup>*Institute of Plant Breeding and Genetic Resources, HAO 'Demeter', Themi, Thessaloniki, 57001, Greece. E-mail: antonios.zamb@gmail.com*

The ascomycete fungus *Monilinia fructigena* is among the most serious causal agents of brown rot in deciduous fruit trees. The genome of the pathogen is available providing novel genomic resources for the thorough characterization of particular gene families. WD40 proteins are scaffolding molecules in protein interactions and despite that these proteins were extensively characterized in other organisms, little is known in phytopathogenic fungi about their expansion, structural patterns, as well evolutionary selective pressures acting upon their WD40 repeats. In our study, we identified 62 WD40 proteins in *M. fructigena* genome (MfWD40s) and based on their phylogenetic classification and domain architectures they were categorized into 5 clusters and 17 classes, respectively, indicating their diverse expansion. Gene ontology analysis revealed that MfWD40s are participated in protein binding and involved in various biological processes. Besides, RNAseq data revealed that the highest number of *MfWD40s* genes showed stage-specific expression profiles with the most of them to highly expressed during germination of conidia. Furthermore, we accurately assessed the evolutionary signatures acting upon their WD40 repeats. Our results postulate existence of purifying selection acting across their phylogenies. However, a high majority of amino acid residues sites were positively selected and were localized widely across the WD40 repeats putatively affecting their ligand- binding specificities. These findings would allow us to further decipher the diverse functions of WD40 gene family in *M. fructigena*.

This work has been supported by Hellenic Agricultural Organization HAO 'Demeter', Greece.

**RNA sequencing-based transcriptional profiling of kiwifruit during infection by *Botrytis cinerea*.** A. ZAMBOUNIS<sup>1</sup>, I. GANOPOULOS<sup>1</sup>, D. VALASIADIS<sup>2</sup>, L. KARAPETSI<sup>3</sup> and P. MADEISIS<sup>3</sup>. <sup>1</sup>*Institute of Plant Breeding and Genetic Resources, HAO 'Demeter' Themi, Thessaloniki, 57001, Greece.* <sup>2</sup>*Laboratory of Pomology, Department of Agriculture, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece.* <sup>3</sup>*Institute of Applied Biosciences, CERTH, Themi, Thessaloniki, 57001, Greece. E-mail: antonios.zamb@gmail.com*

*Botrytis cinerea* is a widespread plant fungal pathogen, the causal agent of gray mold disease, infecting a broad range of economically important crops including kiwifruit. In order to decipher the induced defense mechanisms upon infection in kiwifruit at three time points (12, 24, and 48 h after infection), we employed an RNA sequencing (RNA-Seq) approach to explore the transcriptome of the mature affected fruits. Overall, 18 cDNA libraries were produced generating 978,286,445 base pair-end reads. A total of 2,795 differentially expressed genes (DEGs) were identified with the majority of them to being up-regulated at late stages across a broad and time-dependent transcriptional reprogramming during infection. Suppression of photosynthesis-related pathways observed at 12 h after infection (HAI), whereas DEGs involved in specific cell wall modification processes may have facilitated early pathogen colonization. A remarkable shift towards defense responses and a hitherto of induced transcriptional reprogramming was triggered rather in delay and not earlier than 24 HAI. The majority of DEGs were up-regulated mainly at 48 HAI and were predominately involved in the induction of biosynthesis of secondary metabolites, pattern-triggered immunity and signaling transduction cascades, activation of pathogenesis-related proteins, membrane-localized receptor-like kinases, transcription factors, and various sorts of transporters. Furthermore, a subset of *B. cinerea* genes that are linked to pathogen growth or manipulation of particular plant defense responses were also induced. These data would provide novel insights into kiwifruit-*B. cinerea* compatible interaction allowing to expand our knowledge towards the efficient management of this pathogen.

This work has been supported by Hellenic Agricultural Organization HAO 'Demeter', Greece.

**Infection and colonization of grapevine propagation material by pathogens associated with young grapevine decline in Greece.** C. TSOUKAS<sup>1</sup>, A. K. TZIMA<sup>1</sup>, A. TRIANTAFYLLOPOULOU<sup>1</sup> and E. J. PAPLOMATAS<sup>1</sup>. <sup>1</sup>*Laboratory of Plant Pathology, Agricultural University of Athens, Iera Odos 75, Athens, Greece.*

To investigate colonization of propagation material by young grapevine decline associated pathogens, eighty canes from four different varieties of mature mother plants (cvs Ksinomavro, Agiorgitiko, Asirtiko, Roditis) were sampled in July 2018. In December 2018, forty 4-months old vines (cv. Soultanina) grafted on rooted rootstocks (R110 or 41B) were collected. Nested PCR reactions based on amplifications of the ITS region and species-specific primers showed that, grafted young vines were highly infected by the young grapevine decline-associated pathogens. More specifically, fourteen out of forty (35%) grafted cuttings were infected by *Phaeoconiella chlamydospora* and thirty-five out of forty (87.5%) were infected by Black Foot disease-associated species. Multiplex PCR assays revealed that the dominant species was *Dactylonectria macrodidyma*. It is worth noticing that, in July pathogens could not be detected by PCR at the base of growing-season canes used for grafting. For the colonization studies, a *P. chlamydospora* strain was transformed with the green fluorescent protein gene (*gfp*) using ATMT (*Agrobacterium tumefaciens*-mediated transformation). Twenty four 4-months old grafted vines were inoculated with  $10^7$  spores/ml either with the GFP *P. chlamydospora* strain or the wild type, and kept at the greenhouse for three months. Fluorescent microscopy showed that, three months post inoculation the fungus was able to colonize the cells around the xylem vessels, the parenchyma cells and the primary xylem, but had not advanced more than 2 cm above or below the inoculation point.

**Investigation of the disease-associated role, cellular localization and secretion of the thermo unstable translation elongation factor (Ef-tu) encoded by the vascular wilt fungus *Verticillium dahliae*.** G. PATSIS<sup>1</sup>, A. TRIANTAFYLLOPOULOU<sup>1</sup>, D. GKIZI<sup>2</sup>, S. KANG<sup>3</sup>, A. TZIMA<sup>1</sup>, E. PAPLOMATAS<sup>1</sup>. <sup>1</sup>*Laboratory of Plant Pathology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece,* <sup>2</sup>*Department of Food Science and Technology, University of West Attica, 28 Agioy Spiridonos, 12243 Aigaleo, Greece,* <sup>3</sup>*Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, PA 16802, United States.* E-mail: [aliki@aua.gr](mailto:aliki@aua.gr)

Besides its crucial role in protein synthesis, thermo unstable translation elongation factor (Ef-tu) plays multiple other roles (moonlighting effect) in prokaryotes and eukaryotes. The phytopathogenic bacterial Ef-tu triggers the immune system of plants as a PAMP (Pathogen-Associated-Molecular-Pattern) recognized by the specific receptor EFR. In this study the subcellular localization of the orthologous protein was investigated in *V. dahliae* (Vtu) to determine whether it is translocated to the cell membrane or in the extracellular region to interact with the host. The *vtu* gene was fused with the enhanced green fluorescent protein (*egfp*) gene under the control of a strong fungal promoter. This construct was used to transform *V. dahliae* race 1 strain 70V via *Agrobacterium tumefaciens*-mediated transformation. Localization of Vtu-Egfp was detected using a confocal microscope, which showed that the Vtu protein was localized in the cytoplasm and vacuoles. When the transcript level of *vtu* was measured in various transformants with qPCR, it appears that integration of additional copies of the *vtu* gene in *V. dahliae* may have activated the RNA silencing mechanism in some transformants. Selected *V. dahliae* transformants expressing Vtu-Egfp will be tagged with a different fluorescent protein gene to investigate whether the Vtu-Egfp protein is secreted during host infection.

***Bactrocera oleae* as a transmitting agent of olive drupes anthracnose caused by *Colletotrichum acutatum*.** P. ADAMI<sup>2</sup>, P. VELAETI<sup>1</sup>, C. TSOUKAS<sup>1</sup>, S. DERVISOGLOU<sup>2</sup>, E. J. PAPLOMATAS<sup>1</sup>, D. PERDIKIS<sup>2</sup>. <sup>1</sup>Laboratory of Plant Pathology and <sup>2</sup>Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, Iera Odos 75, Athens, Greece.

To investigate possible transmission of *C. acutatum* by the olive fruit fly *Bactrocera oleae* thus contributing to disease spread, pupae and healthy olive drupes were collected in October 2021 from olive orchards in Greece. Drupes were artificially inoculated by an aqueous suspension of  $10^6$  conidia/ml of the pathogen, while control drupes were sprayed with ddH<sub>2</sub>O. Seven days after incubation under appropriate conditions, olive drupes were transferred for 2 days into separate entomological cages, containing adult flies that had emerged from the collected pupae. Subsequently, all drupes were discarded and replaced by new sterilized ones that remained for 2 days with the flies. Thereafter, molecular detection of *C. acutatum*, on both flies and drupes, was performed by Real-Time PCR assays based on TaqMan chemistry, using *C. acutatum* specific primers targeting the *HIS-3* gene. Pathogen DNA was detected in all artificially inoculated drupes and in some of the flies. More specifically, fungal DNA could not be detected in negative control drupes, while in drupes that remained for 2 days with adult flies previously caged with artificially inoculated fruits, pathogen DNA was detected. To investigate the possibility that fungal spores were carried passively by the flies, adult insects were superficially sterilized using either hexane or water. It is worth noticing that besides detection in non-sterilized flies, DNA of the pathogen was also detected in surface sterilized flies. According to these findings, there is initial evidence that *B. oleae* could act as a possible vector of *C. acutatum* anthracnose on olive drupes.



**Manipulation of ACC (1-aminocyclopropane-1-carboxylic acid) deaminase gene in *Verticillium dahliae* revealed a binary role for ACC in regulating virulence and plant defense: two sides of the same coin.** M.-D. TSOLAKIDOU<sup>1</sup>, I. S. PANTELIDES<sup>1</sup>, A. K. TZIMA<sup>2</sup>, S. KANG<sup>3</sup>, E. J. PAPLOMATAS<sup>2</sup> and D. TSALTAS<sup>1</sup>. <sup>1</sup>*Cyprus University of Technology, Department of Agricultural Sciences, Biotechnology and Food Science, 3603 Limassol, Cyprus.* <sup>2</sup>*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 11855, Athens, Greece.* <sup>3</sup>*Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802, USA.* E-mail: [maria.tsolakidou@cut.ac.cy](mailto:maria.tsolakidou@cut.ac.cy)

Some microorganisms that promote plant growth can manipulate the level of ethylene in plants by decreasing ACC, an ethylene precursor, using ACC deaminase (ACCd), making plants more resistant to a variety of abiotic and biotic stresses. Despite the beneficial effect of ACCd-producing microorganisms, the role of ACCd in phytopathogens has been poorly studied. Here, we investigated whether ACCd of the soil-borne pathogen *Verticillium dahliae* is involved in pathogenesis and ethylene production. Interestingly, overexpression and deletion mutants of the *V. dahliae* gene (*ACCd*) encoding this enzyme, produced more ethylene than the wild type strain with deletion mutants producing more ethylene than overexpression strains at most time points of observation. However, overexpression of the *ACCd* gene significantly increased virulence in tomato, while disruption of *ACCd* resulted in reduced disease severity. ACC holds a key position in many plant physiological processes with its main role as direct precursor of ethylene. Recent studies have shown that ACC may act as a potential signaling molecule independent from ethylene. To investigate whether the pathogenicity phenotype observed was due to ACC regulation, roots of WT and *Never Ripe* (*Nr*) tomato plants and Col-0 and *etr1-1* Arabidopsis plants were treated with ACC prior to *V. dahliae* inoculation. Plants pre-treated with ACC displayed less severe symptoms than untreated controls. ACC application on the roots of Col-0 and *etr1-1* plants in vitro triggered root hair formation and induced hormone-dependent defense responses. Collectively, our results suggest a novel role of ACC as a regulator of both plant defense and pathogen virulence.

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**Phenotypic and molecular responses of potato genotypes to infection by pathotype 18(T1) of *Synchytrium endobioticum* (Schilb.) Perc.** T. MARGARITOPOULOU, I. THEOLOGIDIS, D. KIZIS, N. VAKIRLIS, C. KRITIKOS, D. TSIROGIANNIS and I. VLOUTOGLOU. *Benaki Phytopathological Institute, Scientific Department of Phytopathology, Laboratory of Mycology, 8 St. Delta Street, 145 61 Kifissia, Attica, Greece. E-mail: i.vloutoglou@bpi.gr*

The obligate biotrophic, soil-borne fungus *Synchytrium endobioticum* (Schilb.) Perc., the causal agent of potato wart disease, is regarded as one of the most important quarantine pests of cultivated potato (*Solanum tuberosum* L.) worldwide. As chemical control is not effective in eradicating the pathogen, strict phytosanitary measures combined with the use of resistant potato varieties is the only successful strategy to prevent the disease from spreading. However, the pathogen can overcome host resistance by developing new pathotypes. More than 40 pathotypes have been reported so far of which, pathotypes 2(G1), 6(O1) and 18(T1) are the most aggressive and widespread in Europe. Previous studies conducted under natural and controlled environment conditions have shown that only two out of the 50 European potato genotypes tested exhibited phenotypic resistance to infection by pathotype 18(T1) of *S. endobioticum* known to be present in Greece. The potato varieties Kuba (resistant), Novano (tolerant) and Spunta (very susceptible) were selected to further study their molecular response to infection by pathotype 18(T1) using RNA-Seq transcriptomic analysis. Raw data genome-guided transcriptome assembly was performed using STAR2.5.3.a and HTSeq. Differential Gene Expression (DGE) was performed in R using DESeq2 1.32.0, followed by DEGs functional classifications using topGO in R and G-profiler. Many genes related to cell division, lipid catabolism, hormone transportation and peroxidase function were identified. GO-term analysis revealed enrichment regarding terms related to pathogen resistance, membrane bound organelle, cell cycle, defense response, structural molecule activity and response to external biotic stimulus, in intra- and inter-varieties (infected or not) comparisons.

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***BAM3* plays a significant role in host resistance against *Fusarium oxysporum*.** E.N. KALOGEROPOULOU<sup>1</sup>, K.A. ALIFERIS<sup>2</sup>, M.D. LYKOGIANNI<sup>1,2</sup>, S.E. TJAMOS<sup>3</sup>, I. VLOUTOGLOU<sup>1</sup> and E.I. PAPLOMATAS<sup>3</sup>. <sup>1</sup>Benaki Phytopathological Institute, Scientific Directorate of Phytopathology, Laboratory of Mycology, 8 St. Delta str., GR-145 61 Kifissia, Athens, Greece. <sup>2</sup>Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos, GR-118 55 Athens, Greece. <sup>3</sup>Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, GR-118 55 Athens, Greece. E-mail: [e.kalogeropoulou@bpi.gr](mailto:e.kalogeropoulou@bpi.gr)

Fusarium wilt, a vascular disease caused by phytopathogenic strains of the soil-borne *Fusarium oxysporum* (*Fo*) species complex, is responsible for serious yield losses in >120 economically important crops. Because of the soil-borne nature and the wide host range of the pathogen, Fusarium wilt management has been a challenge. Breeding for resistance has shown positive results in reducing the pathogen spread and increasing crop yields. In the present study, the role of  $\beta$ -amylase (*BAM*) genes upon plant resistance against *Fo* was investigated *in planta* using *Arabidopsis thaliana* ecotype Columbia-0 (wild-type, wt) and its mutants *bam1*, *bam2*, *bam3* *bam4* and their combinations inoculated with *F. oxysporum* f. sp. *raphani* (*For*). The pathogenicity tests showed that *bam3* plants had significantly lower susceptibility to *For* infection compared with the wt plants. Disease intensity and fungal biomass in the plant vascular system, as quantified by qPCR, were significantly lower in *bam3* mutants compared to the wt plants. Transcriptomic and metabolomic analyses performed using DNA microarrays and gas chromatography-mass spectrometry (GC-MS), respectively, showed that the resistant phenotype of *bam3* plants is interpreted by differential gene expression and differential production of metabolites related to the cell wall integrity, the metabolism of carbohydrates, amino acids and plant hormones.

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**Evaluation of foliar resistance of Greek wine grape varieties to downy mildew by phenotyping methods and comparative transcriptomic and proteomic analyses.** E. N. KALOGEROPOULOU<sup>1</sup>, D. TSIROGIANNIS<sup>1</sup>, G. TSIOLAS<sup>3</sup>, D. KIZIS<sup>1</sup>, E. BAIRA<sup>2</sup>, I. THEOLOGIDIS<sup>2</sup>, E. MARGARITIS<sup>1</sup>, C. KRITIKOS<sup>1</sup>, P. BATAKIS<sup>2</sup>, A. ARGIRIOU<sup>3</sup>, E. MARKELLOU and I. VLOUTOGLOU<sup>1</sup>. <sup>1</sup>*Benaki Phytopathological Institute, Scientific Directorate of Phytopathology, Laboratory of Mycology, 8 St. Delta Street, 145 61 Kifissia, Attica, Greece.* <sup>2</sup>*Benaki Phytopathological Institute, Scientific Directorate of Pesticides' Control & Phytopharmacy, Laboratory of Toxicological Control of Pesticides, 7 Ekalis Street, 145 61 Kifissia, Attica, Greece.* <sup>3</sup>*Centre for Research and Technology Hellas, Institute of Applied Biosciences, 6<sup>th</sup> km Charilaou-Thermi Road, P.O. Box 60361, Thessaloniki, Greece. E-mail: i.vloutoglou@bpi.gr; e.markellou@bpi.gr; d.kizis@bpi.gr*

The obligate biotrophic oomycete *Plasmopara viticola* (Berk & Curt.) Berl. & de Toni, the causal agent of grapevine downy mildew (DM), is one of the most serious threats to viticulture worldwide. Sustainable and environmentally friendly DM management strategies rely mainly on host resistance. However, no information is available on the response of Greek wine grape varieties to *P. viticola* infection. In the present study, the resistance level of 11 native wine grape varieties was assessed in leaf disc bioassays. The response of the grape varieties to DM was evaluated based on the host necrotic reaction and the intensity of pathogen's sporulation over a period of 15 days post-inoculation (dpi). Results showed that the varieties exhibited different responses to DM, while none of them was found to be resistant to the pathogen. The least susceptible and the most severely affected varieties were selected for transcriptomic and proteomic analyses to gain an insight into the molecular aspects of host-pathogen interactions. Libraries prepared from total RNA and protein extracts of grapevine leaf disc samples (inoculated and non-inoculated) collected five dpi, were analyzed with the Illumina NextSeq500 and the Thermo Q-Exactive Orbitrap nanoHPLC-HRMS/MS platforms, respectively. Transcriptomic analysis was performed according to the "New Tuxedo" pipeline. Differential expression and functional classifications of the DEGs revealed genes involved in disease resistance, modulation of pathogen defense and leaf cell death, downy mildew specific resistance, and plant hormone regulation. Proteomic analysis conducted using R programming revealed significant differences between the two varieties.

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**Exploring the selective signatures upon LRR-containing genes towards their functional diversification... the cases of cherries and mulberries.** A. ZAMBOUNIS<sup>1</sup>, I. GANOPOULOS<sup>1</sup>, A. TSAFTARIS<sup>2</sup> and P. MADEISIS<sup>3</sup>.<sup>1</sup>*Institute of Plant Breeding and Genetic Resources, HAO 'Demeter', Themi, Thessaloniki, 57001, Greece.* <sup>2</sup>*Perrotis College, American Farm School. Thessaloniki, 57001, Greece.* <sup>3</sup>*Institute of Applied Biosciences, CERTH, Themi, Thessaloniki, 57001, Greece. E-mail: antonios.zamb@gmail.com*

Cherry (*Prunus avium* L.) and mulberry (*Morus notabilis*) crops are plagued by several phytopathogenic fungi. Gene families that contain leucine-rich repeat (LRR) domains are key components of plant immune responses. Particularly, the subfamily of LRR receptor-like serine threonine kinases is playing a pivotal role in defense responses against phytopathogenic fungi. Besides, resistance gene analogs (RGAs) which are closely related to the resistance (*R*) genes are widely employed as functional molecular markers in breeding programs towards disease resistance against pathogens. The objective of this study was to gain insights about the evolutionary profiles of these genes (173 RGAs and the 142 LRR receptor-like serine threonine kinases of cherry and mulberry, respectively), hypothesizing that successive episode of diversifying selection might contribute in the acquisition of novel pathogens recognition repertoires. These genes were subjected to strong positive selection, while the majority of the positively selected amino acid residues are localized widely across genes sequences. These residues might have originally conferred specificity to a hypothetical ligand which was being apparently altered repeatedly to provide novel binding functions. The clustered distribution of these genes might also be pronounced of high birth and death rates with diversifying episodes acting on their functional domains, putatively affecting their ligand-binding specificities. Such evolutionary insights shed light on how these genes are being evolved, assigning them as the foremost surveillance mechanism for durable resistance against rapidly evolving pathogens.

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**Genetic and structural diversity of disease resistance genes through whole genome re-sequencing of sweet cherry (*Prunus avium* L.) cultivars.** A. ZAMBOUNIS<sup>1</sup>, I. GANOPOULOS<sup>1</sup>, A. TSAFTARIS<sup>2</sup>, P. MADESIS<sup>3</sup>, A. MOLASSIOTIS<sup>4</sup> and A. XANTHOPOULOU<sup>1</sup>.<sup>1</sup>*Institute of Plant Breeding and Genetic Resources, HAO 'Demeter' Themi, Thessaloniki, 57001, Greece,* <sup>2</sup>*Perrotis College, American Farm School. Thessaloniki, 57001, Greece.* <sup>3</sup>*Institute of Applied Biosciences, CERTH, Themi, Thessaloniki, 57001, Greece.* <sup>4</sup>*Laboratory of Pomology, Department of Agriculture, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece. E-mail: antonios.zamb@gmail.com*

Sweet cherry is plagued by many pathogens that are a major threat to crop production. Nowadays, as climate change alters dramatically the susceptibility of sweet cherry cultivars to rapidly evolved and emergent pathogens, it is more than crucial to thoroughly investigate the repertoire of highly impact structural variations in disease resistance genes among cultivars in order to facilitate the selection of superior genotypes. In this study, whole-genome resequencing (WGRS) of 21 sweet cherry cultivars representing the majority of wild and cultivated Greek germplasm, was employed to characterize the genetic and structural variation among the 119 defense-related genes (such as *RPM1*, *RPP13*, *RGA2* homologues). The total number of structural variants were 2,468 and they were mapped on 107 disease resistance genes. The majority of these variants was heterozygous and were assigned as missense or synonymous variants. Forty-four NBS-LRR genes had SNPs (single nucleotide polymorphisms) mutations in their coding sequences with high impact across the sweet cherry genotypes. These data of our genome-wide analysis provide promising resources for expanding our knowledge on the diversity and evolution of these variations at disease resistance genes across the sweet cherry cultivars. On top of that, screening and genetic analysis of these genes would facilitate to identify the functional variations contributing to disease resistance variation among the sweet cherry cultivars. This approach is essential for the evaluation of natural resistance resources towards building up durable disease resistance against pathogens in sweet cherry.

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**Transcriptome analysis and gene expression profile in response to drought stress in *Citrus macrophylla* stem tissues.** M. SILVA<sup>1</sup>, P.I.S. PINTO<sup>1</sup>, A. DUARTE<sup>2</sup>, S. DANDLEN<sup>2</sup>, R. GUERRA<sup>3</sup>, D. POWER<sup>1</sup>, N.T. MARQUES<sup>3</sup>. <sup>1</sup>*Comparative Molecular and Integrative Biology, Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.* <sup>2</sup>*Mediterranean Institute for Agriculture, Environment and Development (MED), Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.* <sup>3</sup>*Center for Electronics, Optoelectronics and Telecommunications (CEOT), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. E-mail: nmarques@ualg.pt.*

Drought is a major abiotic stress with adverse effects on citrus. In grafted plants drought tolerance is mainly determined by the rootstock. *Citrus macrophylla* Wester, a rootstock used in the Mediterranean region, is known to confer an enhanced tolerance to drought compared to other citrus rootstocks. Transcriptional changes of *C. macrophylla* under water deficit was studied by Illumina RNA-seq technology and responsive genes were identified. A total of 2745 differentially expressed transcripts (DETs, fold change >2), were identified of which 631 (23 %) were up-regulated and 2114 (77%) were down-regulated. Gene Ontology analysis identified “phenylpropanoid biosynthesis”, “amino sugar and nucleotide sugar metabolism” and “photosynthesis” as overwhelmingly down-regulated in response to drought stress (DS). Up-regulated processes in DS were soluble carbohydrates and amino acids, which contribute to osmotic balance and protect against oxidative damage. Glycolysis and photorespiration were also strongly up-regulated in *C. macrophylla* as well as transcripts for low molecular weight proteins such as late embryogenesis abundant protein (LEA). In the environment citrus plants face both abiotic and biotic stresses and to assess the interplay of DS and *Citrus tristeza virus* (CTV) infection, twelve genes were profiled by quantitative PCR (qPCR) analysis in control and CTV-infected CM plants, with or without DS. The twelve analyzed transcripts were significantly correlated ( $r = 0.82$ ,  $p < 0.001$ ) with the RNA-Seq results and gave insight into the response of *C. macrophylla* to drought and/or to infection with CTV. Transcriptome results unveiled highly responsive genes to DS in stem tissue, which may be candidates for genetic selection of high drought tolerant plants of *C. macrophylla*.

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**Efficiency of different proactive measures in the management of *Verticillium* wilt of potato in Lebanon.** F. BAROUDY<sup>1</sup>, L. SAADÉ<sup>2</sup>, Z. MAHFOUZ<sup>3</sup>, C. SAAB<sup>1</sup>, E. GERGES<sup>1</sup>, W. HABIB<sup>1</sup> \*. <sup>1</sup>Laboratory of Mycology, Department of Plant Protection, Lebanese Agricultural Research Institute, P.O. Box 90-1965, Fanar, Lebanon. <sup>2</sup>Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, P.O. Box 55530, Dekwaneh, Lebanon. <sup>3</sup>Faculty of Agricultural and Food Sciences, Holy Spirit University of Kaslik, P.O. Box 446, Jounieh, Lebanon.

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*Verticillium* wilt caused by *Verticillium dahliae* Kleb. is an economically important disease producing severe losses on potato crop in Lebanon. The management of the disease is difficult and requires the integration of several measures because the pathogen is able to survive in soil as microsclerotia for up to 15 years. In the present study, two field experiments were carried in artificially inoculated soil over two years in order to evaluate the effect of different preventive measures on wilt incidence and severity, soil inoculum density, tubers yield and plant height. In the first trial, the synthetic fumigant allyl-isothiocyanate significantly reduced microsclerotia density by 41.3% and wilt incidence by 17.5%. The application of fungicides (azoxystrobin + mefenoxam) at sowing reduced wilt incidence by 20%. In the second trial, inoculum density was significantly reduced by soil solarization (58%) and the integration of solarization and incorporation of broccoli residues (56.8%). Soil solarization decreased the disease severity by 25% whereas the integration of fresh broccoli residues and soil solarization significantly reduced disease incidence by 23%. Although the incorporation of fresh broccoli residues before sowing did not significantly affect the disease parameters in both trials, it promoted growth parameters resulting in an average total tuber yield of 15.1 t ha<sup>-1</sup> compared to 7.9 t ha<sup>-1</sup> in the untreated control. The results of both experiments suggest that the integration of measures, particularly soil solarization and the incorporation of fresh residues of broccoli can be a promising approach to manage *Verticillium* wilt of potato in Lebanon.



**Endophytic bacteria as potential biocontrol agents against *Phaeomoniella chlamydospora*, the dominant causal agent of Petri disease in grapevines.** C. TSOUKAS<sup>1</sup>, A. K. TZIMA<sup>1</sup>, E. J. PAPLOMATAS<sup>1</sup>. <sup>1</sup>*Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece.* \*e-mail: [epaplom@aua.gr](mailto:epaplom@aua.gr)

Petri disease of grapevines is considered as the most destructive disease worldwide, and its occurrence in grapevine nurseries is widely reported. Due to the lack of agrochemicals to control the disease, the utilization of effective biological control agents (BCAs) becomes imperative. The aim of this study was to screen the grapevine root bacteriome for its biocontrol potential against the fungal pathogen *P. chlamydospora*, and to further develop microbial communities capable of suppressing the disease. Bacteria were isolated from roots derived from vines of different ages (5 to >50 years) with no apparent symptoms of trunk diseases. In total, twenty-three bacterial isolates were selected, based on colony morphology. The antagonistic ability of the bacterial strains was assessed in *in vitro* experiments, by applying a modified dual culture technique. Inhibition was estimated as the percentage of mycelial growth compared with the control. In total, four isolates significantly inhibited the growth of *P. chlamydospora*, compared to the rest of the isolates. Sequencing of the 16S rRNA region revealed that one strain belonged to *Bacillus halotolerans* while all other isolates were grouped to *Bacillus* sp. To develop efficient microbial communities, interaction assays between the most effective bacterial strains based on antagonism is underway. These preliminary findings show that, the endophytic microbiome has the potential to be utilized in disease control.

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**Monitoring of Copper persistence on Plants by Active Thermography.** M. RIPPA<sup>1</sup>, V. BATTAGLIA<sup>2</sup>, M. CERMOLA<sup>2</sup>, P. MORMILE<sup>1</sup>, E. LAHOZ<sup>2</sup>. <sup>1</sup>*Institute of Applied Sciences and Intelligent Systems “E. Caianiello” of CNR, 80072 Pozzuoli (Na), Italy.* <sup>2</sup>*Research center for Cereal and Industrial Crops - Council for Agricultural Research and Economics (CREA-CI) Via Torrino 3, 81100 Caserta (CE). E- mail: [valerio.battaglia@crea.gov.it](mailto:valerio.battaglia@crea.gov.it)*

Optimization of agricultural practices represents an objective of great worldwide interest, especially in the period of post-covid resilience that awaits us. Copper (Cu) has been used in agriculture as a fungicide for over a century playing a key role in integrated pest management. Because of accumulation in the soil the use needs to be monitored and controlled, also by using modern technologies to minimize the amount of Cu per season used. Here, we propose a novel approach based on the Active Thermography (AT) to monitor the persistence of Cu on leaf surfaces and the use of new polysaccharide-based adjuvant to prolong the persistence of Cu fungicides. We monitored the thermal response of different treatments of both vine and tobacco plants over a three-week period. Spatial maps of the thermal recovery times of leaves were calculated and compared. The monitoring carried out shows that the new adjuvant can prolong the persistence on the leaves of the Cu-based fungicide used. Moreover, from our measurements we estimated the percentage of the Cu on the leaves. According to our results, the maximum difference in the Cu percentage between the plants treated with Cu alone and with Cu+adjuvant (after two weeks for both species) is respectively about 29% for vine and 32% for tobacco plants. Our achievements demonstrate that the new approach based on AT can be an effective tool to evaluate in real-time the presence of Cu on treated plants allowing to optimize its use in the agricultural practices.

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**Fungicide resistance of *Botrytis fabae* population isolated from faba bean in Morocco.**  
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Chocolate spot, caused by *Botrytis* spp., is one of the most economically important diseases of faba bean worldwide. Management of this disease in Morocco is largely depending on chemical treatments using several classes of fungicides, especially the site-specific fungicides. The pathogen presents a high risk for the development of resistance to fungicides. The objective of this study was to evaluate the sensitivity of *Botrytis fabae* population to fungicides fludioxonil, fenhexamid and boscalid, currently used for disease control in Morocco. Thirty- one isolates of *B. fabae* were collected from seven bioclimatic regions, where faba bean production is important. The sensitivity of isolates to fludioxonil, fenhexamid and boscalid was evaluated with an automated quantitative test (AQ) using 96-well microtitre plates. The growth inhibition percentage (GIP) of pathogen was calculated using the absorbance data at 492 nm of each well after 72h of incubation to determine sensitive isolates to each fungicide. Results showed that isolates with resistance to Fludioxinil and Boscalid were widespread. Seven resistant profiles were determined in the population (Flu<sup>R</sup>Fen<sup>R</sup>Bos<sup>R</sup>, Flu<sup>S</sup>Fen<sup>S</sup>Bos<sup>S</sup>, Flu<sup>R</sup>Fen<sup>R</sup>Bos<sup>S</sup>, Flu<sup>R</sup>Fen<sup>S</sup>Bos<sup>R</sup>, Flu<sup>R</sup>Fen<sup>S</sup>Bos<sup>S</sup>, Flu<sup>S</sup>Fen<sup>S</sup>Bos<sup>R</sup> and Flu<sup>S</sup>Fen<sup>R</sup>Bos<sup>R</sup>). Among them, Flu<sup>R</sup>Fen<sup>R</sup>Bos<sup>R</sup> was widely distributed and present in 58% of isolates, in almost all of the prospected locations. The prevalence of resistant profiles found in this study can be useful in designing proper management strategies against faba bean chocolate spot disease in Morocco.

**Integrated management of *Aspergillus carbonarius* and ochratoxins in vineyards in Greece.** M.K. ILIADI<sup>1</sup>, C.S. LAGOIANNI<sup>1</sup>, M.D. KAMINIARIS<sup>1</sup>, E-F.N. VARVOUNI<sup>1</sup>, M.N. VARVERI<sup>1</sup>, E.G. POULAKI<sup>1</sup>, C.K. KAVROUMATZI<sup>1</sup>, A.X. VARYMPOPI<sup>1</sup>, E.I. MARGARITIS<sup>1</sup>, N.S. MASTRODIMOS<sup>1</sup>, K.E. POLITIS<sup>1</sup> and D.I. TSITSIGIANNIS<sup>1\*</sup>. <sup>1</sup>*Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Athens, Greece.*  
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*Aspergillus spp.* can cause severe pre- and postharvest berry rots in vine cultivation such as the sour rot of grapes. Specifically, *A. carbonarius* is responsible for ochratoxin A production, a carcinogenic mycotoxin which affects both humans and animals. The control of *Aspergillus spp.* is quite difficult as the application of many fungicides is required in vineyards. The control of the fungus is particularly difficult and requires multiple applications of systemic fungicides. The increasing use of pesticides in vine cultivation during the last decade, combined with the low maximum residue levels, demands to find new more sustainable methods to control sour rots and ochratoxin A contamination in vineyards. The aim of this study was to develop an integrated management control strategy for sour rots caused by *Aspergillus spp.* and OTA production in vineyards. Several biological and chemical plant protection products were evaluated to control sour rot and OTA production: 1) Grape yeasts from the collection of Laboratory of Plant Pathology of Agricultural University of Athens isolated from Greek vineyards, 2) Botector®, 3) Triatum®, 4) Tusal®, 5) Serenade Max®, 6) Vacciplant®, 7) EM-Effective Microorganisms®, 8) Remedier®, 9) Mevalone®, 10) Switch®, 11) Quadris®, 12) Geoxe®, 13) Chorus®, 14) Cantus® and 15) Scala®. Experiments were carried out in commercial vineyards for four years during the 2016-2019 years on two Greek varieties of vine, Malagousia and Savvatiano. A successful integrated ochratoxin management system combining biological and synthetic PPPs was developed and will be presented.

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**IKOPROTECTA - Agricultural composted products as plant protection and growth regulators. M. VARVERI<sup>1</sup>, A. BAKOS<sup>1</sup>, P. GIANNIOTIS<sup>2</sup> and D.I. TSITSIGIANNIS<sup>1\*</sup>.**

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Composts are natural products known for their beneficial properties in plant nutrition by enriching the soil with organic matter and nutrients. Composts contain high microbe populations, that are mainly non-pathogenic and a number of studies have highlighted their capacity to suppress or control a variety of plant diseases. The aim of this study was to assess the biopotential of two composted products, (IKORGANIC), based on disease assessments in greenhouse and *in vitro* experiments. The bacterial/fungal strains that were isolated from the composted products, were evaluated on their ability to inhibit the growth of phytopathogenic fungi and bacteria such as *Verticillium dahliae*, *Colletotrichum acutatum* and *Pseudomonas syringae* pv tomato. The antagonistic activity of the isolates was investigated, *in vitro*, by employing dual culture technique. Subsequently, the efficiency of the composted products concerning the plant growth promotion and the reduction of disease severity against the above-mentioned pathogens, was evaluated in greenhouse, on tomato plants and *in planta*, on olive fruits according to the host of each pathogen. The results of the *in vitro* experiments as well as the comparative study of the two different composted products regarding their ability to control both foliar and soil- borne plant diseases will be presented.

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**Investigation of DMI-fungicides sensitivity and resistance in grape powdery mildew (*Erysiphe necator*) populations in Hungary.** Z., SPITZMÜLLER<sup>1</sup>; X., KARÁCSONY-PÁLFI<sup>1</sup>; A., PINTYE<sup>2</sup>; O., MOLNÁR<sup>2</sup>; M. Z., NÉMETH<sup>2</sup>; Á. N. HORVÁTH<sup>2</sup>; L., KISS<sup>2,3</sup>; G. M., KOVÁCS<sup>2,4</sup>; K. Z.,

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Powdery mildew is one of the most widespread fungal diseases associated to grapevine (*Vitis vinifera* L.) caused by the obligate biotrophic fungus *Erysiphe necator*. The continuous and intensive use of sterol demethylation inhibitor (DMI) fungicides results in a gradual development of sensitivity by alterations in several genes. The occurrence of resistant *E. necator* populations is generally low and it has been previously shown that pathogens with reduced sensitivity to one certain DMI fungicide could be still sensitive to other DMIs. The main goal of our research was to detect single nucleotide polymorphisms (SNPs) in genes causing DMIs resistance and to describe fungicide sensitivity of Hungarian populations of *E. necator*. We adapted a commonly used method to test resistance of *E. necator* conidia collected from the vineyard of Eszterházy Károly Catholic University to 4 DMI fungicides with propiconazole; tebuconazole; myclobutanil and penconazole active ingredient. The fungicides were tested at different concentrations as triplicates. The ratio of the germinating and non-germinating spores and the length of germ tubes were determined. We observed reduced sensitivity except in the case of the fungicide with propiconazole content. With gene sequence analysis we identified conserved nucleotide variation in the coding region of 14  $\alpha$ -demethylase (*CYP51*), the target of the commonly used DMI fungicides. SNP in *CYP51* causing tyrosine to phenylalanine substitution amino acid position 136 (Y136F) of the protein is associated with DMI resistance in *E. necator*. In our further studies we would like to test fungicides with other mechanism of action.

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**Markers of resistance to succinate dehydrogenase inhibitor fungicides in *E. necator* populations in Hungary.** D. SERESS<sup>1</sup>, F. MATOLCSI<sup>1,2</sup>, O. MOLNÁR<sup>1</sup>, A. PINTYE<sup>1</sup>, Á.N. HORVÁTH<sup>1</sup>, G.M. KOVÁCS<sup>1,2</sup> and M.Z. NÉMETH<sup>1</sup>. <sup>1</sup>*Plant Protection Institute, Centre for Agricultural Research, ELKH, Herman Ottó út 15., 1022 Budapest, Hungary.* <sup>2</sup>*Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary.* E-mail: [seress.diana@atk.hu](mailto:seress.diana@atk.hu) (presenter), [nemeth.mark@atk.hu](mailto:nemeth.mark@atk.hu) (corresponding author)

Fungicides of the succinate dehydrogenase inhibitor type (SDHIs) are widely used to control grapevine powdery mildew (GPM) caused by *Erysiphe necator*. Resistance to SDHIs was observed in *E. necator*, and it is often caused by mutations in the genes encoding subunits of the succinate dehydrogenase enzyme (*sdhB*, *sdhC*, *sdhD*). To effectively manage GPM, knowledge on fungicide resistance is crucial. Our aim was to assess whether markers of SDHI resistance are present in *E. necator* populations in Hungary. We collected powdery mildew infected grapevine leaves from two Hungarian wine regions (Eger and Szekszárd) in 2018 and 2019. DNA was extracted from field samples, as well as from newly established *in vitro* *E. necator* isolates, and the *sdhB* and *sdhC* genes were amplified and sequenced. The presence of two mutations indicating resistance to SDHIs was confirmed. We have identified an A-G nucleotide change (A794G) at position 794 of the coding region of the *sdhB* gene, which results in an amino acid substitution in the protein (H242R). This mutation is the marker of resistance to boscalid, a commonly used SDHI. In nucleotide position 724 of the coding region of the *sdhC* gene, we identified a G-A nucleotide change (G724A), resulting in an amino acid substitution (G169D). This mutation was not reported previously from Hungary. This mutation leads to reduced efficacy of several different SDHIs (e.g., boscalid, fluopyram, fluxapyroxad). The monitoring of fungicide resistance is fundamental for the successful control of GPM and for the long-term maintaining of the effectiveness of SDHIs.

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**Expression of *Tomato spotted wilt virus* genes in antisense orientation affects virus progression in *Nicotiana benthamiana*.** V. PIRES<sup>1§</sup>, S. A. DANDLEN<sup>2§</sup>, G. NOLASCO<sup>2</sup>, M. R. FÉLIX<sup>3</sup>, P. MATERATSKI<sup>4</sup>, C. VARANDA<sup>4</sup>, N. MARQUES<sup>1</sup>. <sup>1</sup>CEOT Centro de Eletrónica, Optoeletrónica e Telecomunicações, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro. <sup>2</sup>MED Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. <sup>3</sup>MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal. <sup>4</sup>MED - Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal. Email: nmarques@ualg.pt

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*Tomato spotted wilt virus* (TSWV) is a member of the *Tospoviridae* family that infects a wide range of hosts. TSWV genome has three linear negative-sense or ambisense RNA segments. Segment S RNA encodes the silencing suppressor NSs, and the nucleocapsid protein N. Segment M RNA encodes the cell-to-cell movement protein NSm and two glycoproteins (Gn and Gc). In the present work, antisense transcripts of the N, NSs and M viral genes were expressed in leaves of *Nicotiana benthamiana* infected with TSWV to assay for its ability to silence virus progression. Constructs in the binary vector pK7WG2 were transiently co-expressed with p7WG2-GFP in *N. benthamiana* leaves through agroinfiltration, followed by inoculation with TSWV after 48h. Inoculated leaves were harvested 5 days after agroinfiltration for RNA extraction. Antisense transcripts of partial sequences of the above-mentioned genes were also expressed throughout the plant using the *Tobacco rattle virus* viral vector (pTRV). New leaves were harvested 10 days after agroinfiltration of the pTRV viral vector. TSWV detection and absolute quantification was performed by a TaqMan real-time RT-PCR assay. Inoculated leaves with TSWV alone and new leaves showed a low viral titer, a result that indicates host plant resistance to TSWV infection. In both assays, antiviral M mRNA transcripts appeared to limit TSWV genomic RNA accumulation and propagation compared to antiviral transcripts of N or NSs genes. These studies allowed us to conclude that the M gene transcripts in the antisense orientation is a preferable target to limit virus progression in *N. benthamiana* plants.

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**Polysaccharides and plant protection against *Verticillium dahliae*.** P. NEOFYTOU, D. GKIZI, and S.E. TJAMOS. Agricultural University of Athens, School of Crop Science, Laboratory of Plant Pathology. Iera Odos 75, 11855, Athens, Greece. E-mail: sotiris@aau.gr

*Verticillium dahliae* is a destructive soil fungus distributed all over the world, infecting a wide range of economically important crops. The lack of efficient chemically based disease management strategies has oriented the research on the development of resistant cultivars or root stocks, biological control agents, or compounds to induce resistance of the host plant. Towards this direction, we investigated the plant protective activity of starch and cellulose against *Verticillium* wilt of eggplants. It was observed that both polysaccharides reduced symptom development in plants compared to controls. In a split root system, it was shown that the starch and cellulose treated plants were less infected than controls. Transcriptomic analysis revealed the higher expression levels of *PR1* and *PR4* in the cellulose treated plants compared to the controls at 5 days post inoculation (dpi) of *V. dahliae*; while the starch treated plants exhibited higher than controls *PR4* expression levels at 10 dpi. Pathogenicity experiments on plants grown in sterilised soil revealed the efficacy of starch and cellulose to protect plants against *V. dahliae*; while, the polysaccharides failed to protect plants in a split root system. Therefore, starch and maltose may positively affect a microbial population that triggers ISR in plants and also both polysaccharides may have a direct effect against *V. dahliae*.

**Soil solarisation and biological control of soil borne pathogens in strawberry plantations.** A. TZIONIS<sup>1</sup>, I. KEFALOGIANNI<sup>2</sup>, I. CHATZIPAVLIDIS<sup>2</sup> and S.E. TJAMOS<sup>1</sup>.  
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Soil solarization is one of the most appealing methods to control soilborne pathogens in Mediterranean countries. Strawberry is a high value crop that is susceptible to various fungal and oomycete pathogens. Throughout the years, it has been shown that fumigants have low efficacy against soilborne pathogens. In the present study, we evaluated next generation polyethylene foils for soil solarization, in strawberry plantations under greenhouse conditions. It was observed that a totally impermeable 7-layer film (KRITIFIL® TIF, Plastika Kritis SA, Greece) was the most efficacious among the tested soil disinfection films. The main soil pathogens were *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *fragariae*, *Phytophthora* sp., *Rhizoctonia solani* and *Macrophomina phaseolina*. In the TIF covered soil the diseased plants were 4-10 times less than in the Metam sodium treated soil. Analysis of the microbial community of the different treatments revealed the presence of eighteen prevailing and distinct bacterial isolates. Dual culture tests revealed that six of the isolated bacteria reduced *V. dahliae* and *Fusarium oxysporum* growth. The six isolates were tested as single and mixed treatments against Verticillium wilt of eggplants. It was observed that the consortium of the isolates was the most efficacious treatment. Further analysis of the six isolates revealed the presence of biocontrol and plant growth promoting traits such as, cellulolytic activity, indole acetic acid and siderophore production, phosphorus dissolving activity and swarming motility.

**The multiple effects of the biocontrol agent *Pseudomonas putida* Z13 against *Botrytis cinerea* in tomato fruits.** L. AMPNTELNOUR, E. G. POULAKI, and S.E. TJAMOS. *Agricultural University of Athens, School of Crop Science, Laboratory of Plant Pathology. Iera Odos 75, 11855, Athens, Greece. E-mail: litsaabd@gmail.com*

Grey mold, caused by *Botrytis cinerea*, is an important postharvest disease on fresh-market tomatoes. Although fungicide treatment has been one of the main methods for controlling gray mold, there is increasing international concern over the heavy use of fungicides on crops because of the possible harmful effects on human health and the emergence of pathogen resistance to fungicides. Therefore, there is a need to develop alternative disease control methods, such as the use of plant beneficial microorganisms. The *Pseudomonas* genus encompasses various species with biocontrol activity against various plant pathogens. Recently, we reported the isolation and identification of strain *Pseudomonas putida* Z13, a potent biocontrol agent against *Verticillium dahliae*. *Pseudomonas putida* is a bacterium commonly used in environmental studies because it is able to degrade many aromatic compounds. It degrades cellulose and chitin, and also produces a large number of different antibiotics. The aim of the present study was to evaluate the biocontrol activity of strain Z13 against *Botrytis cinerea* in tomato fruits. The application of Z13 in tomato fruits reduced disease severity and incidence by 50% compared to controls. This plant protective activity can be attributed to antibiotic production, since Z13 reduced the growth of *B. cinerea* in vitro and also to the priming of the plant defense responses. The qPCR analysis revealed that expression of the defense associated genes *PR1* and *WRKY70* was upregulated in the Z13 treated fruits; this upregulation was most prominent upon *B. cinerea* infection. Therefore, Z13 may constitute a biocontrol agent targeting multiple pathogens in different plant species, as it has been shown in our experiments against *V. dahliae* and *B. cinerea* in eggplants and tomato fruits, respectively.

**Characterization of rhizobacteria from Cyprus indigenous wine grape cultivars bearing antagonistic traits against grapevine trunk pathogens.** C. OPLOS<sup>1</sup>, S. EFSTATHIOU<sup>1</sup> and L. I. KANETIS<sup>1</sup>. <sup>1</sup>*Department of Agricultural Sciences, Biotechnology, and Food Science, Cyprus University of Technology, Limassol, 3036, Cyprus. E-mail: [loukas.kanetis@cut.ac.cy](mailto:loukas.kanetis@cut.ac.cy)*

In recent years, there is an intense activity of the Cyprus wine industry, where the local winemakers focus mainly on the exploitation of indigenous grape varieties. Grapevine trunk diseases (GTDs) form the most destructive biotic complex, limiting vineyard productivity and longevity globally. Based on recent data, the Cyprus vineyard is seriously affected by GTDs. The present study represents the first attempt to decipher microbial communities of rhizobacteria from indigenous varieties, *Vitis vinifera* cv. Giannoudi, Maratheftiko, Promara, and Xinisteri, in order to select effective biocontrol agents (BCAs), that could be used for GTD management. The antagonistic activity of 499 bacterial isolates was screened *in vitro* against six important GTD pathogens (*Phaeoemoniella chlamydospora*, *Diplodia seriata*, *Neofusicoccum parvum*, *Eutypa lata*, *Ilyonectria liriodendri*, *Dactylonectria alcacerensis*, and *Dactylonectria torresensis*). Eighty-two isolates exhibiting significant fungal inhibition ( $\geq 40\%$ ) were selected and further characterized. Based on 16S sequencing, isolates were assigned to the genera *Acinetobacter* (1.2%), *Bacillus* (17.1%), *Bordetella* (2.4%), *Chryseobacterium* (3.7%), *Paenibacillus* (1.2%), *Pantoea* (6.1%), *Pseudomonas* (19.5%), *Olivibacter* (2.4%), *Rhizobium* (14.6%), *Serratia* (22%), *Streptomyces* (2.4%) and *Variovorax* (7.4%). Enzymatic traits of the selected isolates related to antifungal activities were also examined. More specifically, lipase, esterase, protease, amylase, xylanase, pectinase, cellulase, chitinase, HCN activity, as well as biofilm formation was evident for the 41.5, 63.4, 78.1, 36.6, 0, 36.6, 46.3, 31.7, 11, and 34.1% of the isolates, respectively. Antibiotic production, VOC activity, and growth promotion traits are underway to complete the characterization of a novel, indigenous pool of promising BCAs for successful GTD management.

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**A newly reported bacteriophage against *Pseudomonas syringae* pv. tomato and its plant protective activity.** PAPAZOGLOU, P.,<sup>1</sup> SKLIROS, D.,<sup>1</sup> PARASKEVOPOULOU, E.G.,<sup>1</sup> GKIZI, D.,<sup>4</sup> GOUMAS, D.E.,<sup>3</sup> TJAMOS, S.E.,<sup>2</sup> AND FLEMETAKIS, E.<sup>1</sup>. <sup>1</sup>*Agricultural University of Athens, Biotechnology Department, Lab of Molecular Biology.* <sup>2</sup>*Agricultural University of Athens, Crop Science Department, Lab of Phytopathology.* <sup>3</sup>*Hellenic Mediterranean University, School of Agricultural Sciences, Department of Agriculture, Lab of Phytopathology.* <sup>4</sup>*University of West Attica, Department of Wine, Vine and Beverage Sciences.*

The biology of bacteriophages has been extensively studied in recent years to explore new and environmentally friendly methods of controlling phytopathogenic bacteria. *Pseudomonas syringae* pv. *tomato* (Pst) is responsible for the bacterial speck disease in tomato plants. Plant symptoms include black or brown spots with chlorotic margins on leaves and fruits. The most common disease management strategy is the application of copper based pesticides. The biological control of Pst with the utilization of bacteriophages could be an alternative environmentally friendly approach to diminish the detrimental effects of PSt in tomato cultivations. In order to examine the potential preventive efficacy of bacteriophages under greenhouse conditions, the lysogenic bacteriophage Medea1 was used. Medea1 has been isolated from a tomato cultivation and has been fully characterized. This newly reported bacteriophage is a ds-DNA bacteriophage with a genome size of 58.919 bp having a strong lytic activity against many strains of Pst, suppressing the bacterial populations *in vitro* for at least 10 hours. The efficacy of Medea1 against Pst was further evaluated *in planta*, under greenhouse conditions. The Pst and Medea1 populations were monitored at 1 and 3 days post-infection, by RT qPCR. Furthermore, plant gene expression patterns involved in defense against Pst were studied. Results showed a statistically significant reduction of the bacterial population up to 85%. Additionally, disease symptoms were delayed up to 6-times fold. These results suggest that the Medea1 phage could pave the way for an effective alternative way to control Pst in tomato plantations, under greenhouse conditions. Further isolation of bacteriophages against Pst and the design of a smart phage-based cocktail, could render an efficient disease management strategy.

**Evaluation of biological and synthetic plant protection products for the management of downy mildew in grapevines.** C.K. KAVROUMATZI<sup>1</sup>, M.K. ILIADI<sup>1</sup>, M. VARVERI<sup>1</sup>, D. AKRIVOPOULOU<sup>1</sup> and D.I. TSITSIGIANNIS<sup>1\*</sup>. <sup>1</sup>*Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece.* \*Email: dimtsi@aua.gr

Grape downy mildew caused by the oomycete *Plasmopara viticola* is the most serious threat of grapevines in virtually all viticultural regions worldwide, where rainfall occurs regularly during the late spring and summer. The pathogen can destroy a vineyard if no measures are taken to control it. The main strategies to restrict the pathogen are mainly based on fungicides that result in soil accumulation and environmental hazard. High amounts of a fungicide must be applied many times through the season to keep the pathogen under control. Current strategies for the control of grapevine downy mildew are mainly based on the use of preventive copper or mancozeb treatments from the beginning of the period during which plants are susceptible to infection. The purpose of this study was to evaluate the efficacy of different plant protection products (PPPs) in grape leaves after artificial infection of the plants with zoospores of the oomycete *P. viticola*. Nine commercial bio-PPPs and five synthetic PPPs were tested against the grape downy mildew. The selected sensitive grape cultivars were *Moschato* and *Agiorgitiko*. The most effective bio-PPPs for both tested cultivars were *Amylo-X*®, *Remedier*®, *Trianum*® and *Vacciplant*® but were not as effective as copper formulations. All tested synthetic PPPs were able to successfully control the disease in grapevine plants on both cultivars and the most efficient was *Bion-MX*® that combines a biostimulant (acibenzolar-S-methyl) and a classical chemical active ingredient (metalaxyl). The results of the comparative study of the different PPPs will be presented.

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**Antifungal and phytotoxic properties of essential oil isolated from three spontaneous Lamiaceae species from Morocco against the main chickpea pathogens., S. KRIMI**

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Investigation to develop biological product become important regarding the environment impact. The aim of this study was to evaluate the chemical composition of some aromatic plants belonging to the Lamiaceae family and its antifungal activity against the causal pathogens of Ascochyta blight and Fusarium wilt of chickpea. Three species that are grown spontaneously in Morocco were selected: *Origanum compactum*, *Thymus vulgaris* and *Mentha pulegium* L. Results showed that Oregano was the most effective against *Fusarium oxysporum* (MIC - 0,5µl/ml) in inhibiting mycelium growth and spore germination followed by thyme and pennyroyal (MIC - 1,5 µl/ml). An inhibition of 100% of *A. rabiei* was also observed using Oregano at 0.15 µl/ml. No phytotoxic effect of essential oil was observed on chickpea seed germination using oregano and thyme oils at low doses, while pennyroyal oil can affect the germination rate of seeds. Thymol and carvacrol were found as major compounds of oregano and thyme oil using GC/MS analysis. These elements can present the main active ingredient of pathogens control. Further experiments were carried out in greenhouse to evaluate the efficacy of oregano and thyme oils against *A. rabiei* as protective or curative treatment. In curative treatments, 0.15µl/ml of oregano displayed a significant decrease ( $P < 0.05$ ) in disease severity of Ascochyta blight of 65.78% similar to chemical fungicide (Azoxystrobine 250g/l). The thyme oil had also a significant protection effect by reducing 52.63% of disease severity at 0.5µl/ml. These essential oils or their major compounds could be investigated as natural antifungal products against chickpea pathogens.

**Effects of the leafy liverwort extract on plant pathogenic fungi causing olive fruit rot and gray mold of strawberry.** J. LATINOVIC<sup>1</sup>, M. SABOVLJEVIC<sup>2</sup>, M. VUJICIC<sup>2</sup>, N. LATINOVIC<sup>1</sup> and A. SABOVLJEVIC<sup>2</sup>. <sup>1</sup>University of Montenegro, Biotechnical Faculty, Mihaila Lalica 1, 81000 Podgorica, Montenegro. <sup>2</sup>University of Belgrade, Institute of Botany and Botanical Garden, Faculty of Biology, Takovska 43, 11000 Belgrade, Serbia. E-mail: [jelenalat@ucg.ac.me](mailto:jelenalat@ucg.ac.me)

Bryophytes were among the first land plants and they faced harsh terrestrial environment and interacted with different organisms during their long evolutionary history. They cope well with fungal cohabitants in mutualistic, parasitic or neutral relationships. Due to interesting chemical content they are assumed not to be easily infested by fungal organisms. This feature is used to test the effects of selected bryophyte extract to plant pathogenic fungi that cause plant diseases. The methanol extract of leafy liverwort *Scapania nemorea* (L.) Grolle was used to test growth inhibition of two fungi: *Botryosphaeria dothidea* isolated from rotten olive fruits and *Botrytis cinerea* isolated from diseased strawberry fruits. Treatments were performed in amounts of 5, 10 and 15 µl while distilled water served as a control. The results obtained showed that selected bryophyte extract affects the growth of *B. dothidea* and *B. cinerea*. The extract applied at the highest dosage of 15 µl caused statistically significant inhibition ( $P < 0.01$ ) of *B. cinerea* in regard to control. *B. dothidea* mycelium growth was inhibited when extract of *S. nemorea* was applied at the dosages of 10 and 15 µl with clearly recorded statistically significant differences compared to control ( $P < 0.01$ ). The inhibitory effects of tested extract indicate the potential use of certain bryophytes as sources of natural active substances that can be used for development of environmentally friendly alternatives in control of some important plant diseases.

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**The phenolic responses in callus to the scion sanitary status and disinfectants allowed in the biological grapevine nurseries in Slovenia.** D. RUSJAN, S. GAČNIK and M. MIKULIČ PETKOVŠEK. *Chair for fruit growing, viticulture and vegetable, Department of Agronomy, Univeristy of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. E-mail: denis.rusjan@bf.uni-lj.si*

The impacts of sanitary status of the scions infected by grapevine trunk diseases (GTD) and five different disinfectants (one combined with thermotherapy (TT)) on the phenolics in callus during grapevine propagation were studied. The study included scions from healthy vines (HLT), GTD infected asymptomatic vines (ASYM) and GTD infected symptomatic vines (SYM) of 'Cabernet Sauvignon' variety. Scions were treated separately with Beltanol, Serenade, sodium bicarbonate, Remedier, BioAction and with combination of Beltanol and TT. Calluses were studied soon after callusing and later after classification in autumn. The results showed that different sanitary status of the scions has an impact on the content of phenolic compounds in callus. After callusing the significantly highest content of TAP (363-378 µg/g FW), flavanols (FLA; 297-310 µg/g FW) and stilbenes (STB; 25,7-27,0 µg/g FW) in callus was measured from SIM and ZDR scions, while after classification the significantly highest contents of TAP, FLA and STB were measured in callus from ASIM scions. However, the significantly highest content of TAP (599-783 µg/g FW) was measured in callus from ZDR and SIM scions treated with BioAction. After classification, the significantly highest content of TAP (6951-7110 µg/g FW) was measured in callus from ASIM scions treated with Remedier in Beltanolom and from ZDR scions (3231- 3262 µg/g FW) with Remedier and sodium bicarbonate and from SIM scions (5024±40 µg/g FW) with sodium bicarbonate. The study contributes to the knowledge of the impact of biocontrol agents on the phenolics alterations in the callus from scions with different GTD status.

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***Melia azedarach* induces the expression of marker genes involved in tomato defense responses against nematodes.** A. KROKIDA<sup>1</sup>, D. KIZIS<sup>2</sup>, M. SAMARA<sup>3</sup>, N. NTALLI<sup>3,\*</sup>.

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Tomato is the second most common vegetable produced globally and Root-Knot Nematodes (RKN) consist one of its most notorious agricultural pests worldwide. To date the environmental and health concerns impose the use of plant secondary metabolites as ecofriendly alternatives to the conventional nematicides. In that frame, we previously proved that *Melia azedarach* ripe fruits yield nematocidal extracts against *Meloidogyne* sp, with substantial activity under field conditions, and that these extracts trigger plant defense mechanisms on cucumber. However, the molecular processes by which these extracts activate plant defense against RKN remain largely unclear. To unravel the molecular mechanism of this induced resistance, we used qRT-PCR to monitor the expression of selected key defense genes and the H<sub>2</sub>O<sub>2</sub>-degrading enzymes of catalase and ascorbate peroxidase in tomato roots, 5 and 30 days after RKN inoculation. *M. azedarach* aqueous extract induced the expression of salicylic acid pathway genes (*PR-1*, *PR-5*), *Mi1* gene, that confers resistance against RKNs, and proteinase inhibitor *Pi1*. An overexpression of *ACO* and *RAB18* gene, markers of ethylene and ABA signaling pathways respectively, was also observed. A lower induction of jasmonic acid pathway genes (*LOXD* and *SAM*) was detected, whilst the expression of *CCD7*, involved in strigolactones biosynthesis, was reduced in 30 days after RKN inoculation. Finally, the expression profiles of catalase and ascorbate peroxidase genes were altered 30 days after RKN inoculation. In conclusion, data presented herein provide evidence that *M. azedarach* extract can modulate the expression of genes and metabolites associated with plant defense responses in tomato.

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***In vitro* and *in vivo* antifungal properties of Thymol against *Ascochyta rabiei*** Fatima Zahrae IBN EL MOKHTAR<sup>1,2</sup>, HAJAR ERRATI<sup>1</sup>, HAFSA HOUMAIRI<sup>1</sup> SANAE KRIMI BENCHEQROUN<sup>2</sup> <sup>1</sup>University Hassan 1<sup>st</sup>, Faculty of Science and Techniques, P.O. Box 577, Settat, Morocco. <sup>2</sup>National Institute of Agriculture Research (INRA, CRRRA-Settat), P.O. Box 589, Settat, Morocco. Email: [sanae.krimibencheqroun@inra.ma](mailto:sanae.krimibencheqroun@inra.ma)

Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. is an economically important disease of chickpea worldwide. With growing organic farming practices, there is a need to reduce chemical inputs in agricultural systems, and to develop new effective biological products. Thymol is a natural monoterpene phenol, found mainly in the essential oil of thyme (*Thymus vulgaris*) and other aromatic and medicinal plants. The aim of this work is to evaluate the antifungal properties of thymol *in vitro* and *in vivo* against *A. rabiei*. The *in vitro* test was performed on chickpea medium CPMA amended with six concentrations of thymol (0 to 0.3 µl/ml). The minimum inhibitory concentration (MIC) was estimated by calculating the percentage of inhibition of mycelium growth (MGI). The fungistatic or fungicidal property of thymol was determined using Thompson method. Further experiments were performed in the greenhouse to evaluate the efficacy of thymol as a curative or preventive treatment in the control of ascochyta blight. Results showed that radial growth of pathogen was completely inhibited using thymol at low concentration of MIC= 0.15 µL/mL. The thymol was classified as fungicidal against *A. rabiei* at concentrations superior to 0.3 µL/mL. The application of thymol on plants was significantly effective in controlling the disease as preventive or curative treatments at 0.4 µl/ml by reducing the mean of disease index (MDI) by 39% and 32% respectively. Therefore, the use of thymol can be investigated as natural antifungal products against Ascochyta blight.

**Antifungal and phytotoxic properties of essential oil isolated from three spontaneous Lamiaceae species from Morocco against the main chickpea pathogens.** SANAE KRIMI BENCHEQROUN<sup>1</sup> AMAL ENNOURI<sup>1,2</sup>, LAMYAE ET-TAZY<sup>1,2</sup>, HAJAR ERRATI<sup>1,2</sup>, ABDESSALAM LAMIRI<sup>2</sup>. <sup>1</sup>*National Institute of Agriculture Research (INRA, CRRRA-Settat), P.O. Box 589, Settat, Morocco. E-mail: [sanae.krimibenchegroun@inra.ma](mailto:sanae.krimibenchegroun@inra.ma)* <sup>2</sup>*University Hassan 1<sup>st</sup>, Faculty of Science and Techniques, Laboratory of Applied Chemistry and Environment, P.O. Box 577, Settat, Morocco.*

Investigation to develop biological products becomes important regarding the environmental impact. The aim of this study was to evaluate the chemical composition of some aromatic plants belonging to the Lamiaceae family and their antifungal activity against the causal pathogens of Ascochyta blight and Fusarium wilt of chickpea. Three species that are grown spontaneously in Morocco were selected: *Origanum compactum*, *Thymus vulgaris*, and *Mentha pulegium* L. Results showed that Oregano was the most effective against *Fusarium oxysporum* (MIC - 0,5µl/ml) in inhibiting mycelium growth and spore germination followed by thyme and pennyroyal (MIC - 1,5 µl/ml). Inhibition of 100% of *A. rabiei* was also observed using Oregano at 0.15 µl/ml. No phytotoxic effect of essential oil was observed on chickpea seed germination using oregano and thyme oils at low doses, while pennyroyal oil can affect the germination rate of seeds. Thymol and carvacrol were found as major compounds of oregano and thyme oil using GC/MS analysis. These elements can present the main active ingredient of pathogens control. Further experiments were carried out in the greenhouse to evaluate the efficacy of oregano and thyme oils against *A. rabiei* as a protective or curative treatment. In curative treatments, 0.15µl/ml of oregano displayed a significant decrease (P<0.05) in disease severity of Ascochyta blight of 65.78% similar to chemical fungicide (Azoxystrobin 250g/l). The thyme oil had also a significant protection effect by reducing 52.63% of disease severity at 0.5µl/ml. These essential oils or their major compounds could be investigated as natural antifungal products against chickpea pathogens.

**BIOVEXO, a BBI-JU-H2020 project on biocontrol of *Xylella* and its vector in olive trees for integrated pest management.** S. COMPANT<sup>1</sup> and the BIOVEXO Consortium<sup>2</sup>. <sup>1</sup> AIT Austrian Institute of Technology, Center for Health and Bioresources, Bioresources Unit, Tulln, Austria. <sup>2</sup> BIOVEXO Consortium: RTDS Group (Austria), AIT Austrian Institute of Technology (Austria), CNR – Istituto per la protezione sostenibile delle piante (Italy), Centro di Ricerca, Sperimentazione e Formazione in Agricoltura “Basile Caramia” (Italy), Universidad de Sevilla (Spain), Universiteit Antwerpen (Belgio), Globachem NV (Belgium), Domca SA (Spain), Acies Bio (Slovenia), Aimerit SL (Spain) and ASAJA (Spain). E-mail: [stephane.compant@ait.ac.at](mailto:stephane.compant@ait.ac.at)

*Xylella fastidiosa* is increasingly causing diseases on olive trees and various other crops in the Mediterranean region. It wiped out a number of olive groves in Italy and Spain in only a few years. Unfortunately, the climate of the southern European Union is ideal for *Xylella*. Due to its rapid transmission across cultivation areas, *Xylella fastidiosa* is projected to cause yield losses of 35% - 70% in olive harvests and 13% in almond harvests. Currently, there are no products available on the market proven to be effective against *Xylella fastidiosa*, which is spread by xylem-feeding insects – notably the spittlebug *Philaenus spumarius* – present worldwide and in different climatic conditions. Only chemical insecticides are authorized to control xylem sap-feeding insects, but this is not allowed in organic production. In the BBI JU-H2020 project BIOVEXO (1), 11 partners from 5 different countries and belonging to industries, SMEs, RTOs and universities aim to develop environmentally sustainable and economically viable plant protection solutions that can be deployed as a method of integrated pest management to control *Xylella fastidiosa* and its spittlebug vector. At least, the two best performing solutions will be brought forward, closer to the market, at the end of the project (Technical Readiness Level 7-8). Integrated pest management measures will be applied to the existing, and the newly planted orchards during the large-scale pilot in Apulia (Italy) and Mallorca (Spain) — the two most dominant *Xylella* outbreak regions in Europe. In the conference, we will present the overview of the project as well as the objectives and the research plan of BIOVEXO.

For more information about the BIOVEXO project: [www.biovexo.eu](http://www.biovexo.eu).

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## **An emerging threat to chestnut nuts: *Gnomoniopsis castanea* in the Northern Apennines**

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*Gnomoniopsis castanea* (syn. *Gnomoniopsis smitholgyvi*) is an emerging fungal pathogen that damages sweet chestnut (*Castanea sativa*) nuts and may cause twig and leaf necroses on both sweet chestnut and hazelnut (*Corylus avellana*). Nut rot of sweet chestnut has long been observed in Northern Italy, but was only recently associated to the presence of *G. castanea*, which is considered responsible for heavy pre- and post-harvest nut losses that chestnut growers are currently facing in the area. Little is known about the epidemiology of this fungus that is often reported to be a natural endophyte in chestnut and was found associated to galls of the Asian gall wasp *Dryocosmus kuriphilus*. The aim of this work was to investigate the presence of *G. castanea* in the Northern Apennines (regions Liguria and Toscana) where sweet chestnut has historically been grown for nut production and as coppices. Nuts and wasp galls were sampled and *G. castanea* was isolated with high frequency at all sites including those where symptoms were moderate, revealing a common and massive presence of the fungus. In addition, we have assessed temperature response for mycelial growth and spore germination to assist modeling of disease spread and incidence at sites with different climatic conditions. Since application of management measures relies on accurate and rapid identification of the pathogen before occurrence of symptoms, we are also developing a specific diagnostic assay based on Loop mediated isothermal AMPLification (LAMP) technology to serve as a field-deployable tool for helping risk assessment, damage prevention and disease control.

This activity received funding in the frame of the Agreement for Scientific Cooperation between Regione Toscana and University of Florence - DAGRI on quarantine organisms harmful to agriculture and forestry

**Wheat rusts monitoring in Tuscany and Sicily: re-emergence of stem rust on both durum and common wheat cultivars.** M. NOCENTINI<sup>1</sup>, B. RANDAZZO<sup>2</sup>, M. S. HOVMOLLER<sup>3</sup>, M. PATPOUR<sup>3</sup>, A. F. JUSTESEN<sup>3</sup>, L. MUGNAI<sup>1</sup>. <sup>1</sup>*Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Piazzale delle Cascine 28, I-50144, Firenze, Italy.* <sup>2</sup>*As.A.R. – Ciminna, Palermo, Italy.* <sup>3</sup>*Department of Agroecology, Aarhus University, Flakkebjerg 4200, Denmark*

Wheat stem rust has not been reported in Western Europe for several decades, but in the last ten years an outbreak occurred in central Europe first, mainly in Germany, and, even if only with sporadic infections, in Denmark, Sweden and UK. The presence of *Puccinia graminis* f. sp. *tritici*, causal agent of wheat stem rust, was also recorded in Sicily with an epidemic outbreak in 2016. Stem rust symptoms were also reported in Tuscany during the last three years, and during the 2021 crop season with 100% of monitored varieties showing symptoms. In Sicily stem rust was found in 13 of the 14 monitored sites. Hence, monitoring and sampling wheat varieties represents an important tool to provide a better understanding of the epidemic and to detect the rust races present in the different countries and regions respectively. For this purpose some varieties were sown both in Tuscany and Sicily 1) to follow the appearance of rust symptoms in different climatic and soil conditions 2) to evaluate the perspectives of an early warning system coordinated between Tuscany and Sicily, the region where the symptoms appearance is recorded well ahead than in the central-northern regions, and 3) to assess the races present on the same varieties in so different regions. First results from molecular analysis of collected samples from both regions showed that clade III-B and clade IV-F were detected in both of the regions and race TTRTF (belonging to clade III-B) and TKKTF (belonging to clade IV-F) were detected in Sicily.

**Characterization and distribution of *Pseudomonas syringae* pv. *syrinae* on wheat in Syria.** MOHAMMAD KASSEM<sup>1</sup>, NADER ASAAD<sup>2</sup>, SAFAA G. KUMARI<sup>3</sup> and ABDUL RAHMAN MOUKAHEL<sup>3</sup>. <sup>1</sup>*Plant Protection Department, Faculty of Agriculture, University of Aleppo, Aleppo, Syria.* <sup>2</sup>*General Commission for Scientific Agricultural Research (GCSAR), Al-Ghab, Hama, Syria.* <sup>3</sup>*International Center for Agricultural Research in the Dry Areas (ICARDA), Terbol Station, Beqa'a, Zahle, Lebanon. E-mail: s.kumari@cgiar.org*

Leaf blight bacterial disease caused by *Pseudomonas syringae* threaten wheat crop in many production areas over the world. Around 500 wheat leaf samples showing symptoms suggestive of bacterial disease infection were collected from 107 fields in north, central and coastal areas of Syria during 2021 growing season. Disease symptoms consisted of water-soaked dark green to brown lesions on expanding leaves which become necrotic and turn from grey-green to tan- white. A total of 56 isolates were selected using a semi-selective medium and characterized further by morphological, biochemical (LOPAT), pathogenicity tests to identify the virulent properties of isolates, and molecular characterization by PCR using specific primers to syringomycin production gene. The field observation showed that the overall severity was 54% approximately, whereas the coastal area isolates had high disease severity (61.5%) followed by the central area isolates (56%). The pathogenicity test revealed that all these isolates were pathogenic to wheat under greenhouse conditions with 63% of the isolates ranked as highly virulent. The PCR results confirmed that the isolates characterized were *P. syringae* pv. *syrinae*. Thus, the findings of this study help to better understand the ecology, distribution and severity of the microbial wheat communities.



***Diatrype stigma* and *D. whitmanensis* associated with canker and dieback of Russian olive (*Elaeagnus angustifolia* L.) trees in Iran.** F. AHMADYOUSEFI-SARHADI, H. MOHAMMADI and S. PANAHANDEH. *Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, P.O.Box: 76169-14111, Kerman, Iran. E-mail: saleh.panahandeh@yahoo.com*

Russian olive (*Elaeagnus angustifolia* L.) is a shrub or small deciduous tree in the family Elaeagnacea that can grow in various provinces of Iran. During 2018–19, a number of surveys for fungi associated with canker and dieback of Russian olive trees were conducted in Southern provinces of Iran. Samples were collected from branches of trees showing disease symptoms and fungal isolations were made from necrotic wood tissues on potato-dextrose-agar (PDA) supplemented with streptomycin sulphate. In this study 15 isolates of Diatrypaceous fungi were obtained from symptomatic branches. Isolates were placed onto 2% water agar amended with autoclaved pine needles and incubated under a 12-h daily photoperiod for 3-5 weeks to induce sporulation. Based on morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS) nrDNA and a partial sequence of the  $\beta$ -*tubulin* gene, isolates were identified as *Diatrype stigma* (8 isolates) and *D. whitmanensis* (7 isolates). Pathogenicity of these species was verified by inoculation of detached shoots of Russian olive trees under controlled conditions. Both species were pathogenic and caused significant wood discoloration on inoculated shoots 40 days post-inoculation. *Diatrype stigma* was more virulent, based on the mean lengths of wood discolorations, than those of *D. whitmanensis*. Our study is the first report on the occurrence and pathogenicity of *D. stigma* and *D. whitmanensis* on Russian olive trees in the world.

**Identification of *Venturia asperata* on scab-resistant apple cultivars in South Tyrol (Italy).**  
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Reports of atypical fruit spots on *Vf*-resistant cultivars from South Tyrolean apple orchards increased in August 2020. Symptomatology reminded of apple scab caused by *Venturia inaequalis*, however the spots were less pronounced and of light grey color. Microscopic examination of mycelia scraped off the spots from the fruit peel revealed rounded-tip conidia of 10-14  $\mu$ M length and were morphologically assigned to *Venturia asperata*. Molecular analysis of the ITS (internal transcribed spacer)-region by species-specific primers (Stehmann et al. 2001) resulted in amplicons only for the atypical scab lesions but not for typical leaf or fruit spots caused by *V. inaequalis*. Additionally, sequence analysis on the amplicons confirmed the presence of *V. asperata*. Up to now, the atypical scab symptoms were observed only on *Vf*-resistant cultivars like Bonita, Topaz, CIVM49 and Ipador where low-impact plant protection strategies were applied. Further monitoring on the occurrence of *V. asperata* is necessary to develop more targeted fungicide management also for scab-resistant cultivars to prevent emergence of new fungal diseases.

**Occurrence of *Monilinia* species in South Tyrolean (Italy) sweet cherry orchards.**  
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Production of sweet cherry may present an interesting niche for small farmers in the mountainous province of South Tyrol (Italy), therefore commercial orchards have increased within the last 10 years. The most important fungal pathogens of stone fruit are *Monilinia fructigena*, *M. laxa* and *M. fructicola*, causing brown rot. However, to date nothing has been known on the occurrence of these species in South Tyrol. Thus, in 2020 a collection of fruit mummies from 17 orchards of the main cherry growing areas was analyzed by real-time PCR (modified according Guinet et al. 2016). On 30

% of the mummified fruit samples at least one *Monilinia* species was revealed. The most common species was *M. laxa* detected on 22 % of the samples, while *M. fructigena* as single species was identified only on 4 % of the samples. Mixed infections with these species were identified on 6 % of the samples. *M. fructicola* was found on four samples from two bordering cherry orchards. This is the first detection of the former quarantine organism *M. fructicola* in South Tyrolean sweet cherry orchards.

**Chemical management of *Colletotrichum acutatum* causing olive anthracnose. M. VARVERI<sup>1</sup>, M.K. ILIADI<sup>1</sup>, C.K. KAVROUMATZI<sup>1</sup> and D.I. TSITSIGIANNIS<sup>1\*</sup>. <sup>1</sup>*Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece.*  
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Infections of olive fruit (*Olea europaea* L.) by fungi of the genus *Colletotrichum* are very frequent and particularly destructive worldwide. *C. acutatum* is considered to be the causal agent of olive anthracnose (OA) in Greece. OA causes fruit rot leading to its drop or mummification, resulting in significant yield losses and the degradation of oil quality. The control of olive anthracnose is extremely difficult and is usually achieved through a combination of methods including chemical control. The first objective of the current study was to evaluate, *in planta*, several chemical plant protection products (PPPs) for the control of *C. acutatum* in two different greek olive varieties, cv. Koroneiki and cv. Kalamon, using 12 commercial fungicides. Fruits were surface sterilized and then immersed in a solution of each PPP according to the maximum certified dose of each synthetic fungicide. Two days later, treated fruits were sprayed with a conidial suspension of a greek *C. acutatum* isolate. Several of the PPPs were able to effectively inhibit the colonization and sporulation of *C. acutatum* on olives fruits. Subsequently, nine *C. acutatum* strains from different regions of Greece, were characterized, *in vitro*, in terms of their sensitivity/resistance to seven active substances of the most effective synthetic fungicides. All isolates were considered sensitive to the selected sterol-inhibiting fungicides (SBIs), while on the other hand, most of the isolates were relatively resistant to copper compounds.

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**Fungicide sensitivity and genetic diversity of *Botrytis cinerea* populations from conventional and organic tomato and strawberry fields in Cyprus and Greece. G. MAKRIS<sup>1</sup>, A. SAMARAS<sup>2</sup>, N. NIKOLOUDAKIS, G. S. KARAOGLANIDIS<sup>2</sup>, and L. I. KANETIS<sup>1</sup>.**

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*Botrytis cinerea* is a complex species prone to fungicide resistance and characterized by enormous genetic diversity. Unraveling pathogen population diversity facilitates the development of more efficient disease management schemes. During 2016-2017, 360 *B. cinerea* isolates were collected from conventional and organic, tomato and strawberry farms in Greece and Cyprus. Our scope was to analyze the genetic diversity and population structure of *B. cinerea* from different hosts and farming systems, in terms of agrochemicals-input. Results highlighted widespread resistance, with 61.9 % of the total population found resistant to at least a botryticide, while 15.5 and 85.1% of the isolates from conventional and organic farms, respectively, were sensitive to all tested active ingredients. More specifically, resistance frequencies of the isolates collected from conventional and organic fields were, 67.7 and 8.2% to boscalid, 65.9 and 29.1% to cyprodinil, 7.3 and 0% to fludioxonil, 18.6 and 3% to fenhexamid, 18.6 and 0% to iprodione, 67.3 and 9.7% to pyraclostrobin, and 61.4 and 15.7% to thiophanate-methyl, respectively. Fludioxonil and fenhexamid resistance was not detected in any tomato farm or country, while resistance to iprodione was not recorded in organic fields. Multiple fungicide resistance was highly recorded, with fungicide sensitivity classes ranging from >3 and <1 for conventional and organic farms, respectively. A lambda measure of association revealed a significant symmetric value ( $0.563 \pm 0.065$ ;  $p < 0.001$ ), suggesting that farming systems correlate with fungicide resistance, across crops and countries. Furthermore, *B. cinerea* populations were genotyped using a set of seven microsatellite markers. *B. pseudocinerea* was not detected, while the populations exhibited high genetic diversity and a mixed-mode of reproduction. Genetic variance among strawberry and tomato populations was evident, ranking host specificity higher than other selection forces. Finally, AMOVA suggested significant associations of population structure and resistance to fungicides thiophanate-methyl, pyraclostrobin, boscalid, and cyprodinil.

**Exploration of non-enzymatic wood degradation pathway in *Fomitiporia mediterranea*, the historical Esca agent.** S. MORETTI<sup>1</sup>, M. L. GODDARD<sup>1,2</sup>, J. LALEVÉE<sup>3</sup>, S. DI MARCO<sup>4</sup>, L. MUGNAI<sup>5</sup>, C. BERTSCH<sup>1</sup> and S. FARINE<sup>1</sup>. <sup>1</sup>*Laboratoire Vigne Biotechnologies et Environnement UPR-3991, Université de Haute Alsace, 33 rue de Herrlisheim, 68000 Colmar, France.* <sup>2</sup>*Laboratoire d'Innovation Moléculaire et Applications, Université de Haute-Alsace, Université de Strasbourg, CNRS, LIMA, UMR 7042, 68093 Mulhouse cedex, France.* <sup>3</sup>*Université de Haute-Alsace, CNRS, IS2M UMR 7361, F-68100 Mulhouse, France.* <sup>4</sup>*Institute of BioEconomy, National Research Council, Bologna, Italy.* <sup>5</sup>*Plant Pathology and Entomology Section, Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), University of Florence, Florence, Italy. E-mail: samuele.moretti@uha.fr*

*Fomitiporia mediterranea* (Fmed) is a basidiomycete genetically described as a white rot agent, historically associated to Esca in grapevine.

Although described as a white rot agent, its biomolecular mechanisms of wood degradation are not yet fully understood. Indeed, like all white rot agents, it has an enzymatic pool (Laccases, Manganese Peroxidases, Endoglucanases and  $\beta$ -glucosidases) potentially capable of attacking and depolymerizing all components of lignocellulosic biomass (cellulose, hemicellulose and lignin). However, comparative genomics studies as well as other experimental observations on the importance of iron in the pathogenesis of Fmed retrieved in the literature, allowed us to formulate the hypothesis that Fmed could adopt both non-enzymatic and enzymatic mechanisms of wood degradation in grapevine.

Our hypothesis is based on the CMF (Chelator Mediated Fenton) model, proposed in the late 1990s by American researchers for brown rot fungi. According to our results, under appropriate experimental conditions and as close as possible to the physiological conditions of grapevine wood, Fmed demonstrates in vitro the ability to: *i*) produce low molecular weight chelating metabolites, *ii*) reduce ferric iron to ferrous iron, *iii*) produce radical species such as hydroxyl radical, thus satisfying all the conditions to adopt non-enzymatic wood degradation mechanisms. Moreover, the CMF model seems to be strain-dependent in Fmed.

Further research is ongoing to study its effectiveness *in lignum* and *in planta*.

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**Protection of grapevine pruning wounds against natural infections by trunk disease fungi.** R. BUJANDA<sup>1</sup>, B. LÓPEZ-MANZANARES<sup>1</sup>, S. OJEDA<sup>1</sup>, O. ONEKA<sup>2</sup>, L.G. SANTESTEBAN<sup>2</sup>, J. PALACIOS<sup>3</sup> and D. GRAMAJE<sup>1</sup>. <sup>1</sup>*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain.* <sup>2</sup>*Dpto de Agronomía, Biotecnología y Alimentación, Universidad Pública de Navarra (UPNA), Campus Arrosadia, 31006 Pamplona, Spain.* <sup>3</sup>*Viticultura Viva S.L., Cabmesado 4, 31390 Olite, Spain. E-mail: [david.gramaje@icvv.es](mailto:david.gramaje@icvv.es)*

Infection of grapevines by grapevine trunk disease (GTD) fungal pathogens primarily occurs through annual pruning wounds made during the dormant season. This study aimed to evaluate and compare the efficacy of various liquid formulation fungicide (pyraclostrobin + boscalid) and paste treatments as well as biological control agents (*Trichoderma atroviride* SC1, *T. atroviride* I- 1237, and *T. asperellum* ICC012 + *T. gamsii* ICC080), for their potential to prevent natural infection of grapevine pruning wounds by trunk disease fungi in two field trials over one growing season. Vineyards were located in Samaniego, Northern Spain (19-years-old; cv. Tempranillo) and Madiran, Southern France (24-years-old; cv. Cabernet Franc). Wound treatments were immediately applied after pruning in February 2020. Untreated controls were mock treated with sterile distilled water. In February 2021, canes were harvested from vines and brought to the laboratory for *Trichoderma* spp. and fungal trunk pathogens assessment. A total of 1,848 and 1,179 fungal isolates associated with five GTDs were collected from the Samaniego and Madiran vineyards, respectively. In some cases, it was not possible to establish significant differences about treatments due to the low incidence of some GTD in the untreated control. The efficacy of each product varied according to the GTD fungi and the grape-growing region. *Trichoderma* recovery percentages ranged from 16.7 to 67.5% in Samaniego, and from 32.5 to 89.6% in Madiran. The experiment will be repeated during the 2021-2022 season.

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## **Fungal Trunk Pathogens Associated with *Juglans regia* in the Czech Republic**

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The aim of this study was to unveil the spectra of fungal trunk pathogens associated with the wood necrosis of the English walnut trees in the Czech Republic. In 2016 the field surveys of the walnut trees were carried out in the region of Moravia. Branches of the walnut trees showing the typical symptoms of dieback were selected for the sampling. In total, 138 of the fungal isolates were obtained from the six orchards. Based on the morphology and sequencing data, fungal isolates were divided to the groups representing 10 species of trunk pathogens: *Cadophora novi-eboraci*, *Cadophora spadicis*, *Cryptovalsa ampelina*, *Diaporthe eres*, *Diplodia seriata*, *Dothiorella omnivora*, *Eutypa lata*, *Eutypella* sp., *Peroneutypa scoparia*, and *Phaeoacremonium sicilianum*. The most predominant fungal taxa belonged to the genus *Cadophora* followed by the groups of species belonging to the Botryosphaeriaceae and Diatrypaceae families. The pathogenicity tests revealed the isolates of *Cadophora* spp. and *Eutypa lata* as highly virulent to the walnut branches.

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**Preliminary observations on the interaction among *Neocosmospora solani* (syn. *Fusarium solani*), *Neofusicoccum batangarum* and *Opuntia ficus-indica*.** S. BURRUANO<sup>1</sup>, S. GIAMBRA<sup>1</sup>, G. GUSELLA<sup>2</sup>, G. CONIGLIARO<sup>1</sup>, G. SURICO<sup>3</sup>.  
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*Opuntia ficus-indica* (L.) Mill. (*Cactaceae*) is a xerophilous plant native to Mexico and naturalized throughout the Mediterranean Basin, where it is a common landscape species. *O. ficus-indica* is cultivated on many continents. In Italy, production is mostly concentrated in certain Sicilian hilly areas. Recently, a severe disease of cactus pear was reported on minor Sicilian islands: Lampedusa and Linosa (Pelagie archipelago), Favignana (Aegadian archipelago), and Ustica. The disease has been named “scabby canker” and its causal agent has been identified as *Neofusicoccum batangarum*, (*Botryosphaeriaceae*, *Ascomycetes*). Scabby cankers appears as radially expanding, concentric, necrotic cankers on cladodes. They begin as early as April following first rains, and continue to develop during summer. Occasionally, also *Neocosmospora solani* isolates appear to be associated with symptomatic cladodes. A study was started to investigate, both *in vitro* and *in planta*, the interactions between *N. solani* and *N. batangarum* and between the two fungi and the host plant. Dual growth *in vitro* assays showed *N. solani* overgrowing *N. batangarum*, and a dark reaction zone was observed at the point of contact of the two colonies, suggesting an antagonism. *In planta* assays carried out by inoculating the cladodes with *N. solani*, individually or in association with *N. batangarum* (which was applied after 30 days) never showed lesions. On the other hand, lesions were clearly visible on the cladodes when they were inoculated with *N. batangarum* only, or simultaneously with *N. solani*. *In vitro* and *in planta* observations suggest that *N. batangarum* has a neutral interaction with *O. ficus-indica*, and that it has antagonistic activity towards *N. batangarum*. Furthermore *N. batangarum* is confirmed as the only causal agent of scabby cankers.

*This work is dedicated to the memory of Dr Gaetano Conigliaro*

**An innovative protocol for the monitoring and onsite detection of *Erwinia amylovora* in Sicily.** F. VALENTINI, F. SANTORO, M. GALLO, L. OUALGUIRAH and A. M. D'ONGHIA.

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*Erwinia amylovora* (*Ea*), the causal agent of the fire blight bacterial disease of pomefruits, is still a quarantine agent (list A2) despite its wide spread in the EU. Effective control of the bacterium is based on the use of resistant varieties, the application of reliable tests for pathogen detection, the knowledge of climatic conditions that can promote the infection, the adoption of specific agronomic practices (e.g. pruning) and/or chemical treatments to reduce the inoculum of the pathogen. Therefore, the objective of this study is to develop an effective protocol for monitoring *Ea* in order to detect infection early before the onset of symptoms, without moving infected plant material from the outbreak area of the infection. To this aim the infection was monitored in pomefruits groves in the *Ea* outbreak of Sicily. Visual inspections for fire blight symptoms and detection of the pathogen by real time LAMP with an onsite device were carried out during 2 vegetative seasons (March- October 2018-2019). The results were correlated to the parameters of an agroclimatic station located in the study area, thus implementing the Maryblight model. The combination of the forecasting model and the onsite detection by real time LAMP is an innovative approach for the official monitoring of this pathogen.

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**Development of an algorithm of recognition for the automatic reading of nitrocellulose membranes processed by DTBIA (Direct Tissue Blot Immunoassay).** S. GUALANO<sup>1</sup>, E. PANTALEO, F. SANTORO<sup>1</sup>, D. FRASHERI and A. M. D'ONGHIA<sup>1</sup>. <sup>1</sup>*Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Via Ceglie, 9 - 70010 Valenzano (BA), Italy.*  
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Serological tests are simple and rapid methods of detecting many plant pathogens. ELISA (Enzyme- Linked ImmunoSorbent Assay) and DTBIA (Direct Tissue Blot ImmunoAssay) are commonly used for screening a large number of plant samples with good efficiency for most pathogens. Compared to ELISA, DTBIA is easy to use, shorter lasting, cheaper and can also be used as on-site test. On the contrary, the reading phase of the DTBIA under stereomicroscope is longer and manual, while an automatic optical reader is available for the ELISA plates. In this work the first attempt was therefore made to develop an automatic reading procedure for the DTBIA. The main components of variability of the processed membranes (e.g. the membrane commercial brand, the storage period of the processed membrane) were analysed with regard to the print color and the membrane background using plant material infected by *Citrus tristeza virus* (CTV) and *Xylella fastidiosa* (Xf). A significant reading variability correlated to some components was observed, confirmed by the optical characteristics. On the basis of these results, an algorithm was developed for the recognition of positive prints using normalized high resolution images.

This research was financially supported by the Project Multitrace - Advanced Information Technologies and Systems for Traceability in Phytopathology (PON MISE H2020).

**Phenometabolomics of olive quick decline syndrome using nuclear magnetic resonance, hyperspectral reflectance and integrative chemometrics analysis.** F. SANTORO<sup>2</sup>, A. ELHUSSEIN M.F.M.H.<sup>1,2</sup>, S. GUALANO<sup>2</sup>, B. MUSIO<sup>1</sup>, A.M. D'ONGHIA<sup>2</sup>, V. GALLO<sup>1,3</sup>. <sup>1</sup>*Department of Civil, Environmental, Land, Building Engineering and Chemistry (DICATECh), Polytechnic University of Bari, Via Orabona, 4, I-70125, Bari, Italy.* <sup>2</sup>*International Centre for Advanced Mediterranean Agronomic Studies of Bari (CIHEAM Bari), Via Ceglie 9, 70010, Valenzano (BA), Italy.* <sup>3</sup>*Innovative Solutions S.r.l. – Spin-off company of Polytechnic University of Bari, Zona H 150/B, 70015, Noci (BA), Italy.*

*Xylella fastidiosa* (Xf) subsp. *pauca* sequence type ST53 has severely affected olive groves in the Apulia region (Southern Italy), infecting around 11 million olive trees over an area of more than 50,000 hectares. The disease caused by Xf is known as olive rapid decline syndrome (OQDS) and produces phenotypic and metabolomic changes in the host plant. Currently, the control strategy is the destruction of infected plants that are detected on the basis of symptoms and diagnostic tests. The ability to detect infection before symptoms develop can provide a great advantage against the spread of the disease, allowing preventive action.

The combination of hyperspectral reflectance (HSR) and nuclear magnetic resonance (NMR) can offer a non-destructive method to detect the phenotypic and metabolic fluctuations caused by Xf. To this aim, young plants of the susceptible variety Cellina di Nardò, artificially infected with Xf, were grown in a temperature-controlled environment and co-inoculated with additional xylem-inhabiting fungi. Asymptomatic leaves were exposed to HSR acquisition while their extracts were subjected to an untargeted metabolomic study in order to select diagnostic signals and wavelengths associated to Xf-infected plants.

Then, covariance matrices between these methods were used as a guide to link HSR spectral features with some NMR and HRMS diagnostic signals. Furthermore, these matrices revealed different wavelength ranges with different levels of association to the corresponding metabolites, a few regions within the visual range of 420-520 nm, 570-720 nm, and more within the near-infrared range of 1000-1830 nm.

**Evaluation of resistance of grape varieties to *A. carbonarius* and ochratoxin contamination.** M.K. ILIADI AND D.I. TSITSIGIANNIS\*. *Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece. \*Email: dimtsi@aua.gr*

*Aspergillus carbonarius* causes grape berry rots in vineyards and is responsible for the production of the mycotoxin, ochratoxin A (OTA) which affects the organoleptic characteristics of the wine and the grape and wine quality and safety. OTA poses a serious risk to consumer safety because of its nephrotoxic, hepatotoxic and carcinogenic properties. The goal of the present study is the evaluation of 21 different Greek vineyard varieties (Razaki, Mandilaria, Roditis, Malagouzia, Savatiano, Fraoula, Athiri, Sideritis, Mavrodafni, Asyrtiko, Xinomavro, Debina, Agianiwitiko, Pavlos, Agiorgitiko, Aidini, Lagorthi, Limnio, Georgiana, Kydonitsa and Stafida) concerning their resistance on *A. carbonarius* rots and OTA contamination. *In vitro* artificial infections were carried out on grape berries of the aforementioned varieties with a mixture of four ochratoxigenic strains of *A. carbonarius* isolated from Crete, Peloponnese, Attica and Macedonia. The evaluation of resistance was performed by assessment of *A. carbonarius* grape rot spots, conidia production and OTA production in berries. The results showed a significant variation among the grape varieties.

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**Characterization of *Alternaria* species associated with black point of wheat kernels in Lebanon.** M. MASIELLO<sup>1</sup>, W. HABIB<sup>2</sup> \*, R. EL GHORAYEB<sup>3</sup>, E. GERGES<sup>2</sup>, C. SAAB<sup>2</sup>, A. SUSCA<sup>1</sup>, G. MECA<sup>4</sup>, J.M. QUILES<sup>4</sup>, A.F. LOGRIECO<sup>2</sup>, A. MORETTI<sup>2</sup>. <sup>1</sup>*Institute of Science of Food Production - ISPA, Research National Council – CNR, Via Amendola, 122/O, 70126 Bari, Italy.* <sup>2</sup>*Laboratory of Mycology, Department of Plant Protection, Lebanese Agricultural Research Institute, P.O. Box 90-1965, Fanar, Lebanon.* <sup>3</sup>*Faculty of Agricultural and Food Sciences, Holy Spirit University of Kaslik, P.O. Box 446, Jounieh, Lebanon.* <sup>4</sup>*Department of Preventive Medicine, Nutrition and Food Science Area, University of Valencia, Avenida Vicent Andres Estelles s/n, 46100 Burjassot, Valencia, Spain.*  
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*Alternaria* is a common fungal genus that may infect wheat crop worldwide in the field, causing harvest and postharvest decay of wheat grains and symptoms on the heads, such as dark brown discoloration of the grain embryos, known as black point disease. Several *Alternaria* species are able to produce a wide range of mycotoxins, associated to a number of different toxic activities to human and animals. The aims of the present study were: i) to assess the incidence of black point disease of wheat in Lebanon and isolate the *Alternaria* species causing the disease; ii) to evaluate the level of contamination by *Alternaria* mycotoxins in 33 samples of grains; iii) to identify the main occurring *Alternaria* species, using a molecular approach, and study their phylogenetic relatedness. The disease occurred in the majority of sites (97.2%) with the highest and the lowest average incidence in Akkar (55.3%) and in Baalbeck (2.2%) districts, respectively. Chemical analyses, performed by HPLC-DAD, showed that altenuene, alternariol, alternariol monomethyl ether, and tenuazonic acid were not detected in any sample. The phylogenetic analyses, based on DNA sequences of  $\beta$ -tubulin, glyceraldehyde-3-phosphate dehydrogenase, allergen alt-a1, and calmodulin genes, showed that isolates were grouped in two main clades: 37 strains belonged to section *Infectoriae* and 42 strains to section *Alternaria*. The present research showed that although black point disease of wheat kernels is widespread in Lebanon, the risk of contamination by *Alternaria* mycotoxins remains low.

**Cultivar-dependent differences in the phyllosphere-associated mycobiome of grapevine (*Vitis vinifera*)** A. MOLNÁR<sup>1</sup>, J. GEML<sup>1,4</sup>, A. GEIGER<sup>1,3</sup>, C. M. LEAL<sup>3,4</sup>, G. M. KGOBE<sup>3,4</sup>, A. M. TÓTH<sup>2</sup>, SZ. VILLANGÓ<sup>2</sup>, L. MÉZES<sup>1</sup>, A.M. CZEGLÉDI<sup>1</sup>, ZS. ZSÓFI<sup>2</sup>.  
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The perennial crop grapevine naturally hosts a reservoir of fungi, affecting grape production, quality and plant health. The above-ground tissues of grapevine, the so-called phyllosphere represents a dynamic and harsh habitat for microbial colonizers due to the exposure to various environmental factors. It is an important question, what drivers shape its fungal composition, thereby contributing to plant health and possibly to crop quality and quantity. Our aim was to investigate the potential differences of the phyllosphere-associated mycobiome inhabiting healthy leaves and berries of three different cultivars, *V. vinifera* cv. Furmint, cv. Kadarka and cv. Syrah, grown under identical environmental conditions in the Eger wine region. We addressed the question whether the grapevine cultivar had an effect on the richness, relative abundance and composition of the fungal communities using high-throughput DNA sequencing. To better clarify the background of the community differences, we also tested the physiological parameters, the macro-and microelement composition of leaf samples and the sugar content of the berries. While richness and relative abundance of fungal functional groups did not statistically differ among cultivars, we did find strong compositional differences among cultivars. In contrast, berry samples differed significantly in terms of richness and relative abundance among cultivars, but not community composition, suggesting the existence of a core grape microbiome at least at terroir level.

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**Selecting tolerant strawberry and melon germplasm to the fungus *Macrophomina phaseolina*. S. A. YOUSSEF<sup>1</sup>, N. DAI<sup>3</sup>, R. COHEN<sup>4</sup>, S. FREEMAN<sup>2</sup> AND A.A. SHALABY<sup>1</sup>,**

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*Macrophomina phaseolina* is a soilborne fungal pathogen causing crown and root rot in strawberry and affects melon causing vine decline worldwide and considered one of the most destructive soilborne pathogens of strawberry and melon in the Mediterranean region. *M. phaseolina* was isolated from different cultivars of strawberry showing crown rot, foliage wilting, charcoal rot and plant mortality, while vine wilting and plant mortality was observed in melon. Similar disease symptoms were recorded in both melon and strawberry cultivation areas in Egypt and Israel. The most effective approach for managing the disease in strawberry and melon relies on resistant germplasm to be selected using reliable techniques. Therefore, pathogenicity tests of representative *M. phaseolina* isolates were conducted for virulence and viability using the toothpick method, whereby, microsclerotia are produced aseptically on toothpicks that are subsequently inoculated into stem and crown tissues of tested plants. In addition, artificial soil inoculation method was also tested by inserting plants in pots containing a soil mix of  $2.5 \times 10^3$  sclerotia/ml. All inoculated plants were grown at 30°C under greenhouse conditions. In strawberry, complete plant mortality was observed approximately 3 weeks after inoculation, using the toothpick method. However, the microsclerotia inoculation method was more accurate with disease symptoms appearing 2 weeks after inoculation. Using the microsclerotia method, disease symptoms progressed faster in the more susceptible cultivar 'Festival' compared to the more resistant 'Fortuna' cultivar showing the least disease progress over time. In melon, disease symptoms using the toothpick treatment in the greenhouse and in the field produced similar symptoms to plants growing in naturally infested soils. Thus, the toothpick method was very reliable for differentiating between susceptible and resistant melon germplasm in contrast to strawberry. In summary, disease screening of resistant germplasm to *M. phaseolina* should rely on an accurate and reliable inoculation technique.

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**Identification and characterization of *Fusarium solani* and *Dematophora necatrix* on hemp (*Cannabis sativa* L.) in southern Italy.** R. SORRENTINO<sup>1</sup>, V. BATTAGLIA<sup>1</sup>, F. RAIMO<sup>1</sup>, D. CERRATO<sup>1</sup>, G. PICCIRILLO<sup>2</sup>, A. MEROLA<sup>1</sup> and E. LAHOZ<sup>1</sup>. <sup>1</sup>Research center for Cereal and Industrial Crops - Council for Agricultural Research and Economics (CREA-CI) Via Torrino 3, 81100 Caserta (CE) – <sup>2</sup>Servizi di saggio di Agrotecniche (SESAT) Via IX Novembre, 81055 Santa Maria Capua Vetere (CE) Italy. E-mail: [roberto.sorrentino@crea.gov.it](mailto:roberto.sorrentino@crea.gov.it)

Since 2016 the cultivation of industrial hemp is allowed again in Italy according to the law n. 242/2016. Industrial hemp can be grown for various purposes such as: fiber, seed, oil, cannabinoids. In a survey activity started from 2018, two new diseases affected hemp. Two new soil-borne agents have been identified as *Fusarium solani* in indoor crops and *Dematophora necatrix* in outdoor crops. In the first case, symptoms consisted of the development, at soil line, of brownish to dark brown areas along the main stem followed by wilting and dropping of leaves. In correspondence with the lesions, the stems swelled thus creating a canker. Removal of the bark revealed that the inner part of the cortex was affected too. In the second case, symptoms of root rot were observed followed by death that generally occurred within 2 to 3 weeks after the outbreak symptoms, about 10% of plants showed yellowing, canopy wilt, and signs of roots covered with white mycelium and fan-like mycelium under the bark. For both pathogens these were the first reports in Europe. Since the interest in hemp is increasing, it is necessary to implement appropriate disease management tools.

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## **Behavior characterization of durum wheat varieties to distinct strains of *Pyrenophora tritici-repentis* (Tan spot) in field and controlled con**

**ditions.**S. TISSAOUI<sup>1</sup>, A. MOUGOU-HAMDANE<sup>1</sup>, N. OMRI-BENYOUSSEF<sup>2</sup> and B. NASRAOUI<sup>1</sup>. <sup>1</sup>LR14AGR02 Laboratory of Bio-aggressors and Integrated Pest Management in Agriculture, National Agronomic Institute of Tunisia, University of Carthage, Tunis, Tunisia. <sup>2</sup>Field Crop Laboratory, National Agronomic Research Institute of Tunisia, Tunisia. E-mail: salmatissaoui2@gmail.com

Tan spot, caused by the Ascomycetes *Pyrenophora tritici-repentis*, is considered a great economic importance of wheat worldwide, and an emerging in Tunisia. Necrosis with chlorosis, and chlorosis on wheat leaf were the distinct symptoms induced. The necrotrophic pathogen produces distinct host-specific toxins responsible of symptoms variation on susceptible genotypes. The reaction of fourteen durum wheat varieties to *P. tritici-repentis* was evaluated in field and controlled conditions by inoculating with three characterized pathotypes. The susceptibility of used varieties was determined by percentage of leaf Area infection. Tan spot lesion developed on all inoculated wheat genotypes which showed behavior variation in the two trials conditions. A significant difference ( $P < 0.05$ ) was recorded in strain virulence. The varieties were also differed significantly in disease reaction to distinct strains. The most virulent was strain I1 inducing chlorosis on varieties which shown reaction variation. Khiar, Sculture and Monastir varieties were moderately resistant in controlled conditions but susceptible in field. A significant isolate x variety interaction was significantly different, that which shown specificity between strain and varieties. The global reaction of varieties indicated that the most resistant varieties was Monastir, while the most susceptible varieties were Maali and Razzak. The use of distinct pathotypes could help to evaluate behavior of wheat varieties in order to select resistant varieties and develop management program of tan spot disease.

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**Effect of rootstock and viroid infection on yield and composition of peel's essential oil of the Tunisian sweet orange (*Citrus sinensis*) cv. Maltaise.** G. ZOUAGHI<sup>1</sup>, A. NAJAR<sup>2</sup>, A. AYDI<sup>3</sup>, C. CLAUMANN<sup>4</sup>, A. ZIBETTI<sup>5</sup>, A. JEMMALI<sup>2</sup>, F. MOUSSA<sup>6</sup>, M. ABDERRABBA<sup>1</sup> and N. CHAMMEM<sup>7</sup>. <sup>1</sup>Laboratory materials, molecules, applications, IPEST (Preparatory Institute for Scientific and Technical Studies), University of Carthage, La Marsa 2070, Tunis, Tunisia. <sup>2</sup>Laboratory of plant protection, National Institute of Agronomic Research of Tunisia, University of Carthage Rue Hédi Karray, 1004 El Menzah, Tunisia <sup>3</sup>Department of Chemical and Materials Engineering, College of Engineering, Northern Border University, P.O. Box 1321, Saudi Arabia. <sup>4</sup>Laboratório de Controle de Processos, Departments of Chemical Engineering and Food Engineering, Universidade Federal de Santa Catarina (UFSC), P.O. Box 476, Florianópolis, SC, Brazil. <sup>5</sup>Department of Informatics and Statistics (INE), Universidade Federal de Santa Catarina (UFSC), P.O. Box 476, Florianópolis, SC, Brazil. <sup>6</sup>Laboratory for the study of molecular analysis techniques and instruments, IUT d'Orsay - Moulon plateau - Building 602 - 91400 ORSAY. <sup>7</sup>Laboratory of Microbial Ecology and Technology, Department of Biological and Chemical Engineering, National Institute of Applied Sciences and Technology (INSAT), Carthage University, B.P. 676, 1080 Tunis, Tunisia. E-mail: asmanajara@yahoo.fr

Besides agronomic performances, some attention is accorded to yield and composition of essential oils (EO) of citrus peels that is very used in some industries. Citrus bioactive components are reported to be influenced by environment factors. In this regard, attention is focused on the effect of rootstocks in presence and in absence of viroid infection on yield and composition of peel's (EO) of the tunisian sweet orange Maltaise. For this purpose, *Citrus exocortis viroid* (CEVd) and *Hop stunt viroid* (HSVd) or Cachexia viroid have been artificially inoculated to eight different rootstocks. Preliminary results show that the yield varies between 0.4 and 0.8 %. However, infection with Exocortis and Cachexia caused a yield decrease of 50% in the case of *Citrumelo swingle* but seemed without significant effects on other rootstocks. Regarding the composition of EO, healthy *Citrus macrophylla* gave the better rate of oxygenated monoterpenes (17.23%). We notice that infection with exocortis enhances considerably this class of monoterpenes in *Citrus macrophylla*, *Citrango carrizo* and *Citrus volkameriana* in which it respectively reached 31.84, 21.87 and 10.56%, while it is significantly reduced by Cachexia infection in *Citrus macrophylla* from 17.23 to 2.90%.

This research is part of PRF (Federated Research Project) on Citrus, entitled "Principal Citrus Viruses: Characterization and Integrated Management" and was financially supported by Ministry of Higher Education and Scientific Research of Tunisia (MESRST)

**Decision Support Systems to improve crop protection, productivity and sustainability: piloting and internationalization.** M. RUGGERI<sup>1</sup>, P. MERIGGI<sup>1</sup>, A. KARTSIAFLEKIS<sup>2</sup> and S.E. LEGLER<sup>1</sup>. <sup>1</sup>*Horta srl, Spin-off Università Cattolica Sacro Cuore, Piacenza, Italy.* <sup>2</sup>*Fondagro, Larissa, Greece.*

Since 2008, Horta has implemented Decision Support Systems (DSSs) for sustainable crop management following the “super consultant” approach (Magarey *et al.* 2002) for achieving high acceptance by agricultural stakeholders. Horta’s DSSs are web-based platforms that collect site-specific weather and crop data in real-time through remote and proximal sensors, organize these data in cloud systems and analyse them via advanced modelling and big data techniques, provide automated agronomic interpretation of model outputs, and consequently alerts and decision supports. Process-based mathematical models are the core of the DSSs; they provide advice to decision maker for overall crop management: pests/diseases management, weed control, fertilization, and irrigation. The DSS supports (not replaces) farmers in the practical implementation of Integrated Crop Management. Benefits are related to all sustainability pillars: economic (e.g., reduction of direct costs), environmental (e.g., reduction of GHG emissions) and social (e.g., reduction of exposure to pesticides, workers’ satisfaction).

To date, DSSs are available for wheat, barley, wine and table grapes, olives, legumes, tomatoes, corn, potatoes and others are in-progress. Users are farmers, technicians, private/public advisors, producers’ organizations, and agri-food companies. DSSs are used in Italy and, increasingly, in other Countries. For instance, vite.net® (grapevine) is widespread in Spain, grano.net® (wheat) and orzo.net® (barley) in Greece and Bulgaria, mais.net® (corn) in Hungary. To quantify, the DSS in Europe for wheat and barley was used by more than 6,300 farms, yielding more than 575,100 tons of grains in 2019.

Internationalization of DSSs is a resources-demanding process, which include customization for each country and demonstration of the multifold benefits rising from DSSs in relevant agricultural contexts to local stakeholders.

**Metagenomics analysis of fungal communities associated with postharvest diseases in pear fruits.** A. ZAMBOUNIS<sup>1</sup>, I. GANOPOULOS<sup>1</sup> and P. MADESIS<sup>2</sup>. <sup>1</sup>*Institute of Plant Breeding and Genetic Resources, HAO 'Demeter', Themi, Thessaloniki, 57001, Greece.* <sup>2</sup>*Institute of Applied Biosciences, CERTH, Themi, Thessaloniki, 57001, Greece. E-mail: antonios.zamb@gmail.com*

An amplicon metagenomic approach was employed in order to identify the composition of fungal communities associated with postharvest diseases of pear fruits. The fruits were harvested at an orchard using routine usage of management practices involving various chemical fungicides and transferred to a storage packinghouse. Effective tag sequences clustered into OTUs and Ascomycota was the dominant phyla (83.4%) followed by Basidiomycota (15.8%). Pear fruits supported a high diversity of microbial organisms, but four genera, *Penicillium*, *Rhodotorula*, *Alternaria* and *Cladosporium* were the most abundant representing 59-95% of the relative abundance of all effective sequences. Pear fruits displayed significantly different fungal communities accordingly to diversity analyses among untreated and treated samples. Particularly, the interruption of chemical treatments for the last one month before harvest resulted in a significant modification in the structure of the fungal community of fruits. We assume that various antagonistic episodes might be occurred in fruit carposphere among the detected fungal genera whose relative abundances were affected by fungicides treatments.

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**DNA-based comparison of plant pathogenic fungi between grapevine and wild woody Rosaceae with a focus on trunk diseases.** L.A. LEPRES<sup>1,2</sup>, J. GEML<sup>1,3</sup>, Z. KARÁCSONY<sup>1</sup>, A. GEIGER<sup>1,2</sup>, A. TÁNCICS<sup>2</sup> and K.Z. VÁCZY<sup>1</sup>. <sup>1</sup>*Food and Wine Research Center, Eszterházy Károly Catholic University, 3300 Eger, Leányka u. 6., Hungary.* <sup>2</sup>*Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Páter Károly u. 1., Hungary.* <sup>3</sup>*ELKH-EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, 3300 Eger, Leányka u. 6., Hungary.*

Grapevine (*Vitis vinifera*) is one of the major cultivated plants worldwide and is naturally colonized by commensal, beneficial or pathogenic microorganisms. Many of these can influence plant health condition and disease incidence and severity. Grapevine microbiome studies have primarily focused on grapevine plant parts and rhizosphere, while the influence of semi-natural ecosystems on the composition of the grapevine microbiome is practically unknown. In this project, we focus on trunk diseases that can be caused by a wide variety of fungi in grapevine, e.g., species of *Phaeoacremonium*, *Phaeomoniella*, *Eutypa*, *Diplodia* etc. Many fungi in these genera can also infect wild and cultivated fruit species, particularly in Rosaceae, and can cause symptoms that are similar to those of grape trunk diseases. Here, we explored possible connections between grapevine microbiome and that of wild woody Rosaceae present in the surrounding landscape. Specifically, we assessed the compositional overlap of plant pathogenic fungi associated with grapevine and nearby wild Rosaceae shrubs and trees. Plant samples were taken from the living trunk parts of the studied plants with and without symptoms of trunk diseases. Based on the obtained data, we found several pathogens present in all studied plants, including *Botryosphaeria*, *Phaeoacremonium*, and *Phaeomoniella* species that are known to cause grape trunk diseases in the vineyards. This overlap suggests that wild Rosaceae fruit species living near vineyards may serve as an inoculum source of plant pathogenic fungi capable of infecting grapevine.

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## **Grapevine environmental DNA provides insights into temporal succession of plant pathogenic fungi under organic and conventional management**

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Plant growth and health are highly dependent on plant-associated microbes and despite recent advances, we still lack a systematic overview of the diversity of grapevine-associated fungi and how the grapevine microbiome is influenced by cultivation methods. In this study, we present the diversity and composition of plant pathogenic fungal communities occurring in grapevines under an organic and conventional management. We hypothesized that the type of plant protection used in conventional and organic vineyards would have a great impact on the microbial, and especially the fungal, community structure associated with grapevine plants. We sampled leaves of the cultivar Bianca at the Eszterházy vineyard situated in Eger, Hungary (2020). After DNA extraction and Illumina NextSeq sequencing, the quality-filtered and rarefied dataset reads were assigned to functional groups, of which 911 ASVs were considered plant pathogen. At genus level, *Phaemoniella* showed the highest number of ASVs, followed by *Alternaria*, *Epicoccum* and *Diplodia* from the 88 different genera found (ASV richness). Differences in fungal richness were apparent among months, but not between organic and conventional management types. Similarly, community structure was primarily influenced by sampling month, as shown by NMDS and PERMANOVA analyses. Beside the strong temporal turnover of leaf-associated fungi, the organic vs. conventional management did not seem to affect leaf fungal communities in a significant manner. It is unclear how much of this community turnover is driven by periodic fungicide applications and how much by seasonality. The lack of significant differences among cultivation types suggest that leaf-associated fungi undergo a seasonal succession.

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**Biocontrol by atoxigenic *Aspergillus* strains and *Trichoderma* spp.** C. ALTOMARE, and A. LOGRIECO.  
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Aflatoxins are a group of mycotoxins produced by moulds of the genus *Aspergillus* in the course of infection of agricultural crops and spoilage of stored food and feeds. Aflatoxins exhibit carcinogenic, mutagenic and hepatotoxic effects and are found in a number of crops, such as cereals, oilseeds, spices, tree nuts, maize, peanuts, pistachios, dried fruit and figs, and derived products. Studies on the genetic and aflatoxigenic diversity in *A. flavus* have led to the development of a biological control strategy based on competitive exclusion, which relies on the inundative introduction of non-toxigenic strains that with time displace the native aflatoxigenic genotypes. The strategy has proven to be effective and capable of reducing contamination with aflatoxin by more than 90% in valuable crops, such as maize, peanuts, pistachios and cottonseed. Nevertheless, the method has limits and drawbacks. First, the non-aflatoxigenic *A. flavus* strains are not necessarily non-pathogenic, and they might still cause disease and yield loss. Also, the possibility exists that the capability to produce aflatoxins is transferred to the progeny of non-toxigenic strains by mating with aflatoxigenic strain. Finally, other toxic metabolites beside aflatoxins may be still produced by non-aflatoxigenic *A. flavus* strains. Recently the capability of members the well-renown biocontrol genus *Trichoderma* to control *A. flavus* and reduce aflatoxins biosynthesis has been pointed out and is raising increasing interest. Using *Trichoderma* spp. for biocontrol of aflatoxigenic fungi may overcome some limits and offer a few advantages in respect to the use of non-aflatoxigenic *A. flavus* strains. As broad-spectrum biocontrol agents, *Trichoderma* can at a time protect the plants from the attacks of other plant pathogens besides the mere *A. flavus*. In addition, *Trichoderma* have indirect favourable effects that are related to the enhancement of plant resilience to drought stress and to the prevention of insect pest damages, which are factors that facilitate aflatoxin occurrence, particularly in a climate change and global warming scenario. In this presentation, an overview of the current technologies based on non-aflatoxigenic strains and prospects of the use of *Trichoderma* spp. for biological control of *A. flavus* and mitigation of aflatoxins risk is given.

**Round Table Discussion: “Biopesticides & Biostimulants: the new reality”, April 7, 2022**