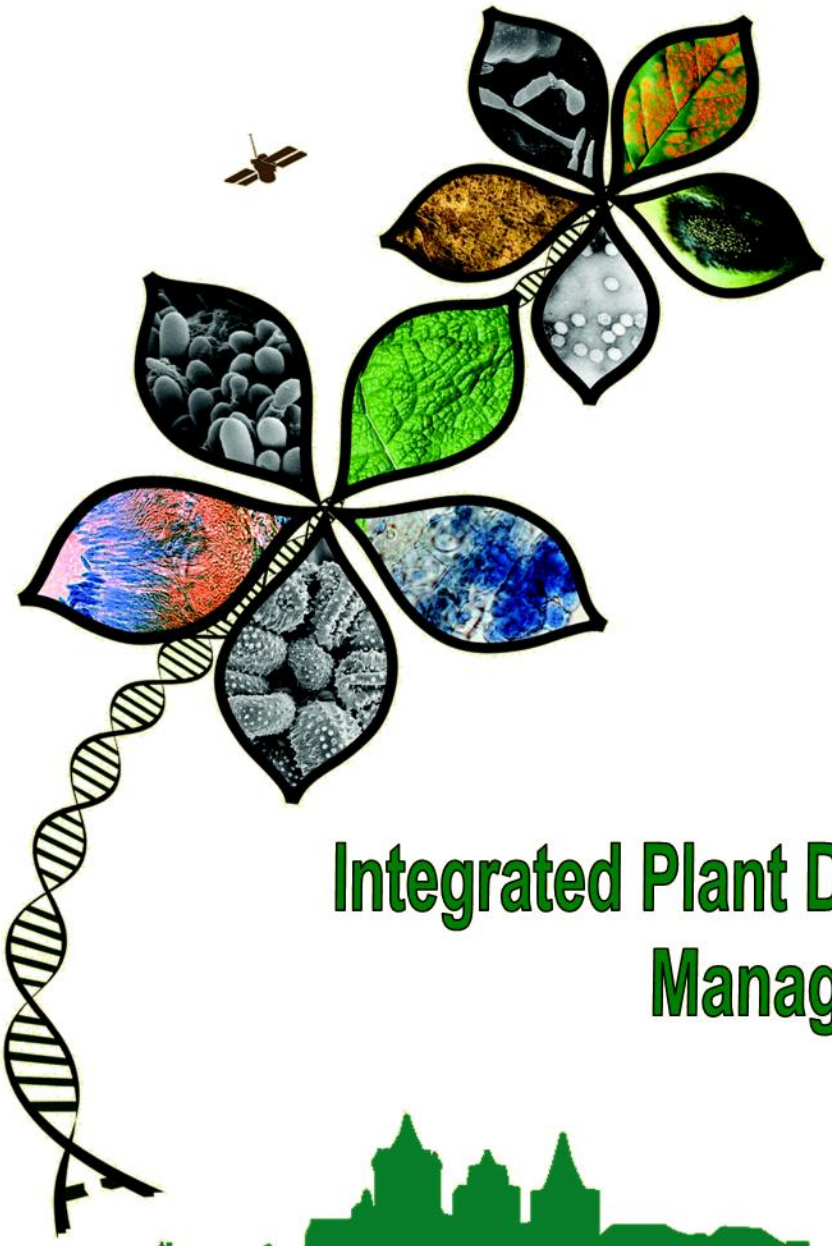
Book of Abstracts

9th Conference of the European Foundation for Plant Pathology

6th Congress of the Sociedade Portuguesa de Fitopatologia

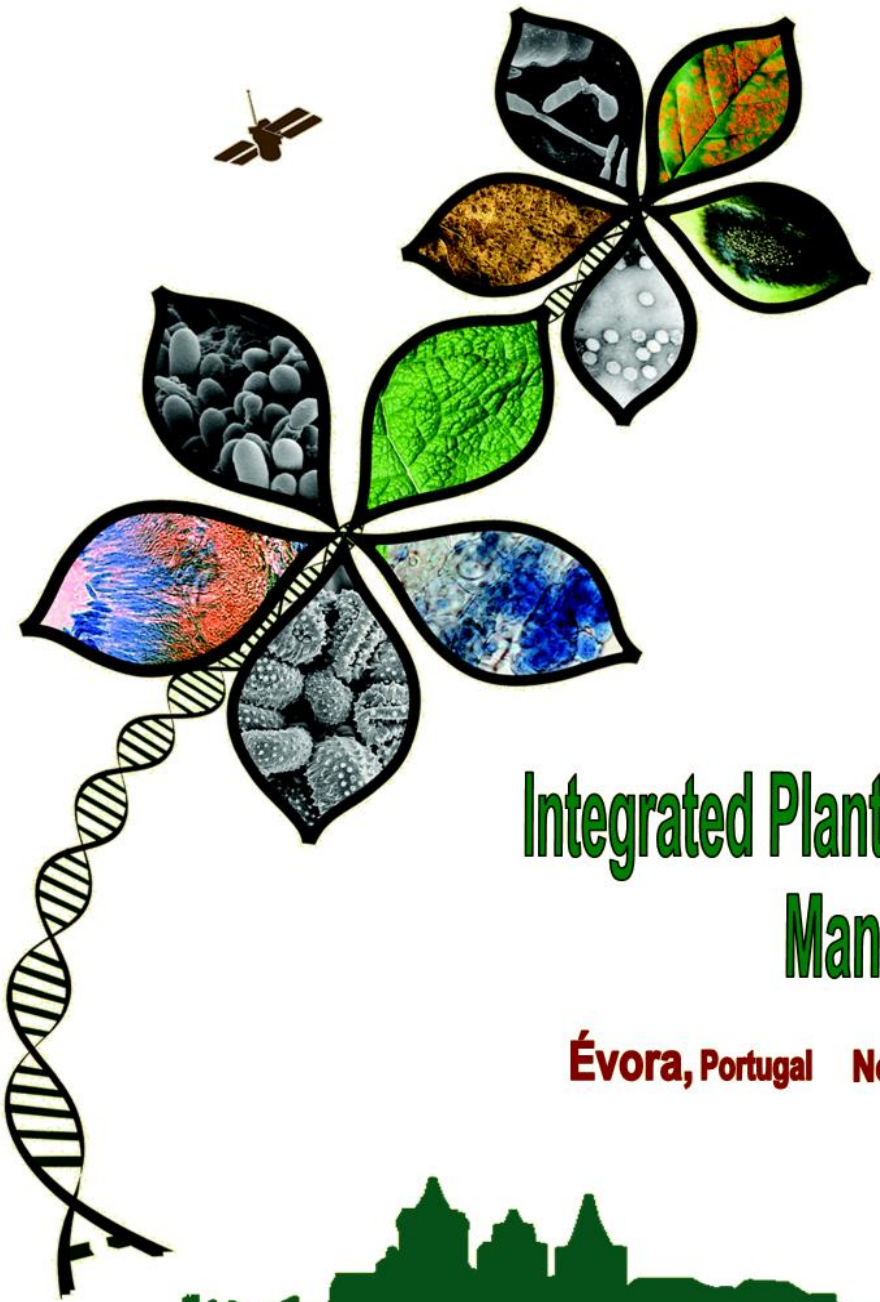


Integrated Plant Disease Management

Évora, Portugal Nov 15-18, 2010

9th Conference of the European Foundation for Plant Pathology

6th Congress of the Sociedade Portuguesa de Fitopatologia



Integrated Plant Disease Management

Évora, Portugal Nov 15-18, 2010

Fountain in the main cloister of the University of Évora – Colégio Espírito Santo.
Photo: S. Rodrigues

First page of the document (Bula) enacted by Pope Paul IV who founded the University of Évora in 1559 (the original Papal document is in the Vatican).
Photo: S. Rodrigues

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Copia Bullæ uniuersitatis Eborensis

Paulus Epis copus ser mus ser rorum dei dilecto filio nostro Henrico tituli sanctorum quatuor coronatorum Sanctæ Romanæ Eccliæ Presbitero Cardinali Infanti Portugaliæ salutem & Aplicam benedictionem. Cum a nobis petit quod iustum est et honestum tam ingor equitatis quam ordo exigit rationis, ut id per solicitudinem officij mi ad debitum perducat effectum Sane pro parte tua nobis nuper exhibita petitio continebat quod dilectus filius pro parte tua dilecto filio Raimundo Tituli Sancti Angeli Sanctæ Romanæ Eccliæ Presbitero Cardinali & maiori penitentiario nro exposito quodtu qui Eccliæ Boren. ex dispensatione Aplica praesse dignoscens pro diuini cultus augmento ac animarum salute unum collegium seu uniuersitate in ciuitate Eborensi erexeras, illudq; sic erectum dilectis filijs presbiteris seu patribus nuncupatis Societatis de I E S V tradideras, concesseras & donaueras, ut in ipsa ciuitate, in qua sedes Metropolitana consistit, & quæ una ex insignioribus ciuitatibus totius regni Portugaliæ ac aduniuersitatem Studij Gnralis tenendam capax & idonea existit, legeq; a ciuitate Conimbricensi, in qua Studium Gnrale uiget, distat; tam ciues eiusdem ciuitatis q; eius districtq, aliorumq; circumuicinorum locorum incolæ a quibus longe itineris dispendio Ires incumbere posset, ut per hæc diuinus cultus augeatur, aliaq; bona ex literarum studio pluementa procederent in omnipotentis dei Laudem & honore cupiebas in dicta ciuitate Eborensi uniuersitatem Studij Gnralis, in qua omnes scia seu facultates præter medicinam, & iuris ciuile, acca partem iuris Canonici, quæ ad forum contentiosum pertinet doceri legi, ac interpretari, ac omnes gradus etiam magisterij, & Doctoratus conferri posset, ac quæ cura regimini et administroi ipsorum presbiterorum seu patrum dicta Societatis de I E S V tuis uero ac tui uicarij Iurisdictioni & correctioi salus tamen eiusdem Societatis priuilegijs quoad eius personas tua uita durante & deinde pro tempore existentis Archiepiscopi Eborensis successoris tui, uel charissimi in Chro filij regis Portugaliæ Illis. prout magis hbi expedire uideretur subiaceret Aplica. autoritate erigi, ipse Raimundus Cardinalis & maior penitentiarius tuum pium & Laudabile propositum desuper confouere uolens tuis supplicationibus inclinatus autoritate nra cuius penitentiariæ curam gerit & de speciali mandato sibi per nos nuna uocis oraculo facto in ciuitate Eborensi prædicta uniuersitatem Studij Gnralis, in qua omnes Licita

9TH CONFERENCE OF THE EUROPEAN FOUNDATION FOR PLANT PATHOLOGY AND 6TH CONGRESS OF THE SOCIEDADE PORTUGUESA DE FITOPATOLOGIA

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WELCOME MESSAGE

The European Foundation for Plant Pathology and the Sociedade Portuguesa de Fitopatologia are very proud to host of the 9th Conference of EFPP and the 6th Congress of SPF, which takes place from 15-18 th of November 2010 at the University of Évora, Portugal. We are pleased to invite you to join us in Évora, a World Heritage Site, dating back more than two thousand years, where Romans and Moors have lived, and finally Christians, since the XII century when Portugal became an independent country. Thus this city is an ideal meeting place for people of different countries who gather together to report about the newest scientific achievements in the diverse fields of Pant Pathology.

The theme of this meeting **'Integrated Plant Disease Management'** (IPDM) aims to bring together people interested in a thorough understanding of plant pathogens and diseases in general, and in the development of new tools and approaches that may offer solutions for the integrated control of diseases in the ecosystems with the least cost and lowest impact on the environment.

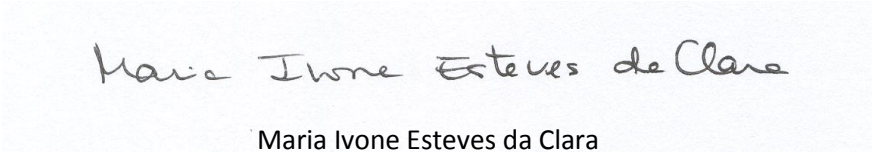
The scientific contributions so far received cover a variety of interdisciplinary topics concerning recent progress on integrated management of major diseases affecting crops including specific topics as: Strategies for disease resistance; Plant pathogen interactions; Advances in biological and in chemical control of pathogens; Epidemiology, modeling and forecasting of plant diseases; Global climate changes and plant diseases; Molecular techniques for detection of pathogens; Molecular variability of pathogens; Precision agricultural technologies in IPDM; Knowledge and technology transfer to IPDM.

More than one hundred participants coming from 25 different countries, from four continents, have confirmed their presence, at the time of writing this.

Throughout the duration of this meeting we hope to create an atmosphere where everyone, scientist, student, professional, can share their experience in their field of expertise, exchange ideas so as to facilitate establishing working partnerships. To achieve this we count on your presence here in Évora, in 2010. Your contributions will certainly make this meeting a real success.

We look forward to welcoming you to Évora!

On behalf of the Organizing Committee



Maria Ivone Esteves da Clara
(President of the EFPP and of the SPF)

SCIENTIFIC PROGRAMME

Sunday 14 November 2010	
Time	**Programme**
17:30 - 21:00	Registration at University of Évora room 124; Poster placement in room 129

Monday 15 November 2010	
Time	**Programme**
8:30 - 9:30	Registration and Poster exhibition
9:30-10:30	**CONFERENCE OPENING**
10:30 - 11:00	**Coffee break**
	SESSION 1: EPIDEMIOLOGY, MODELLING AND FORECASTING OF PLANT DISEASES
11:00 - 11:30	**KN1.1 Population biology and epidemiology of plant virus disease** **Michael J. Jeger, Imperial College of London, London, UK**
11:30 - 11:50	**O1.1 Modelling pathogen competition and displacement** Jonathan Yuen, Swedish University of Agricultural Sciences, Sweden
11:50 - 12:10	**O1.2 *Rhizoctonia* spp. in two forest nurseries *versus* soil fungi communities** Marta Belka and Malgorzata Manka, Poznan University of Life Sciences, Poland
12:10 - 12:30	**O1.3 Epidemiological studies on *Puccinia recondita* causing Leaf Rust of Wheat in Punjab (Pakistan)** S. Ahmad, Z. Iqbal and Y. Iftikhar; University of Sargodha, Pakistan
12:30 - 14:30	**Lunch and poster viewing**

14:30 - 14:50	**O1.4 The epidemiological importance of asymptomatic infection of winter barley by *Rhynchosporium secalis* and its consequences for crop protection and breeding** Adrian C. Newton, Simon D. Atkins, Bruce D.L. Fitt, Bart Fraaije and Mark Looseley, Scottish Crop Research Institute, Dundee, UK
14:50 - 15:10	**O1.5 Understanding the peculiarities of the development of winter oil-seed rape stem canker as the basis for successful disease control** Biruta Bankina, Zinta Gaile and Oskars Balodis; Latvia University of Agriculture, Latvia
	SESSION 2: STRATEGIES FOR DISEASE MANAGEMENT AND DISEASE RESISTANCE
15:10 - 15:40	**KN2.1 Information network and plant disease management** **Karen A. Garrett; Kansas State University, USA**
15:40 - 16:00	**O2.1 Effect of *Neotyphodium lolii* on perennial ryegrass defence reaction against infection by pathogens** Dariusz Panka, Malgorzata Jeske, Mikolaj Troczynski; University of Technology and Life Sciences, Bydgoszcz, Poland
16:00 - 16:30	**Coffee break**
16:30 - 16:50	**O2.2 Compost amended with bactericidal plant materials reduced bacterial diseases and increased the yield of tomato in Abeokuta, Nigeria** Akin R. Popoola, Sikiru A. Ganiyu, Emily I. Ayo-John, Oluwatoyin A. Babalola; University of Agriculture, Abeokuta, Ogun State, Nigeria
16:50 - 17:10	**O2.3 Searching for soil fertilizing microorganisms** Margarida M. Santana, Maria C. Portilllo, Juan M. Gonzalez, Maria Ivone E. Clara; Universidade de Évora, Évora, Portugal
17:10 - 17:30	**Poster viewing**

17:45	**Welcome reception at the City Hall**
Tuesday 16 November 2010	
Time	**Programme**
9:00 - 9:20	**O2.4 Reprogramming the viral disease resistance gene N from tobacco** Julia Niemeyer, Fabian Machens, Dietmar Stahl and Reinhard Hehl; Technische Universität Braunschweig, Braunschweig, Germany
9:20 - 9:50	**O2.5 Exploring feasibility and reliability of rating for resistance to sugarcane yellow leaf virus (SCYLV) in a core collection of sugarcane** Sarah Débibakas, Solen Rocher, Jean-Yves Hoarau, Jean-Heinrich Daugrois; CIRAD, Guadeloupe SESSION 3: ADVANCES IN BIOLOGICAL, CHEMICAL AND CULTURAL CONTROL OF PATHOGENS
9:50 - 10:20	**KN3.1 Targeting carbohydrates: a novel paradigm for pathogen control** **Ricardo B. Ferreira, Regina Freitas, Sara Monteiro; Instituto Superior de Agronomia, Universidade Técnica de Lisboa, ITQB, Portugal**
10:20 - 10:50	**Coffee break**
10:50 - 11:20	**KN3.2 Trapping nematodes with *Solanum sisymbriifolium*** **Luci M. Conceição and Isabel Abrantes; Coimbra, Portugal**
11:20 - 11:40	**O3.1 Novel peptide elicitor of plant defence responses derived from collagenous waste** Vladimir Sasek, Lenka Burketová, Jan Martinec, Vera Kaspárkova and Karel Kolomaznik; Institute of Experimental Botany, Praha, Czech Republic

11:40 - 12:00	**O3.2 Elicitation of phenylalanine ammonia-lyase activity in postharvest apple and tomato leaves by ulvan and glucuronan isolated from *Ulva lactuca*** Elfaïza Abouraïcha, Mohamed E. Gadda, Sanâa Wahbi, Redouan E. Boutachfaiti, Emmanuel Petit, Zainab E.A. Talibi, Bernard Courtois, Josiane Courtois and Cherkaoui E. Modafar; Université Cadi Ayyad, Marrakech, Maroc
12:00 - 12:20	**O3.3 Assessment of combination effects of Pyraclostrobin and Pyrimorph against *Phytophthora infestans*, employment gene expression analysis and microscopic evaluations** Yousef Yari Kamrani, Egon Haden, Gerd Stammler, Andreas Koch, John-Bryan Speakman and Karl-Heinz Kogel; BASF SE, Limburgerhof, Germany
12:20 - 14:20	**Lunch and poster viewing**
14:40 - 15:00	**O3.4 Control of foliar diseases of barley using a combination of resistance elicitors** Dale R. Walters, Neil D. Havis, Linda Paterson and Cecile Sablou; Scottish Agricultural College, Edinburgh, UK
15:00 - 15:20	**O3.5 Effect on yield and disease of interactions between barley cultivars and with soil tillage treatments** Adrian C. Newton, Glyn A. Bengough, David C. Guy, Blair M. McKenzie and Paul D. Hallett; Scottish Crop Research Institute, Dundee, UK
15:20 - 15:40	**O3.6 Production of a postharvest biocontrol agent with food industry by-products** Teresa Manso, Cristiana Maia, Carla Nunes; Universidade do Algarve, Faro, Portugal
	SESSION 4: GLOBAL CLIMATE CHANGES AND PLANT DISEASES
15:40 - 16:10	**KN4.1 Effect of climate change on scenarios of plant diseases and management** **Piet Boonekamp and Kees Booij, Wageningen The Netherlands**

16:10 - 16:30	**O4.1 Effects of climate change on plant health** Bolette Lind Mikkelsen, Cb Gowda Rayapuram and Michael Lyngkjaer; University of Copenhagen, Copenhagen, Denmark
16:30 - 17:00	**Coffee break**
	SESSION 5: PRECISION AGRICULTURAL TECHNOLOGIES IN INTEGRATED PLANT DISEASE MANAGEMENT
17:00 - 17:30	**KN5.1 Precision crop protection - the challenge and the use of heterogeneity** **Erich-Christian Oerke, Ulrike Steiner and Heinz-Wilhelm Dehne; Inres-Phytomedicine, University of Bonn, Germany**
17:30 - 18:00	**SESSION 6 (SPECIAL SESSION): 7th European Union Research Framework Programme Funding Opportunities for Member States associated countries and third countries** **Ana Mafalda Dourado; Ministério da Ciência, Tecnologia e Ensino Superior, Portugal**
18:30	**EFPP meeting with delegates of member societies**
20:30	**Conference Dinner at M'Ar d'Ar Hotel Aqueduto**

Wednesday 17 November 2010	
Time	**Programme**
	SESSION 7: MOLECULAR TECHNIQUES FOR DETECTION OF PATHOGENS
9:00 - 9:20	**O7.1 Molecular detection of apple scab inoculum** Julia C. Meitz, Saskia Von Diest and Cheryl L. Lennox; University of Stellenbosch, South Africa
9:20 - 9:40	**O7.2 PCR-based detection of pathogens in carrot soil samples to predict development of carrot cavity spot and post harvest carrot diseases**

	Arne Hermansen, Grete Lund, Elisa Gausla, Addelhameed Elameen, Ragnhild Naerstad and Sonja Klemsdal; Bioforsk, Hogskoleveien, Norway
9:40 - 10:00	**O7.3 A new *Phytophthora* species causing root rot in pea and other legumes** Fredrik Heyman, Lars Persson and Mariann Wikström; Swedish University of Agricultural Sciences, Uppsala, Sweden
10:00 - 10:20	**O7.4 Development of QOI resistance in *Ramularia collo-cygni* populations** J. M. Fountaine, F.J. Burnett and B.A. Fraaije; SAC, Edinburgh, Scotland
10:20 - 11:00	**Poster viewing and Coffee break**
	SESSION 8: MOLECULAR VARIABILITY OF PATHOGENS
11:00 - 11:20	**O8.1 *Citrus tristeza virus* is a slowly evolving virus** Gonçalo Silva and Gustavo Nolasco; BioFIG, Universidade do Algarve, Faro, Portugal
11:20 - 11:40	**O8.2 Specific aminoacids of *Olive mild mosaic virus* coat protein are determinant for transmission by *Olpidium brassicae*** Carla M.R. Varanda, Maria R.F. Félix and Maria I.E. Clara; Instituto de Ciências Agrárias Ambientais Mediterrâneas, Universidade de Évora, Évora, Portugal
11: 40 - 12:00	**O8.3 What do bacterial genes tell: symbiont or pathogen?** Marta Laranjo, Ana Alexandre and Solange Oliveira; Instituto de Ciências Agrárias Ambientais Mediterrâneas, Universidade de Évora, Évora, Portugal
12:00 - 13:45	**Lunch**
14:00	**Departure to the field trip**

Thursday 18 November 2010

Time	Programme
	SESSION 9: PLANT PATHOGEN INTERACTIONS
9:00 - 9:20	**O9.1 Pine wilt disease: a threat to European forestry** Paulo R. Vieira and Manuel M. Mota; NemaLab-ICAAM, University of Evora, Évora, Portugal
9:20 - 9:40	**O9.2 PathoPlant-assisted prediction of two kinases simultaneously involved in the response to fungal and bacterial pathogens** Fabian Machens, Sarah Pohl, Jeannette Koschmann, Lorenz Bülow, Dietmar Stahl and Reinhard Hehl; Technische Universität Braunschweig, Braunschweig, Germany
9:40 - 10:00	**O9.3 Powdery mildew induced transcription factors HvWRKY1/2 mediate compatibility and repress the defense related gene HvGER4c in barley** Dilin Liu, Gregor Langen and Karl-Heinz Kogel; Justus-Liebig-University Giessen, Giessen, Germany
10:00 - 10:30	**KN9.1 Detecting exotic forest pathogens, and predicting their impact in Mediterranean ecosystems** **M. Garbelotto; University of California, Berkeley, USA**
10:30 - 11:00	**Coffee break**
11:00 - 11:20	**O9.4 Matrix metalloproteinase as factor in plant innate immunity** Puyan Zhao, Dilin Liu, Gregor Langen and Karl-Heinz Kogel; University of Giessen, Germany
11:20 - 11:40	**O9.5 Defence response of oilseed rape against a hemibiotroph pathogen *Leptosphaeria maculans*** Lenka Burketová, Vladimir Sasek, Barbora Korbelová, Károly Bóka and Olga Valentová; Academy of Sciences of Czech Republic, Praha, Czech Republic

11:40 - 12:00	**O9.6 Transcriptomic analysis of *Hemileia vastatrix* in pre and post-penetration stages** P. Talhinhas, H.G. Azinheira, A. Vieira, A. Loureiro, B. Vieira, F. Pina-Martins, D. Batista, E. Tisserant, E. Morin, A.-S. Petitot, O. S. Paulo, S. Duplessis, M.C. Silva and D. Fernandez; CIFC/IICT, Oeiras, Portugal
12:00 - 12:20	**O9.7 Characterization of the activity of phylogenetically distinct silencing suppressors of *Citrus tristeza virus*** Ângela Costa, Natália Marques, Gonçalo Silva, Gustavo Nolasco; Universidade do Algarve, Faro, Portugal
12.20 - 12.40	**O9.8 Identification of a RNA silencing suppressor in the genome of Grapevine leafroll associated virus 3 (GLRaV-3)** Paulo Gouveia, Gustavo Nolasco, Universidade do Algarve, Faro, Portugal
12:40 - 14:20	**Lunch**
	SESSION 10 (SPECIAL SESSION): INTERNATIONAL COOPERATION ON COFFEE RUST RESEARCH - A LEGACY OF PROFESSOR BRANQUINHO D'OLIVEIRA
14:00 - 14:30	**SS10.1 Coffee rusts research center: fifty five years devoted to international cooperation** **Vitor M.P. Várzea, Leonor Guerra-Guimarães, Helena Gil Azinheira, Andreia Loureiro, Ana P. Pereira, Pedro Talhinhas, Dora Batista, Maria do Céu Silva; CIFC/IICT, Oeiras, Portugal**
14:30	Announcement of the winner of the Branquinho d'Oliveira Prize for best MsSci dissertation on Plant Pathology (competition open to MsSci dissertations approved at Portuguese Universities/Polytechnic Institutes) Announcement of the winner of the best poster prize authored by a student presented at the 9th Conference of the European Foundation for Plant Pathology and 6th Congress of the Sociedade Portuguesa de Fitopatologia (competition open to all students presenting a poster)

Pulpit in one of the original classrooms in the University of Évora.
Photo: S. Rodrigues

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ABSTRACTS

SESSION 1: EPIDEMIOLOGY, MODELING AND FORECASTING OF PLANT DISEASES

K1.1 Population biology and epidemiology of plant virus disease

Michael J. Jeger
Division of Biology, Imperial College London, Silwood Park campus, Ascot SL5 7PY. UK
Corresponding author: m.jeger@imperial.ac.uk

Plant virus disease mostly arises from the interactions, at different scales, of a host plant, vector and virus, in a given environment. The epidemiology of plant virus diseases requires an understanding at the population level of host plant dynamics (crop and non-crop hosts, including weeds), the ecology and behaviour of vectors and their interaction with other species, and of factors influencing evolutionary change in the virus and vector. In some cases within-plant and within-vector processes must be taken into account in developing this understanding. In this presentation the complexities of plant virus epidemiology will be illustrated by three topics: (a) the influence of disease management practices (including the use of host resistance based on different mechanisms) on selection for virus virulence; (b) the relationship between horizontal and vertical transmission processes in plant-vector and vector-vector interactions; and (c) the impact of introducing natural enemies such as parasitoids to control herbivorous insect vectors, where plant host and vector signals may play a key role in determining the effects on virus incidence and spread. For each of these topics, mathematical models have been used to dissect and identify the key factors underlying disease epidemiology and may help to improve disease management strategies.

O1.1 Modelling pathogen competition and displacement

Jonathan Yuen
Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Ulls Väg 26A, SE 750 07 Uppsala, Sweden
Corresponding author: Jonathan.Yuen@mykopat.slu.se

Pathogen populations can be subject to immigration by other genotypes. If, in the course of natural selection, the invasive genotypes are more fit, they will displace the original individuals. In order to study this process, a simple epidemic model was developed, based on the Lotka-Volterra model for competition. The model contains two epidemics, each with logistic growth of two separate populations that interact with each other in that they compete for the same resource base (the plant). Routines were added to allow the epidemics to start at different time points with different amounts of inoculum. A numerical solution to the model was developed using the 'ode' package of the open-source statistical program 'R'. The resulting model allows examination of the relative importance of different values for the apparent infection rates and starting parameters for the two sub-epidemics. A case study for potato late blight in Scandinavia is presented, with epidemics corresponding to oospore and tuber sources of inoculum. For this particular pathosystem, where epidemics from oospore infections are assumed to start earlier than those from tuber infections, the delay for epidemic initiation via tuber infection would require extremely high values of 'r' in order for this population to dominate at the end of the season. This could be one reason for the lack of persistent clones in the Scandinavian *Phytophthora infestans* population.

Keywords: ***Phytophthora infestans*, Lotka-Volterra**

O1.2 *Rhizoctonia* spp. in two forest nurseries *versus* soil fungal communities

Marta Bełka and Małgorzata Mańka
Department of Forest Pathology, Poznań University of Life Sciences,ul. Wojska Polskiego 71c, Poznań 60-625, Poland
Corresponding author: mmanka@up.poznan.pl

Rhizoctonia spp. are severe damping-off pathogens of coniferous seedlings in Polish forest nurseries. Every year they cause damage in Oborniki and Lipka forest nurseries (north-western Poland). The aim of this work was to characterize *Rhizoctonia* isolates in the nurseries and to determine the effect of soil fungal communities on the growth of the pathogens. In 2004 soil samples were taken from both nurseries at the end of June, and *Rhizoctonia* spp. were isolated using Scots pine seedlings as baits, while soil fungi were isolated by a soil plate method. For all the *Rhizoctonia* isolates, the number of nuclei per cell and mycelial growth rates were determined. Six isolates of *Rhizoctonia* spp. and four of *R. solani* from Oborniki, and eight *R. solani* and three *Rhizoctonia* spp. from Lipka nursery were considered in further work. The biotic series method was used to check biotic relations between chosen *Rhizoctonia* spp. isolates and saprotrophic soil fungi from each nursery. No correlation between the number of nuclei (binucleate/multinucleate isolates) and growth rate was observed. Pathogenicity of the isolates ranged between 80 and 100%. No correlation between the number of nuclei and pathogenicity was observed. The growth of all *Rhizoctonia* spp. isolates was supported by both soil fungi communities. Both fungal communities supported multinucleate isolates (*R. solani*) to a greater extent than other *Rhizoctonia* spp. No wonder that *Rhizoctonia* spp. are severe damping-off pathogens, as they are strongly supported by soil fungi communities.

Keywords: damping-off, Scots pine, seedlings

O1.3 Epidemiological Studies of *Puccinia recondita* causing Leaf Rust of Wheat in Punjab (Pakistan)

S. Ahmad, Z. Iqbal, and Y. Iftikhar

University College of Agriculture, University of Sargodha, Sargodha, Pakistan
Corresponding author: ahmadyarsalman@gmail.com

One hundred and twenty wheat genotypes were screened against leaf rust virulences to gain an understanding of the epidemiology of leaf rust and to evaluate the resistance level of Pakistani wheat germplasm. Among these genotypes, fifty five were varieties and sixty five were lines. Out of 55 varieties, 10 proved susceptible, 5 moderately susceptible to susceptible, 10 moderately resistant to moderately susceptible and 12 were found to be resistant. Among the 65 wheat lines, 38 were susceptible, 9 moderately susceptible to susceptible, 5 moderately resistant to resistant and 6 were resistant. All other varieties and lines remained asymptomatic. The relationship of epidemiological factors (maximum and minimum temperatures, relative humidity, rainfall, sunshine radiations and wind speed) to leaf rust infections was determined on different wheat genotypes by correlation and regression analysis. Most of the epidemiological factors played an important role in the spread of leaf rust. Maximum and minimum temperature, rainfall and relative humidity were significant while the sunshine radiations and wind speed were non significant for leaf rust spread. Furthermore, maximum temperature, relative humidity and rainfall were positively correlated with leaf rust infections, which means that as the value of these factors increased the severity of leaf rust on different wheat genotypes also increased. However, correlation of minimum temperature with leaf rust severities was negative, which means that as minimum temperature increased leaf rust severity decreased.

Keywords: Screening, varieties, correlation, temperature

O1.4 The epidemiological importance of asymptomatic infection of winter barley by *Rhynchosporium secalis* and its consequences for crop protection and breeding

Adrian C. Newton[1], Simon D. Atkins[2], Bruce D.L. Fitt[2], Bart Fraaije[2], Mark Looseley[1]
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Many microbe-host interactions can easily be categorised as pathogenic, parasitic or mutualistic, but in practice few examples exactly fit these descriptions. For example, several fungi known as plant pathogens are being found to have extensive non-pathogenic phases. Whether they transition to a different trophic relationship with their host such as pathogenic (symptomatic) depends on some trigger, often of unknown nature. We demonstrate this by characterising the epidemiology of leaf scald (caused by *Rhynchosporium secalis*), one of the most economically important diseases of barley, using both visual symptom assessment and molecular detection methods. Two susceptible winter barley cultivars (Sumo and Saffron) and two resistant cultivars (Flagon and Manitou) were sampled at several growth stages (GS) throughout the growing season and visual symptoms assessed. Through quantification of the *R. secalis* DNA by PCR and microscopic observations we demonstrate that the pathogen was able to colonise and sporulate extensively on apparently healthy leaves, and spread to grain without symptoms being seen in the crop. Using a mapping population, we were able to demonstrate that expression of symptoms can be under different genetic control from asymptomatic growth of the fungus. We speculate about how the fungus interacts with the plant, what triggers lesions to develop and the role of asymptomatic infection in pathogen spread, plant defence and crop yield. This will increase our understanding of the true ecological niche of such organisms and the implications of the dynamic state of their trophic interactions with their hosts has for agriculture, including crop rotation, disease control and risk management.

Keywords: symptoms, yield, trophic

O1.5 Understanding the peculiarities of the development of winter oil-seed rape stem canker as the basis for successful disease control

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Stem canker (caused by *Leptosphaeria* spp.) has become one of the most widespread diseases of winter oilseed rape in Latvia. The aim of this investigation was to clarify peculiarities of the disease cycle under Latvian conditions for forecasting and control of the disease. Investigations were carried out in Latvia University of Agriculture (LLU), and development of symptoms was determined in LLU research and study farms "Vecauce" and "Peterlauki". Progress in fruiting bodies was observed in semi-field trials in LLU Institute of Soil and Plant Sciences. First symptoms of the disease (spots with clear pycnidia on the leaves) were observed in September, or about 6 weeks after sowing. Pycnidia with conidia continued to develop on the leaves untill the end of the vegetative season (November-December under Latvian conditions) and also after overwintering untill leaf senescence (end of June). In this time spots with pycnidia developed on the stems. Production of conidia is possible throughout autumn and the following vegetative season. First pseudothecia were observed on the residues of rape stubble during September, but ripe ascospores were found only in late October. Ascospores in the pseudothecia were observed throughout autumn and also in the entire subsequent vegetative season. Infection material of Phoma stem canker existed throughotu the winter rape growing season, but the most crucial periods of infection was not noted untill now. Experiments have to be continued, because thresholds of infection, forecasts of disease development and possible yield losses are necessary to implement integrated disease control.

Keywords: ascospores, forecast, *Leptosphaeria*, thresholds

P1.1 Pest Risk Assessment of *Phytophthora ramorum* in Norway

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The Pest Risk Assessment (PRA) of *Phytophthora ramorum* was initiated by the Norwegian Food Safety Authority as a basis for a possible revision of current phytosanitary regulations. Since the first detection in 2002 *P. ramorum* has been treated as a potential quarantine pest. The pathogen is not widely distributed in Norway, and it is under official control. About half the rhododendrons planted in Norway are domestically produced. The probabilities of entry and establishment of *P. ramorum* are both rated as high with low uncertainty. Known hosts imported for planting is thus the most important pathway. Soil as growing media and contaminant, foliage and cut branches imported for ornamental purposes are other significant pathways. In the absence of statutory control the probability of rapid spread in the PRA area by trade of host plants for planting is rated as high with low uncertainty. A very conservative estimate of the endangered areas is parks and gardens with rhododendron and viburnum and woods with *Fagus sylvatica*. *Phytophthora ramorum* will have moderate economic impact on the nursery industry, parks and gardens in the PRA area with current phytosanitary regulations. Without such regulations, the pathogen is likely to have major economic impact in the best climatic zones of the PRA area. The uncertainties of these assessments are low. The impacts of *P. ramorum* in coniferous and mixed forests in the PRA area are likely to be minor with medium uncertainty. The consequences to natural environments are likely to be moderate with high uncertainty.

Keywords: Phytosanitary regulations, rhododendron, viburnum, beech

P1.2 A study of epidemiology of the barley pathogens *Rhynchosporium secalis* and *Ramularia collo-cygni*

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Two of the most economically important fungal pathogens that attack barley (*Hordeum vulgare)* crops in Scotland are *Rhynchosporium secalis* (leaf scald) and *Ramularia collo-cygni* (Ramularia leaf spot). The epidemiology of these diseases was studied in detail over a number of years in large scale field experiments that combined a number of factors known to influence disease epidemics, such as seed source and fungicide treatment. The movement of fungal spores was monitored by means of a Burkard 7 day spore sampler, which allows a daily quantification of fungal DNA levels in the environment. The environmental conditions that favour the spread of spores were measured using an automated weather station situated close to the field experiments. Analysis of detailed disease assessments indicated that *R. secalis* infection early in the growing season is influenced primarily by seed source and that this should be incorporated into future risk models along with varietal resistance and weather conditions. Analysis of the *R. secalis* DNA recovered from the spore sampler indicated an optimal temperature for spore dispersal of eight degrees Celsius. *Rhynchosporium collo-cygni* spore release events were found to be related to periods of sustained surface wetness in the crop. Seed borne infection was also discovered to be the primary source of *R. collo-cygni* infection in barley crops. Information derived from field experiments has been incorporated into risk assessment models aimed at optimising control of the pathogens. In addition, data from the spore sampler is currently being incorporated into disease forecasting models for the two diseases.

Keywords: disease, DNA, forecasting, leaf scald, models, Ramularia leaf spot

P1.3 Pitch canker disease in Portugal

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Pitch canker disease caused by the fungus *Giberella circinata*, anamorph *Fusarium circinatum*, was recently reported in pine nurseries in Portugal. The disease affecting a large number of pine species in many countries was first detected affecting *Pinus radiata* seedlings in a nursery located in the centre of the country. After the first report, a large survey all over the country has been done. Training for inspectors and formal presentations to develop extension education tools on pitch canker included sampling procedures, disease symptoms, relevant aspects of the biology of the fungus, management recommendations and economic factors. Although the fungus can infect vegetative and reproductive tissues of susceptible hosts at all ages, from seedlings through to mature trees, so far our results show that *F. circinatum* is present only in samples from nurseries and has not been detected in forest stands.

Keywords: *Fusarium circinatum, Giberella circinata*, *Pinus* spp., pine disease

KN2.1 Information networks and plant disease management

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Epidemic networks offer challenges for effective management. Information networks that operate at the appropriate scales to track important changes in epidemic networks can improve disease management. Anticipating the arrival of a pathogen through an epidemic network supports more effective management strategies. The soybean rust epidemic in the USA is an example of a highly coordinated information network operating at a national level. Development of an epidemic network model for the soybean rust epidemic made it possible to identify highly connected locations that are more important sources of information for predicting epidemic progress. These and related strategies for identifying the most important types of information about epidemics can make disease management more efficient. Network models also provide a means to quantify the benefits of 'disease regulation' that may be provisioned by landscapes as an ecosystem service.

O2.1 Effect of *Neotyphodium lolii* on perennial ryegrass defence reaction against infection by pathogens

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Endophytic fungi in the genus *Neotyphodium* often form symbiotic relationships with numerous grass species. The relationship results in the fungus having a strong effect in protecting the host plant from the influence of external stress factors, e.g. infection by pathogens and pest attack. The mechanisms of such beneficial influence of the endophyte are not completely known. Growth chamber trials were conducted to answer the following questions: (i) are endophyte-infected perennial ryegrass plants more resistant to infection caused by *Fusarium poae* and *Rhizoctonia solani* than endophyte-free plants? (ii) is there an effect of endophyte mycelium density in leaf sheaths and blades on development of disease symptoms caused by test pathogens? (iii) is there an effect of endophyte presence on the production of the phenolic compounds by the host plant as a defence mechanism? Three perennial ryegrass genotypes each symbiotic with a different endophyte strain, were inoculated with *F. poae* and *R. solani*. Degree of infection and density of mycelium in leaf sheaths and blades were estimated. The content of total phenolic compounds in tillers and roots of diseased plants was measured. There was a significant effect of the endophyte presence on the intensity of disease symptoms and production of phenolics. Density of mycelium of the endophyte strains differed between cross-sections studied. Results indicate that the mechanism of host protection does not appear to be based on competition between *N. lolii* and pathogen hyphae, but rather through production of phenolic compounds and other defence mechanisms by the host.

Keywords: endophyte, *Fusarium*, grass, phenolic compounds, *Rhizoctonia*

O2.2 Compost amended with bactericidal plant materials reduced bacterial diseases and increased the yield of tomato in Abeokuta, Nigeria

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Field experiments were carried out between August to December 2006 and May to September 2007 on the Teaching and Research Farm of the University of Agriculture, Abeokuta, Nigeria to evaluate the effects of compost amendments and tillage methods on incidence and severity of bacterial spot (*Xanthomonas axonopodis* pv. *vesicatoria*), speck (*Pseudomonas syringae* pv. *tomato*), wilt (*Ralstonia solanacearum*) and yield of two tomato varieties (Beske and UC82B). The experiment was laid out in a randomised complete block design in a split –split plot arrangement with three replications. Bacterial wilt occurred more in unamended (48.9%) than amended compost (19.4%). Incidence of fruit spot and speck were significantly lower ($p<0.05$) in tomatoes planted on amended compost (17.55% for spot and 19.80% for speck) than in unamended compost (46.40% for spot and 37.65% for speck), and no compost (45.80% for spot and 58.95% for speck). Plants grown in amended compost were significantly taller ($P<0.05$), had more leaves per plant, and more flowers per plant than the other two compost applications. Beske also had similar significantly higher growth indices than UC82B. Amended compost resulted in significantly higher yield of 21.56 t/ha at $P<0.05$, compared with 15.28 t/ha for unamended compost and 8.66 t/ha for zero compost. It was concluded that compost amended with bactericidal plant materials had the potential of reducing disease incidence, and increasing the yield of tomato.

Keywords: Beske, field, speck, spot, UC82B, wilt

O2.3 Searching for soil fertilizing microorganisms

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Plants require inorganic compounds for growth, which are supplied by natural or artificial fertilizers; their use is often harmful, leading to soil and water toxicity. It is known that microorganisms have an important role in mineral and nutrient chemical modifications occurring in the soil. Understanding the role of microorganisms on those transformations represents an important line of research towards achieving sustainable and productive crop systems. Here we report on the isolation and characterization of bacteria recovered from soils in Alentejo (Portugal), where during the warmer seasons the superficial soil temperature may vary from 25ºC to 55ºC. These bacteria have an optimal growth between 50ºC-60ºC and possess the ability to produce ammonium and/or to mobilize the organic sulfur as sulfate. The levels of sulfate and ammonium produced vary among the bacterial isolates. Their potential use as soil fertilizers in important crops in Alentejo, grown in abiotic stress, is being evaluated.

Keywords: bacteria isolation, sulfate and ammonium production

O2.4 Reprogramming the viral disease resistance gene *N* from tobacco

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The dominant resistance gene *N* from *Nicotiana glutinosa* confers a hypersensitive response (HR) to plants infected by tobacco mosaic virus (TMV). The helicase domain of TMV is sufficient for induction of an HR. The D-element from the parsley PR2 promoter is known to be induced strongly by bacterial and fungal pathogens. To reprogram the viral disease resistance gene *N* toward a resistance response against bacteria and fungi, the D-element has been linked as a tetramer sequence to a gene encoding the helicase domain of TMV. *Agrobacterium tumefaciens* harbouring a T-DNA vector with this construct were used for *Agrobacterium* infiltration of tobacco plants harbouring the *N* gene. An *Agrobacterium* mediated HR is elicited at the infection site. The promoter-helicase construct was then stably transformed into tobacco plants void of *N*. Offspring of crosses between transgenic lines harbouring the helicase expressing constructs and plants harbouring *N* were analysed (4D-Heli/N). Offspring survived under sterile conditions and showed no visible phenotype after transfer to soil. This shows the high pathogen specifity of the 4D-element and no or low background activity. 4D-Heli/N plants showed an HR after infiltration with *Agrobacterium*, which indicates a successful reprogramming of the *N* gene. An HR could also be observed after Infection with the important tobacco pathogen *Pseudomonas syringae* pv. *tabaci*. The observations highlight the possibility to use the reprogrammed *N* gene as a new versatile disease resistance system.

Keywords: *Agrobacterium tumefaciens*, disease resistance, D-element, hypersensitive reaction, *Pseudomonas syringae* pv. *tabaci*, TMV-helicase

O2.5 Exploring feasibility and reliability of rating for resistance to *Sugarcane yellow leaf virus* (ScYLV) in a core collection of sugarcane.

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Yellow leaf is a worldwide distributed aphid vectored sugarcane disease caused by ScYLV. However, only little information is available on sugarcane resistance to yellow leaf. The objective of the study was to evaluate the feasibility of rating yellow leaf resistance in a naturally infected core collection of 200 sugarcane clones, which will in parallel be subjected to molecular characterization in a perspective to tag alleles linked to useful agronomical traits. ScYLV diagnosis is usually made by RT-PCR or tissue blot immuno-assay of leaves. However yellow leaf is a systemic disease and the ScYLV will circulate within the vascular bundles in the whole plant. We tested therefore tissue blots from basal leave midribs and from one cm core section of bottom stalk to estimate ScYLV incidence and virus titer in leaf and stalk in the 200 varieties. Samples were collected from a three randomized complete block design during two crop cycles. Experimental level broad sense heritability of virus incidence and virus titer, ranged from 0.80 to 0.94 for both crops. This indicates a good control of environmental error in the assessment of yellow leaf resistance. Significant correlations were observed between crop cycles (0.69-0.84 range) even if contamination increased between cycles. Diagnosis obtained from leaf increased the number of varieties in susceptible classes compared to stalk sections. Moreover stalk imprints seem to give a larger segregation on virus titer. Reliability of the collected data needs to be refined. These phenotypic data will be used to look for yellow leaf resistance alleles in sugarcane genome.

P2.1 Classification and genetic diversity of *Rhizoctonia solani* populations causing damping-off of cotton in Iran

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Isolates of *Rhizoctonia solani* were obtained from cotton seedlings showing damping-off symptoms in Iran during 2007–2009. Characterization of various taxonomic groups was done using species-specific primers designed for conserved regions of ribosomal DNA internal transcribed spacer (rDNA-ITS) sequence and restriction fragment length polymorphism analysis of PCR-amplified rDNA-ITS region. Results revealed that from 168 isolates, 84 were AG4 HG-I, 35 were AG4 HG-II, and 49 were AG4 HG-III. All of the isolates were pathogenic on cotton and caused damping-off. Amplified fragment length polymorphism (AFLP) analyzes were used to investigate genetic structure of the pathogen populations collected from various geographic regions in different years. Cluster analysis using different methods and principal co-ordinate analysis (PCO), based on the AFLP data from 489 monomorphic and polymorphic bands generated with seven primer combinations, was performed. This revealed four separate AFLP groups among a total of 168 isolates, which typically showed more than 86% fingerprint similarity. Isolates of the three different intraspecific groups of *R. solani* AG4 were clearly separated in the dendrogram obtained from AFLP data. Within each AFLP group, two or more haplotypes were detected with a genetic similarity of 100%. Analysis of Molecular Variance (AMOVA) revealed that geographic region was the dominant factor determining genetic structure of *R. solani* AG4 populations, but year of sampling had no significant effect.

Keywords: AFLP, rDNA-ITS, seedling, taxonomic groups, *Thanatephorus cucumeris*, variability.

P2.2 Barley Leaf Diseases and Breeding Strategies

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The use of disease resistant varieties is a basic tool for eliminating the quantitative and qualitative damages caused by leaf diseases, which are the most important harmful factors in barley cultivation. Breeding for resistance is concentrated on powdery mildew (*Blumeria graminis f.s.p hordei*), net blotch (*Pyrenophora teres*), leaf rust (*Puccinia hordei*), leaf scald (*Rhynchosporium secalis*) and ramularia leaf spot (*Ramularia collo-cygni*), which is a new disease of barley in the Slovak Republic. The majority of newly released varieties have a high level of resistance to powdery mildew based on mlo gene. In advanced breeding lines sources of resistance from *Hordeum vulgare* subsp. *spontaneum* are extensively used as new allelles of the *Mla* locus, *Mli* and *Mlj* with aim to combine two fully effective resistances. At present breeding for resistance to ramularia leaf spot is at the stage of evaluating the resistance of selected genotypes of barley under field conditions and PCR analyses of diseased leaves. Sources of resistance from *Hordeum vulgare* subsp. *spontaneum* and *Hordeum vulgare* subsp. *agriocrithon* are used as parental stocks for interspecific hybridization with the aim of preparing prototypes that exhibit high resistance to powdery mildew, net blotch, powdery mildew and leaf rust. A variety with a high level of field resistance to leaf scald has been released.

Keywords: *Hordeum vulgare* subsp. *spontaneum,* resistance, variety

P2.3 Evaluation of resistance inducers for the protection of cocoa seedlings against *Moniliophthora perniciosa*

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The efficacy of resistance inducers as alternative products to control cocoa (*Theobroma cacao*) witches' broom caused by *Moniliophthora perniciosa* was evaluated under glasshouse conditions. The following products were tested: Salicylic acid (250 ppm), Methyl-jasmonate (250 ppm), Chitosan (5000 ppm), Rocksil (5000 ppm) and an antimycotic product (250 ppm), composed by salycilic acid and benzoic acid obtained from a pharmacy. Seeds of cocoa a cultivar susceptible to witches' broom disease were peeled and placed on wet blotting paper in a seed germination tray. The germinated seeds were, then, planted singly in conical tubes containing soil and kept in glasshouse. When the seedlings were 40 days old, they were sprayed individually to run off with the products or with water as control eight days before inoculation with basidiospore suspension (2 x 10^5 spores/ml) of the pathogen. Aliquots of 30 μl of spore suspension were placed on the apical buds of the plants. The treatments were arranged in a randomized block design with three replicates of 10 seedlings each/treatment. Forty-five days after inoculation the incidence of the disease was evaluated using Tukey test. Results showed that all the products reduced the disease incidence compared with the uninoculated control. Therefore, the antimycotic solution and chitosan were significantly more effective (P = 0.05) in reducing the number of infected seedlings, indicating a possible effect on the induction of resistance. *In vitro* basidiospore germination was affected only by Rocksil.

Keywords: *Theobroma cacao*, witches' broom disease, control, resistance.

P2.4 Effects of *Trichoderma* isolates and hormonal elicitors application on tomato plant resistance against stem canker disease caused by *Botrytis cinerea*

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Tomato stem canker disease caused by *Botrytis cinerea* is one of the most devastating diseases in tomato production in greenhouse. In this study, possible effect of using hormonal elicitor [salicylic acid (SA)] and bioagent [*Trichoderma* species (*T. asperellum, T. harzianum, T. orientalis, T. koningiopsis, T. atroviride, T. ceramicum, T. brevicompactum, T. koningii, T. viride and T. viridescens*)] in host resistance induction against tomato stem canker was investigated. Tomato seeds (Super Strain B variety), grown in greenhouse, were sprayed by conidia suspensions of *Trichoderma* species (1×10^6 CFU/ml) and salicylic acid with three concentrations (100, 200, 400 µM). After 72 hrs the plants were inoculated with *B. cinerea* conidia suspension (1×10^5 CFU/ml) and disease severity index were evaluated 15 days afterward. SA(100 µM) +*T. viride, T. brevicompactum*, and SA(400 µM) treatments selected for determination of ß1,4-glucanase,and chitinase activities and concentration of total phenolic compounds in tomato leaves at four different stages (0, 24, 48 and 96 hours after inoculation). Treatments with SA (100 µM) +*T. viride+ Botrytis cinerea* increased the activities of ß-1,4-glucanase, chitinase and concentration of total phenolic compounds in tomato leaves and the highest level has achieved at 96 hours after inoculation. The results suggest that the combination between bioagent and hormonal elicitor (either synergistically or antagonistically) played an important role for increasing resistance in tomato plants against Stem canker disease caused by *Botrytis cinerea.*

Keywords: Glucanase, salicylic acid, Chitinase, Phenolic compound.

P2.5 Field and *in vitro* screening for resistance to leaf stripe (*Pyrenophora graminea*) of some barley lines under north Algerian conditions

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Leaf stripe is a seed-borne disease of barley, caused by *Pyrenophora graminea*. The disease causes severe yield reduction in Algerian barley cultivation areas. Twenty barley genotypes (12 doubled haploids, 4 genealogic lines and 4 varieties) were evaluated against two isolates of *P. graminea*. The barley genotype reactions were determined under field conditions. Highly significant differences were observed among barley lines. There were also virulence differences between the two isolates. The lines 3/17/2/a and 3/17/2b showed moderate resistance to the two isolates. These results were confirmed by an *in vitro* test in the laboratory. Data from the field and the *in vitro* test were significantly correlated (r= 0.95, P≤ 0.05). These genotypes can be used as parents in barley breeding programmes against *P. graminea*.

Keywords: double haploids, genealogic lines, *Hordeum vulgare*, genetic resistance, selection.

P2.6 Indian coffee selections with resistance to Coffee Berry Disease

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Coffee is one of the most valuable primary products in world trade being crucial to the economies of many developing countries. Its production is, however constrained by a number of major diseases, including Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae*. This disease may destroy up to 80% of the coffee berries if no control measures are taken. Presently, CBD is confined to Africa and poses a serious threat if it spreads to coffee growing countries of other continents. Selection for resistance to CBD has been based either on seedling inoculation method (pre-selection test) or field expression of resistance on mature trees. At the Coffee Rust Research Center (CIFC) in Portugal, search for new sources of resistance to CBD pathogen has been a focused activity. In the present paper we report the potential of some coffee genotypes from India as sources of resistance to CBD. Some of the interspecific derivatives of Indian origin such as S. 795, Sln 11, Sln 5A and Sln 6 exhibit different levels of resistance against *C. kahawae* isolates from Kenya, Zimbabwe and Cameroon, as compared to the susceptible coffee cultivar Catimor 45. Histological studies of S. 795 and Sln 11 are currently under progress aiming a better characterization of the expression of resistance of these genotypes to *C. kahawae*. The selections Sln 5A and Sln 6 are already under field evaluation in four African countries as part of multi-country programme supported by ICO-CFC (project nº. CFC/ICO/40).

Keywords: *Colletotrichum kahawae*, resistance breeding, CBD

P2.7 Screening tests for evaluation of susceptibility to *Phytophthora cinnamomi* of hybrid clones of *Castanea* species

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Early detection of resistance to *Phytophthora cinnamomi* using non-destructive tests is the main goal of every selection program. Under a breeding program initiated in 2006 for introgression of resistance genes from Asian genotypes (*C. crenata* and *C. mollissima*) into *C. sativa,* the detection of resistance to *Phytophthora cinnamomi* was evaluated to perform genotype/phenotype association studies in the future. In 2006 and 2009, two full-sib progenies were obtained from two crosses, SC (*C. sativa* x *C. crenata*) and SM (*C. sativa* x *C. mollissima*). Four year old hybrid clones (2006 progeny) were used with two types of tests for detection of resistance to *P. cinnamomi* and evaluation of the metrics of resistance, to be used later on for the association studies: whole plant root inoculation and detached leaves. The correlation obtained between the root inoculation and detached leaves tests was not consistent, as well as the results obtained in detached leaf test were not reproducible, suggesting that this test is not suitable for early detection of susceptibility/resistance to *P. cinnamomi*. On the contrary, a good correlation was obtained for the assays done *in vivo:* stem and leaf inoculation in the hybrids of the 2009 cross, suggesting that these may be considered good tests for the evaluation of the metrics of resistance to *P. cinnamomi*. The confirmation of susceptibility/resistance in whole plants, obtained from rooted cuttings from each clone, should be performed in the future for both crosses.

Keywords: Resistance tests, *Castanea* hybrids, *Phytophthora cinnamomi*

KN3.1 Targeting carbohydrates: a novel paradigm for pathogen control

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It is now clear that pathogen control in crops will assume an ever increasing importance in human and animal nutrition. Several major global variables must be taken into account: there is a continuous decrease in the global area of arable land, but a fast increment in world population. When considered independently, these two variables will inevitably lead to a critical point.
Other variables further complicate the present scenario. Thus, the already great but still increasing pressure of public concern over the widespread application of chemical fungicides, the tightening legislation on toxicity over commercially available and newly discovered pesticides, and the development of pathogen resistance against some top pesticides have led some prominent companies in the field to abandon the extremely low cost effective development of new chemical fungicides. The door is definitely open for biological control, with the search based, for the most part, on the molecular interactions between host and pathogen.

Carbohydrates play an essential and direct role in cell-cell recognition, in the distinction between self from non-self, and in the warfare that takes place between host and pathogen before infection is established. After the nucleotide (genetic) code for nucleic acids and the amino acid code for proteins, the sugar code is emerging as the third life code, whose coding capacity greatly exceeds those of the other two codes.
Take fungal pathogens as an example. Their cells are surrounded by a wall, typically composed of polysaccharides such as chitin and β-1,3-glucans. Immediately underneath, the plasma membrane protects the cytoplasm from the extracellular milieu. It contains many *N*-linked glycoproteins, *O*-linked glycoproteins and glycolipids, whose carbohydrate moieties, collectively termed the exoglycome, are projected towards the cell exterior. Thus, to avoid recognition by the plant “immune

system", some pathogens are likely to alter selectively their exoglycome to establish pathogenesis. Such precise alterations may prove extremely useful both in diagnosis and treatment, allowing the development of some of the tools described below for their specific recognition. Such alterations are certainly difficult to detect since many human and plant fungal diseases are diagnosed after *in vitro* incubation, for many days, of samples collected from host infected tissues. This host-free microbial growth will certainly return the exoglycome to its original, host independent, state.

Based on the same principle, a far simpler solution is used by several fungal species to elude and circumvent the host defences. During invasive growth of biotrophic rust fungi, chitin is exclusively present in the cell walls of exterior infection structures, i.e. germ tubes and appressoria. To avoid the degradative action of plant chitinases, instead of this elicitor-active molecule, the hyphae that grow within host leaves contain chitosan, a deacetylation product of chitin supposedly generated by the enzymatic activity of a differentiation-induced fungal-chitin deacetylase: the wolf intrudes in sheep's clothing.

A number of potential applications will be presented, such as (i) the identification of targets in the fungal exoglycome, (ii) the expression of xylem-directed lectins, (iii) the expression of plantibodies in plant tissues, specific for any PAMP or specific feature exhibited by one or a group of pathogens, and (iv) Blad, an edible 20 kDa polypeptide which targets and destroys chitin in fungal cells. This polypeptide exhibits very high efficacies to all fungi tested so far (>40), which are equal to or greater that those presented by the top chemical fungicides commercially available.

Keywords: Blad, exoglycome, fungus, lectin, plantibody

KN3.2 Trapping nematodes with *Solanum sisymbriifolium*

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Potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are a serious problem in potato growing areas. Measures to control PCN were incorporated into several Council Directives including one from 2007 (2007/33/CE). Those directives stipulate that seed potatoes must be grown on land that has been surveyed and found free of PCN. Several measures are available to control PCN: crop rotations; resistant cultivars; trap crops; antagonistic plants; GM potato plants and soil disinfestations with agrochemicals. Crop rotations alone are not effective with the current commercial frequency of potato cropping. Resistance has become ineffective due to selection of new pathotypes/virulence groups by repeated use of (partially) resistant cultivars, and nematicides often fail to prevent the increase of PCN infestations. The use of chemicals is effective only when PCN infestation levels are low and less effective when *G. pallida* is present. Despite the use of all those control measures and Integrated Management Systems, the land infested with PCN has increased. There is a need for other control measures to be developed. Such a measure could be the growth of an effective trap crop that stimulates hatching of second-stage juveniles from cysts. This may decrease considerably the level of soil infestations, destroying the trap crop before the reproduction of PCN, or if the trap crop is resistant to PCN. After extensive screening, a resistant trap crop that produces a high level of hatching agents, *Solanum sisymbriifolium*, seemed an ideal control method. It reduces PCN populations by 80% or more being effective on a commercial scale on a range of soil types. This plant stimulates hatching but is totally resistant to PCN avoiding the risks referred. The introduction of a trap crop needs very careful management especially when it is not a native plant. There is also the necessity to study the plant behavior against other plant diseases in order to know if the plant could become a weed of potato and could act as a reservoir of existing or new pests or plant pathogens. Till now no problems with potato diseases were found in this crop. Recently, root-knot nematodes (RKN) have been found coexisting with PCN in some potato fields, in Portugal. The presence of *Meloidogyne chitwoodi*, a species with quarantine status, was detected and may be an additional problem for farmers and potato growers. This species can cause severe tuber damage that makes

tubers unsuitable for consumption or processing and also affects potato production. In view of the aggressiveness demonstrated by other RKN species, *M. hispanica*, to potato, management of this species should also be considered. It is important to avoid the dissemination of RKN through the potato growing areas of the country. Special care should be taken into account regarding seed potato production. A trap crop that can reduce RKN densities as well as PCN could be an alternative to other methods.

O3.1 Novel peptide elicitor of plant defence responses derived from collagenous waste

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Plants are able to recognize almost all pathogens and defend themselves against the attack. However in a few exceptions the pathogen can disturb plant defence signalling by various effectors that results in plant infection and disease. Resistance to such pathogens can be achieved by pre-treatment with elicitors, which are specifically recognized by the plant and trigger defence responses. Elicitors are various molecules originating from the pathogen or from its activity. We discovered that treatment of oilseed rape plants *(Brassica napus)* with hydrolysed collagen triggers defence responses associated with the plant hormone salicylic acid (SA) and induces resistance against *Leptosphaeria maculans,* the causal agent of blackleg disease. Collagen is a common waste from the tanning industry and slaughter houses and its hydrolysates are already used in agriculture as wetting agents. Hydrolysed collagen induces in *B. napus* cotyledons expression of SA-responsive genes *PR-1, WRKY70* and *ICS1* to a level similar to treatment with benzothiadiazole, the known analogue of SA. Pre-treatment of cotyledons with hydrolysed collagen significantly reduces disease symptoms caused by *L. maculans,* but to lesser extent than benzothiadiazole. We expect that the effect is dependent on an average size of peptides derived from the hydrolysis. Therefore improvement of the hydrolysis process would likely further increase efficiency of the elicitor.

Keywords: *Brassica napus*, *Leptosphaeria maculans,* salicylic acid, gene expression, induced resistance

O3.2 Elicitation of phenylalanine ammonia-lyase activity in postharvest apple and tomato leaves by ulvan and glucuronan isolated from *Ulva lactuca*

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For induction of the natural plant defences, two polysaccharides (ulvan and glucuronan) isolated from the green alga *Ulva lactuca* were tested for their elicitor potential in tomato seedlings and postharvest apple. The elicitor capacities were evaluated by the responses of the activity of phenylalanine ammonia-lyase (PAL) a key enzyme of phenylpropanoid pathways. PAL activity is a sensitive indicator of stress conditions and elicitor treatment. It has been related to lignification as it increased synthesis of specific phenolics required in defence. The obtained results show the differential elicitation according to the polysaccharide tested and the vegetable model used. Thus, in tomato leaves, the glucuronan (unsulfated homopolymer) does not have a significant elicitor effect whereas the ulvan (sulfated heteropolymer sulphated) induces an enhanced response higher than that of laminarin recognized by their elicitor effect and used for comparison. In postharvest apple, the glucuronan induced earlier and more important elicitor potential than that of ulvan which the effect is similar to that of the laminarin. The differential response of the PAL activity of the glucuronan and the ulvan in tomato leaves and postharvest apple will be discussed.

Keywords: Elicitor, natural defence, phenylalanine ammonia-lyase, polysaccharides.

O3.3 Assessment of combination effects of Pyraclostrobin and Pyrimorph against *Phytophthora infestans*, employing gene expression analysis and microscopic evaluations

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We have evaluated and analyzed the synergistic effect of the combination of pyraclostrobin + pyrimorph by an *ex vivo* method with an isobole-based statistical model and consequent screening. In this procedure, small pieces of tomato (*Lycopersicon esculentum*) leaf were cut and put into a 48 multiwell plate and zoosporangia of *Phytophthora infestans* and fungicides were applied to the leaf surface according to a special designed layout. Evaluation of the infected area was performed visually and expressed in percentage of leaf disc area. Regression probit model was used for establishing ED_{50} values for each mixture. Validity of synergism of two fungicides in mixtures was determined by calculating Loewe factor and isobolographic analysis. The highest synergistic effect found was for pyraclostrobin + pyrimorph at a concentration of 1 ppm+0.25 *ppm*, respectively. In addition, we profiled the transcriptional response of *P. infestans* to both solo compounds and also to the combination by quantitative reverse transcription PCR (RT-PCR). The analyzed genes were those for the target of pyraclostrobin (cytochrome *b*) and for pyrimorph (cellulose synthase A3). Total RNA of the mycelia were extracted, cDNA was synthesized and gene expression quantified by real-time PCR with normalization to α-tubulin. A comparison of the gene expression of mycelium grown in fungicide free medium and mycelium grown in medium amended with sublethal doses of pyraclostrobin, pyrimorph or pyraclostrobin + pyrimorph indicate that fungicide treatment did not induce significant changes in the expression of the target genes. Microscopical studies indicated that no morphological changes in the mycelium were induced by the test compounds. However, effects were observed on the motility of zoospores.

Keywords: fungicides, Synergism, isobolographic analysis, Molecular analysis

O3.4 Control of foliar diseases of barley using a combination of resistance elicitors

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A cocktail of the resistance elicitors: *cis*-jasmone, β-aminobutyric acid (BABA) and acibenzolar-S-methyl (ASM) was examined for its ability to induce resistance in barley to foliar pathogens in field studies over two seasons. Treatment with the elicitor combination increased expression of *Pr1b*, a marker gene for systemic acquired resistance, but led to greatly reduced expression of *LOX2,* used as a marker for the jasmonic acid signalling pathway. Plants treated with the elicitor combination also exhibited elevated activities of the defence-related enzymes, peroxidase, β-1,3-glucanase, and cinnamyl alcohol dehydrogenase. Although disease control under glasshouse conditions was good, in the field the efficacy of the elicitor cocktail on its own was poor and variable, depending on both barley variety and season. Greater levels of disease control in the field were obtained using a combination of elicitor and fungicide. However, although a mixture of elicitor and fungicide applied together gave reasonable levels of disease control, most consistent disease control was obtained by treating plants with the elicitor combination at GS24, followed by reduced rate fungicide at GS31 and GS39. Despite the moderate levels of disease control, most elicitor and elicitor + fungicide treatments resulted in increased grain yields.

Keywords: acibenzolar-S-methyl, *cis*-jasmone, β-aminobutyric acid, systemic acquired resistance

O3.5 Effects on yield and disease of interactions between barley cultivars and with soil tillage treatments

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Mixtures of four winter barley cultivars together with their component monocultures were trialled under five tillage techniques (zero and minimum tillage, plough, deep plough, and plough with compaction). The mixtures gave yield increases and disease reduction but there was no interaction with treatments, demonstrating their practicality in a wide range of soil tillage practices. Subsequently, 120 cultivars were trialled on the same tillage plots to detect any differential adaptation to the tillage treatments. Cultivars with contrasting tillage interactions were trialled again to determine the traits responsible for these performance differences. We also determine whether it is necessary to mix cultivars homogeneously to obtain these disease reductions and yield increases.

Keywords: blends, soil disturbance, roots

O3.6 Production of a postharvest biocontrol agent with food industry by-products

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Postharvest biocontrol by means of microorganisms has been developed as an alternative to postharvest fungicides, with the objective to reduce fungicidal residues on fruits and the development of resistant fungal population. A strain of the yeast in the genera *Metschnikowia* isolated from the surface of 'Bravo de Esmolfe' apple was effective in the control of the main postharvest diseases of pome and citrus fruit. Application of biocontrol agents, as an alternative to synthetic fungicides, is quite dependent on its mass production and of the industrial development of a culture medium that can produce effective and high amounts of biomass at low cost Cost of the medium is one of the major operational costs, so the use of cheap and widely available raw materials is an economical requisite. Considerable interest has been shown in the use of agricultural product wastes, and by-products from food industry as nitrogen or carbon sources. The aim of this work was to find wastes or/and by-products from food industry as carbon source that provide maximum biomass production of our biocontrol agent ensuring that it maintained its antagonistic activity against postharvest pathogens of fruits. Different food by-products were tested under different production and scale conditions. A concentration of 10^9 cfu/ml was achieved after 42 h of fermentation with several food by-products, and the higher production and best biocontrol activity was obtained when the yeast was grown on a by-product of the citrus industry.

Keywords: biological control, carbon source, growth, fermentation

P3.1 In vitro screening of bacterial strains isolated from soil for inhibiting effect against phytopathogenic fungi

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Forty-nine bacterial strains isolated from soil were screened in vitro for antagonism of 12 phytopathogenic fungi. About 51% of the bacterial strains were antagonistic against at least one fungus. A strain of *Pseudomonas fluorescens* wasd antagonistic against all the phytopathogenic fungi tested. Strains of *Pseudomonas fluorescens* and *B. subtilis* exhibited a very broad spectrum of action and showed an efficient antagonistic activity against the phytopathogenic fungi strains. *Pseudomonas. fluorescens* induced a 40 mm diameter zone of inhibition against *Sclerotinia sclerotiorum* while *B. subtilis* caused a 45 mm diameter zone of inhibition of *Phytophthora infestans*. Tests with the more antagonistic bacterial strains showed that the bacterial suspension exhibits a stronger inhibitory activity than the bacterial extract. The results show that certain bacterial strains isolated from soil have a pronounced antagonistic activity against a number of phytopathogenic fungi. They exhibit a high antifungal activity, which is due either to the production of very broad-spectrum, non-specific biocides, or to several substances with different potencies.

Keywords: Antagonistic activity, Bacteria, Phytopathogenic fungi

P3.2 Effects of insecticide applications on soil nematode communities: a trait-based approach

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Ecological Risk Assessment, based on ecological/functional traits, is a recent concept and the few data available focus on the aquatic compartment. With the application of this novel approach to soil ecosystems, new perspectives on the evaluation of the toxic potential of chemicals can be achieved. The aim of this work is to investigate the effects of carbofuran sprayings on the abundance and composition of a Mediterranean soil nematode community. These organisms have been widely used as indicators of chemical/ecological stress, are highly abundant and diverse in soils and can be classified according to their feeding habits (a soft life-trait with strong functional implications). Several soil cores were obtained from an uncontaminated agricultural field in Carapinheira (Coimbra, Portugal), mixed and sieved. Part of the soil was defaunated (freeze-heating cycles), while the remaining was used to extract the nematodes community (tray method). The defaunated soil was spiked with four doses of a carbofuran commercial formulation and each of the six replicates was inoculated with a nematode suspension containing ~300 nematodes. Samples from this initial community were obtained and the nematodes were classified into different feeding-groups. After 14 and 28 days of exposure, the nematodes were again extracted, counted and classified. Results showed that nematode abundance was affected only at the highest carbofuran dosages, but important changes occurred in the composition of the feeding groups. Pros and cons of this approach and its implications for a better understanding of response trends towards chemical stressors in soil invertebrate communities are discussed.

Keywords: carbofuran, ecological risk assessment, ecotoxicology, life traits, pesticides.

P3.3 Hatching effects of root exudates from *Solanum sisymbriifolium* on *Meloidogyne* spp.

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Root-knot nematodes (*Meloidogyne* spp.), the most widely distributed and economically damaging group of plant-parasitic nematodes, affect the yield and quality of crops. Nowadays, agricultural practices aiming to reduce the use of pesticides in order to ensure food quality and measures to protect the environment are being developed. Some plants release compounds through the roots which may be used against plant parasitic nematodes. These exudates may act as stimulants or inhibitors of hatching nematode juveniles and/or affect the penetration and development of nematodes. Several studies have shown that many Solanaceae have the ability to produce compounds which act as hatching promoters, of great interest to control nematodes in the field. The main goal of this research is to evaluate the hatching effects of root exudates from *S. sisiymbriifolium* cvs. Pion, Sis 4004, Sharp and Domino and *S. lycopersicum* cv. Easypeel on *M. javanica*, *M. arenaria* and *M. hapla*. Eggs containing second-stage juveniles (J2) (five replicates/plant cv./nematode isolate) were placed, in the dark, at 20ºC, in 2ml exudate obtained by successive root leaching. Hatched J2 were counted daily for a period of 15 days. Tomato plant exudates and distilled water were used as controls. Preliminary results indicate that all exudates did not affect the hatching of *M. arenaria* or *M. javanica* isolates, but the cv. Sharp exudates showed, in the first 5 days, an inhibitory effect on hatching of *M. hapla*. The present work will contribute for the development of an alternative and sustainable strategy to pesticide application against *Meloidogyne* spp.

Keywords: control measures, integrated pest management, nematicides, phytochemicals, root-knot-nematodes, trap crops

P3.4 *In Vitro P*roduction of *Plum Pox Virus* - Free *Prunus Domestica* L. by Combination of Chemotherapy and Thermotherapy

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Plum pox virus is the most harmful virus infecting stone-fruits in the whole Europe. It is the imperative within the European Union, that all the planting material of fruit trees must possess a health certificate. Hence, it is necessary to use only virus-free mother plants for multiplying. The plants of *Prunus domestica* L. cv. Domácí švestka infected by *Plum pox virus* were used for aseptic *in vitro* culture. This culture was sanitated by combination of chemotherapy (Ribavirin® concentration 10, 20 or 40 mg/L) and thermotherapy (at 34 or 37 °C). After 2, 4, 6 or 8 weeks of the treatment various parts of explantants were transferred onto Ribavirin®-free medium and cultivated under common conditions. Subsequently, tests for the presence of *Plum pox virus* (DAS -ELISA a RT-PCR) were carried out. It has been established, that the optimal time for 2 mm apex removal was after 4-6 weeks of treatment. Prolonged thermotherapy resulted in shoots decay. Preliminary testing confirmed that treatment was successful. Re-testing will be carried out in five months. In conclusion, the combination of chemotherapy and thermotherapy seems as a feasible method for sanitation *Prunus domestica L.* cv. Domácí švestka, and virus-free plant production.

Keywords: Ribavirin®, sanitation, virology, i*n-vitro*

P3.5 Nematicidal potential of *Solanum nigrum* extracts against *Pratylenchus goodeyi*

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Pratylenchus goodeyi, the root-lesion nematode, is a banana plant parasite common in Madeira Island (Portugal) that affects the crop, with economical consequences. In order to find less aggressive solutions to the environment and to Man, *Solanum nigrum* and *S. sisymbriifolium* plants are being studied to identify their nematicidal substances against *P. goodeyi*. *S. nigrum* and *S. sisymbriifolium* dried plants were sequentially extracted by dichloromethane, acetone, ethanol and water solvents. Water extractives through cold and hot extractions, from dry and fresh plants, were also studied. To evaluate their effects on *P. goodeyi* mobility and mortality, extract solutions were assayed at a concentration of 10mg/ml. The mortality of *P. goodeyi* was highly significant in all extracts, acetone with water extracts being the the most effective. Water extracts from dry and fresh plants showed the same efficiency on *P. goodeyi* mobility and mortality as the solvent sequence water extracts. The acetone extract of *S. nigrum* showed the greatest effect on mortality and mobility against *P. goodeyi*. Nematode mobility was impaired after the first hour of exposure and reached absolute mortality after 24 hours. According to the results, nematotoxic and nematicidal substances were obtained with acetone from *S. nigrum* plants. Thus, *S. nigrum* has potential as a natural and environmentally friendly nematicide to control the root-lesion nematode *P. goodeyi*. Additional studies are underway in order to identify the active components present in the acetone extract.

Keywords: *Pratylenchus goodeyi*; *Solanum nigrum*; acetone extracts

P3.6 Nematicidal activity of carvone against the root-knot nematode *Meloidogyne javanica*

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Root-knot nematodes, *Meloidogyne* spp., are among the most damaging plant-parasitic nematodes causing significant losses to a wide range of plant species. The demand for alternative strategies for nematode control, such as non-chemical, natural and environmentally-friendly nematicides has been increasing. Carvone, the main compound of the essential oil of caraway (*Carum carvi* L.) fruit, was registered by the European Commission to be used as food additive and is present in the "Everything Added to Food in the United States as Generally Recognised As Safe" (EAFUS) database. The aim of this work was to assess the effects of carvone on *Meloidogyne javanica* second-stage juveniles. *In vitro* experiments were carried out in glass blocks, in the dark, at room temperature with different carvone concentrations (10, 100, 2000 ppm and pure carvone). Each treatment consisted of five replicates of 15 juveniles in 0.5 mL of each carvone concentration, distilled water served as control. Nematodes were observed at 2, 18 and every 24 hours, up to 168 hours. Data were submitted to ANOVA analysis. A nematostatic effect was observed after 18 hours, in 100 ppm carvone concentration. One-hundred-percent mortality was achieved in pure carvone after 15 minutes of exposure. The nematostatic and nematicidal effects of carvone on juveniles suggest that this non-toxic phytochemical may be a promising alternative to chemical nematicides. Further experiments should be conducted to evaluate the activity of this compound on hatching and plant infectivity.

Keywords: Biocide, Monoterpene; Nematode control

P3.7 Impact of *Phytophthora pseudosyringae* on Cannock Chase, Staffordshire

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In early 2009 *Phytophthora pseudosyringae* was identified on *Vaccinium myrtillis* in the Cannock Chase area of Staffordshire, UK. Other heathland species have been tested for the infection, however only *Vaccinium myrtillis* has given positive results. The Cannock Chase infection was initially thought to be *Phytophthora ramorum*. However, the pathogen was subsequently identified by the Food and Environment Research Agency (FERA as *Phytophthora pseudosyringae*. In January 2009 the *P. pseudosyringae* infection was confined to the Brocton Coppice area of the Chase and northern parts of the Country Park. Rapid action was taken in an attempt to stem the spread. A programme of cutting and burning of the infected material was implemented and a monitoring programme was also started. By mid-year the infection had spread to the open heath. The system of cutting and burning of infected material does not appear to have been an effective control method as the infection has spread rapidly. Much of Cannock Chase is designated as a Special Area of Conservation and Site of Special Scientific Interest. *Vaccinium myrtillis* is a key component of the heathland community and is currently under threat. A research programme has been developed to determine a suitable means to control the infection. This programme will consider four different methods of control, namely fungicides, herbicides, cutting and removal of infected material, and burning *in situ*. There are also control plots where no treatment is taking place. Research into *Phytophthora pseudosyringae* at Cannock Chase is continuing.

Keywords: *Phytophthora pseudosyringae, Vaccinium myrtillis,* heathland, treatment, Cannock Chase.

P3.8 Biological control of Fusarium yellow wilt in common bean with *Trichoderma harzianum*

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Fungi of the genus *Trichoderma* have been investigated as biological control agents for several plant pathogens. In this study, Brazilian isolates of *Trichoderma harzianum* (CEN287, CEN288, CEN289, CEN290 and CEN316) belonging to Embrapa's biocontrol agent culture collection were tested against the causal agent of yellow wilt (*Fusarium oxysporum* f. sp. *phaseoli*) in common bean. The experiment was carried out in plots artificially infested with the pathogen. Seeds were sown in treated furrows (1.2 x 10^{12} conidia of *Trichoderma* ha^{-1}). Each treatment was comprised of four plots in a randomized block arrangement. Besides *Trichoderma* isolates, there was a negative control (without *Trichoderma* applications) and two positive controls (*T. harzianum* - Trichodermil® SC oil suspension, sprayed in the furrows) and treated seeds (Vitavax®-Thiram at 300 mL 100 kg^{-1} seeds). After 68 days, wilt symptoms were evaluated according to a scale (1 – no visible symptoms; 3 – approximately 10% of the total foliage exhibiting wilt and chlorosis; 5 - approximately 25%; 7 - approximately 50%; 9 - approximately 75% of defoliation and plant death). Total production was harvested when 85 days old. Based on Fusarium wilt and chlorosis symptoms, four isolates out of five represented the best treatments, along with Trichodermil®. The negative control showed the highest value of disease severity. Therefore, these four isolates can be considered in future biological control programs. Bean production was similar among the treatments (averages ranged between 2,878 and 3,664 kg ha^{-1}).

Keywords: biological furrow treatment, biological control, environment.

P3.9 Effects of wild plant essential oils on the growth of *Phytophthora cinnamomi* and *Castanea sativa*

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In regions that have chestnut as the main economic resource such as in Bragança, in the North of Portugal, chestnut ink disease caused by *Phytophthora cinnamomi* causes major economic losses. The prospect of an active natural product agent has a great appeal, especially if it comes from a wild plant common in the region. Essential oils are natural compounds that can have bactericidal, fungicidal and allelopathic effects, and these characteristics can be an interesting tool to control the development of plant pathogenic agents and prevent infections caused by *P. cinnamomi.* The essential oils were tested in different concentrations from 100% to 2% dilutions in 70% ethanol, on mycelial growth of *P. cinnamomi* after 1, 2, 3, and 4 weeks in pure culture. Since the pathogen can be found in water and can be transmitted through water, their growth was also tested in the presence of filter paper imbibed with the same essential oils and concentrations in liquid medium to determine if the essential oil can affect their development in these conditions. The essential oils from *Mentha pulegioides*, growing wild in the Northeast region of Portugal were tested also *in vitro* for their effect on the growth of *C. sativa*, to establish that the oils are not phytotoxic. Preliminary results show that essential oils of *Mentha* species at concentrations of less than 80% can reduce and even stop the growth of *P. cinnamomi*, and in concentrations of less than 90% do not affect drastically the development of *C. sativa in vitro*.

Keywords: *Phytophthora cinnamomi*, *Mentha* essential oils, *in vitro* culture

P3.10 Mycoflora and fungicide resistant isolates in citrus packinghouses in the South of Portugal

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Penicillium digitatum and *Penicillium italicum* are the most common postharvest pathogens of citrus growing countries. Conidia are present throughout the season in the atmosphere of citrus growing areas, particularly in packinghouses. The fungus reproduces rapidly and if sanitation measures are inappropriate, inoculum levels can gradually increase. Packinghouse sanitation is an important practice for the reduction of inoculum and the use of postharvest fungicides is the primary means to control these diseases. However continual use of fungicides has led to the development of resistant strains. The aim of this work was to evaluate *P. digitatum* and *P. italicum* populations and to determine the presence of imazalil and thiabendazol-resistant strains in the environment and surface of equipment and facilities in representative packinghouses in Algarve during 5 seasons. Two methods was used: 1. Environmental populations were sampled by a gravimetric method using Petri dishes opened for 2 min; 2. Superficial sampling by printing contact plates Rodac. PDA was used for both methods. The same methods were used to evaluate resistant strains, except that PDA was amended with thiabendazole or imazalil. *Penicillium digitatum* and *P. italicum* were the most widespread species, with higher percentage of *P. digitatum* in all sampled zones of all packinghouses, and in general followed by *Rhizopus* sp. and *Botrytis cinerea*. Fungicide resistant strains were collected from 9 out of 11 packinghouses, and more strains of *P. digitatum* than *P. italicum* were resistant. Resistance to thiabendazole (20% of the sampled strains) was double that of resistance to imazalil, and this increased every season.

Keywords: imazalil, *Penicillium digitatum, Penicillium italicum,* thiabendazole

P3.11 Sulphur application against powdery mildew on grapevines and its effect on soil

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Sulphur (S) is used in vineyards all over the world against powdery mildew. In the Bairrada Region a field trial with grapevine var. Bical was conducted throughout a growing season, following integrated plant disease management programs, to evaluate the effect of the application of different formulations and concentrations of sulphur, namely in the total and available content of S on soil and on pH. Two formulations were used in different concentrations: dust at 30–50kg/ha (E); micronised wettable granules at 4kg/ha (MB), at 8kg/ha (MM) and at 12,5kg/ha (MA). The samples were collected at "berry touch" and "berry ripe for harvest" at two different positions (rows and between rows) and at two soil depths (0–10cm and 10–30cm). S content and pH values in eight samples for each modality (formulation × concentration × row/between row) were measured in the laboratory. Data were analyzed by comparing averages for the different modalities with the control. Results showed higher values of total S in modality MA at the 0–10cm depth at berry touch in samples collected on grapevine rows, but this did not differ significantly from the control. The values of available sulphur were significantly higher in all modalities (when compared to the control) at berry ripe for harvest on grapevine rows at 0–10cm depth. No significant differences were observed in the 10–30cm depth. pH values did not change significantly and ranged from 5.5 to 6.2. The application of sulphur against powdery mildew of grapevine can increase the available S in soil without contributing to soil acidification in vineyard.

Keywords: concentration, depths, formulations, IPDM, pH, *Vitis vinifera*

P3.12 Combined use of real-time PCR and cytological analysis to study epidemics of *Mycosphaerella graminicola* in wheat

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The accuracy of quantitative real-time polymerase chain reaction (qPCR) to study epidemics of *Mycosphaerella graminicola* (Septoria) leaf blotch in wheat was evaluated. Under field conditions, the copy number of the *M. graminicola* β-tubulin-specific gene was measured using qPCR in spore traps of air samples and in the flag leaf (F1), and the second (F2) and third (F3) leaves below it, of five wheat cultivars. The effect of wheat cultivar on the distribution of sterol 14 α-demethylation inhibitors (DMIs)-resistant genotypes of *M. graminicola* was studied using allele-specific qPCR targeted mutations in the *CYP51* gene. Three-week-old plantlets were used to study the wheat infection process and the efficiency of prothioconazole, under controlled conditions. Results of ascospore traps showed late spore arrival, ranging from 30 to 3000 spores/day in the period April–May, which increased the amount of *M. graminicola* DNA in the upper leaf layers (F1 and F2). Distribution of DMI-resistant populations of *M. graminicola* was not affected by different wheat cultivars. Close correlations were obtained between qPCR analysis and leaf colonization stages as well as with visual observations when the leaf had less than 40% necrotic area. Cultivar resistance determined by qPCR correlated well with the susceptibility rating given by the ARVALIS-Institut du végétal. Direct penetration of leaf tissue was confirmed by electron microscopy and, coupled with qPCR results, prothioconazole showed a significant inhibitive effect against spore germination and postgermination. We concluded that qPCR is an accurate and specific quantitative method for studying plant disease epidemics.

Keywords: Epidemiology; DMI-resistant genotypes; fungicide efficiency; *Mycosphaerella graminicola*, real-time PCR; resistant cultivars.

P3.13 Production of bioactive compounds against wood contaminant fungi: Partial characterization by LC-MS with electrospray ionisation

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The alarming problem of some fungal diseases in forest systems makes the discovery of new compounds an urgent task. The integration of various disease control strategies, including biological control, should be considered to improve the efficacy of control and reduce fungicide levels in the environment. *Bacillus* species produce a wide variety of metabolites with interesting biological activities. Several strains of *Bacillus subtilis* and *Bacillus amyloquefaciens* produce cyclic peptide antibiotics including iturinic compounds, which present a strong antifungal activity. Previous studies developed in our laboratory showed that several *Bacillus* strains isolated from *Quercus suber* in Beja region are innovative lipopeptide antibiotics, with antifungal activity against several contaminant fungi of forestry products. In this work, batch cultivations of three *Bacillus* sp. strains (sp.1, sp.2 and sp.3) were carried out and their ability for lipopeptide production was assessed. Two different culture media and growth temperatures were tested to optimize the lipopeptide production by the *Bacillus* strains. In cell free broth cultures it was possible to identify, by LC-ESI-MS, characteristic peak clusters of lipopeptides (between 700-1500 Da). The results showed the presence of lipopeptides in all cultures, but *Bacillus* sp.2 seems to be the most efficient strain to produce this type of compounds. Additionally, MS^2 analyses in SIM mode enabled the quantification of the lipopeptides produced by each strain. The larger chromatographic peak areas were obtained by the *Bacillus* sp.2 strain. Finally, a sporulation study was performed in order to investigate a possible correlation between sporulation and the lipopeptide antibiotics production.

Keywords: Anti-fungal Activity; *Bacillus*; Lipopeptides; Phytopathogenic Fungi; Spore Formation.

P3.14 Growth inhibition 4 fungi species by *in vitro* activity of antagonistic bacteria

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In recent years increasing attention has been paid to the replacement of chemicals with alternative methods of plant protection. Some antagonistic microorganisms, among them bacteria, could play an important role in the control of fungal diseases. In the present work the biotic interaction of *Bacillus subtilis* and *Pseudomonas fluorescens* with 15 selected isolates of cereal pathogens were studied. The fungi were isolated from wheat, barley, rye, triticale and oat. Bacterial isolates were streaked in a line on potato dextrose agar and after 48 hours, a 5 mm disk of mycelium was placed at 4 cm distance. After 4 and 6 days of incubation the inhibition zone between the bacteria and each of the fungus isolate was measured. The results obtained were statistically analysed using the analysis of variance and Tukey's HSD test. Statistical analysis showed that P*. fluorescens* and *B. subtilis* isolates limited the growth *A. alternata* as compared to others. The highest antagonistic activity against all pathogens was by *P. fluorescens*. Ability of bacterial species to limit the growth of fungi was greatest after 4 days of dual cultivation and decreased after 6 days.

Keywords: biocontrol, cereals

P3.15 *Phytophthora cactorum* – a problem on gooseberry plantations

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Collar rot of gooseberry, caused by *Phytophthora cactorum*, was recorded for the first time in Poland in 2008 on Pax gooseberry cultivar. Wilting and dying of some bushes was observed during spring. Such symptoms were not observed on gooseberry plants of cv Pax grafted on *Ribes aureum* rootstock planted on the same plantation. The affected plants showed brown, water-soaked lesions appear mostly at the base of the stem, typical for collar rot. Rotted lesions also develop lower down and up to 10–20 cm above soil level. The pathogen isolated from symptomatic plants was identified as *P. cactorum*. On LBA (Difco) medium it produces colonies with homothallic mycelium with predominantly paragynous antheridia and markedly papillate, caducous sporangia, typical for this species. This pathogen could be distributed into new plantations on infected plants or it may have existed on the site before and infected a very susceptible tissue of 'Pax' cv. gooseberries. Resistant *Ribes aureum* rootstock seems to be a good solution because it keeps susceptible tissue away from infected soil. Good results in disease control were obtained after use of metalaxyl and fosetyl-aluminium several times during the season. These products drastically reduce new infections of gooseberries.

Keywords: collar rot, soilborne pathogen, 'Pax' cultivar, chemical control

P3.16 Biological activities of *Lavandula luisieri*

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Essential oil of *Lavandula luisieri* belonging to the family *Labiatae* is extensively used in aromatherapy. In the context of biological control, essential oils or extracts of some plants can be used as an alternative to chemical control. Aromatic species, particularly those in the family of *Labiatae* are among the most widely used plants in insect pest control. *Lavandula luisieri* is endemic to the Iberian Peninsula, and is common in central and southern Portugal, especially in the Alentejo province. The aim of this study was to determine biological activity of essential oil of leaves and flowers of indigenous *L. luisieri* collected in Évora (Alentejo). Essential oil was extracted separately from leaves and flowers by hydrodistillation in a Clevenger type apparatus. The chemical composition of oils was determined by gas chromatography with flame ionization detector (GC/FID). Essential oils of leaves and flowers showed antioxidant activity by the DPPH radical scavenging method, however a higher effect was observed through the system β-carotene/acid linoleic. Antifungal activity was evaluated against three filamentous fungi: *Aspergillus niger, Fusarium oxysporum* and *Penicillium sp.* These species, ubiquitous soil inhabitants, may be pathogenic to some plants, especially in agricultural settings. Disc Diffusion Assay and Minimal Inhibitory Concentrations (MIC) were evaluated. Essential oils showed important antifungal activity against the selected strains with MIC ranging from 250 -500µg/mL. On the basis of our results, it would be important to evaluate the use of essential oils of leaves and flowers of *L. luisieri* as a biological control in some plant cultures.

P3.17 Gooseberry cultivars and their susceptibility to fungal diseases

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In commercial gooseberry plantations American powdery mildew (*Sphaerotheca mors-uvae*) and leaf spot (*Drepanopeziza ribis*) are the most harmful diseases. In Poland the old gooseberry cultivar 'White Smith' is commonly cultivated, but it is very susceptible to these diseases. Introduction of new cultivars resistant to American powdery mildew and leaf spot could reduce the number of fungicide applications. In 2007–2009 the susceptibility of 18 gooseberry cultivars to the main fungal diseases was evaluated under field conditions. The tested gooseberry cultivars differed in their field resistance to American powdery mildew and leaf spot. The average level of infection ranged from 1.0 to 5.0 in a 5 grade ranking scale and depended on genotype and weather conditions in each year of studies. In all years of investigation eight cultivars ('Invicta', 'Kamieniar', 'Laskovij', 'Misorskij', 'Pax', 'Pixwell', 'Rochus', 'Rolonda') did not show any symptoms of American powdery mildew. Whereas White Smith', 'Krasnoslawiańskij', 'Puszkinskij' and 'Ruskos' were the most susceptible to this disease. None of the studied cultivars was completely resistant to *D. ribis* causing leaf spot. The most resistant to this disease were 'Misurskij', 'Rochus' and 'Spine, while 'White Smith', 'Pax' and 'Hinonmaki Gelb' were the most susceptible .

Keywords: gooseberry, susceptibility, *Sphaerotheca mors-uvae, Drepanopeziza ribis,* American powdery mildew, leaf spot

P3.18 Pathogenicity study on endophytic fungi isolated from wheat (*Triticum aestivum* L.)

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Endophytes are microorganisms that form symptomless infection with in healthy plants tissues. In order to study fungal endophytes were isolated from leaves, crowns and ears of healthy wheat plants from Central Anatolia and Black Sea region of Turkey. *Alternaria, Stemphylium, Fusarium, Epicoccum, Ulocladium, Septonema, Nigrospora, Pythium,* and *Cladosporium* spp. were the fungi that showed the highest colonization frequency (CF %) in all the tissue and organs analyzed. Of the 52 isolates as 12 fungal genera were tested on Canik cultivar of wheat. All isolates except *Acremonium* (W3-S4) caused some localized discolorations in stem tissue, when inoculation was conducted with the agar piece onto crown method. *Acremonium, Chaetomium, Nigrospora, Melanconium, Epicoccum* and *Septonema* sp. have potential as biological control agents which were in the same group of negative controls.

Keywords: Fungi, endophytes, wheat, healthy, mycoparasites, biological control

P3.19 *Trichoderma viride* effects on lettuce growth and protection against *Sclerotinia sclerotiorum*

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An indigeneous *Trichoderma viride* isolate with known activity against *Sclerotinia sclerotiorum* in laboratory screening tests was evaluated for its ability to control disease caused by *S. sclerotiorum* in lettuce in a glasshouse trial during one growing season. The abilty for the isolate to promote growth was also monitored. Application of *T. viride* was in the form of alginate pellets, in which the fungal wet biomass is entrapped in an alginate matrix. The pellets proved to be a suitable formulation for maintaining and dispersal of *T. viride* inocula into the soil. The *T. viride* treatments promoted growth of the lettuce plants. Furthermore, colonisation of sclerotia of *S. sclerotiorum* by *T. viride* reduced inoculum-producing capacity of the pathogen, which resulted in reduced disease incidence. The results suggest that *T.viride* has potential value as promotor of plant growth and as a biological control agent against lettuce drop caused by *S. sclerotiorum* in glasshouse systems.

Keywords: alginate pellets, biofungicide, *Lactuca sativa*, plant-growth promoter, white mould.

KN4.1 Climate Change and Plant Disease Management

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Various international phytopathological societies have observed that plant diseases are being covered insufficiently in climate change scenarios. Therefore a conference has been organized in Évora, Portugal from 10 – 12 November 2010 with international experts from various research fields in order to bridge this gap between climate change science and plant disease management. The result of this conference will be a 'white paper' on the potential effects of climate change on the spatio-temporal aspects of plant diseases, and on recommendations for required research.
Some of the sections this 'white paper' will contain, are a) From understanding to predictions, b) From prediction to adaptation and control, and c) Approaches for necessary research. The outline of the 'white paper' will be presented, including some details of the recommendations on new fields of research needed for disease management of crops in a changing environment due to climate changes.

O4.1 Effects of climate change on plant health

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In our future climate the mean annual temperature and concentrations of atmospheric CO_2, and tropospheric ozone will increase substantially. Therefore, it is important to understand how these factors, individually or in combination, may influence plant growth and plant-pathogen interactions. Recent studies indicate that CO_2, and temperature can be beneficial for plant growth but their combinations non-beneficial. However, very little is known regarding the effects of multi-factorial climatic change on plant–pathogen interactions and our current study aims to address this. We investigate possible effects of multi-factorial climate change on disease resistance in barley towards the biotrophic fungus *Blumeria graminis* sp. *hordei* (powdery mildew) and the hemibiotrophic fungus *Bipolaris sorokiniana*. Elevated temperature or ozone concentration, but not CO_2 resulted in increased basal resistance against powdery mildew. However, elevated CO_2, either in combination with increased temperature (2 factor) or increased temperature and ozone (3 factor) dramatically compromised basal resistance. For *B. sorokiniana* elevated CO_2 resulted in less disease symptoms, elevated temperature resulted in increased disease symptoms, whereas elevated ozone concentration had no effect. Elevated temperature in combination with increased CO_2 (2 factor) and in combination with increased CO_2 and ozone (3 factor) also lead to increased *B. sorokiniana* disease symptoms. Changes in stomatal conductance and photosynthesis could not explain the changes in disease resistance. We are currently analyzing histological, metabolic and physiological changes to explain the differences. Based on our preliminary results, we conclude that combination of factors can result in unpredictable disease response and only by investigating muliti-factorial changes will it be possible to predict the effects of climate change on plant health.

Keywords: CO_2, ozone, barley, powdery mildew, spot blotch

P4.1 The impact of climatic changes on the behaviour of *Biscogniauxia mediterranea*, the cause of charcoal disease in cork oak

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Charcoal disease, caused by the fungus *Biscogniauxia mediterranea*, is one of the most frequently encountered diseases of cork oak in Portugal. The pathogen has been considered to be a secondary invader that attacks only weakened hosts. Nevertheless, in recent years an increasing number of young vigorous trees exhibiting charcoal disease symptoms have been recorded. Moreover, a variation in fungus behaviour has been noticed in that the anamorphic stage is now more common than the teleomorphic form. These recent changes are probably due to plasticity of this species, which may be affected by temperature effects on its growth (it tends to be thermophilic) and water stress resistance. Such observations might be related to global climate changes, which are particularly severe in the mediterranean region.

Keywords: *Biscogniauxia mediterranea*, anamorphic stage, *Quercus suber*; global warming

SESSION 5: PRECISION AGRICULTURAL TECHNOLOGIES IN INTEGRATED PLANT DISEASE MANAGEMENT

KN5.1 Precision Crop Protection – the Challenge and Use of Heterogeneity

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Agri- and horticultural crops worldwide face the attack of various kinds of pests – additionally abiotic stresses are reducing the quantity and quality of crop production. The incidence of competing organisms impairing plant growth is difficult to identify – their appearance is rather heterogenous in time and space. This variation is due to edaphic factors, climatic conditions and especially due to the biological as well as epidemic specifities of the particular pest.

Innovative precision crop protection measures can be improved by the use of geographic information systems (GIS) – additionally satellite based observation systems provide cross-regional and global data. Innovative techniques can provide very relevant further supporting information for site specific decisions on production systems. These techniques have to be regarded as rather promising also for the decision support, but also for the improvement of plant production as well as disease control measures.

The problems to identify pests are highly subject to heterogeneity. This is a challenge that has been neglected in the past due to the lack of supporting informations. Recently there have been strongly supporting developments. The presentation will give some examples of future applications of these innovative techniques – but also the limitations will be discussed.

To ensure the production of sufficient quantities and high qualities of food and feed as well as to provide healthy, high yielding plant resources for the production of fiber and energy. Precision crop protection and innovative techniques will lead in the future to decision support systems and will improve the development of sustainable agri- and horticulture.

These techniques will be of essential support for further Integrated Pest Management (IPM) Programs – the technical potentials are developing rapidly. These will also be tools to recognize and to demonstrate cross-compliance. By this it will lead to further tools for sustainable crop production.

It will be not only necessary, but essential in the future to make use of the increasing potential of sensing technologies and informatics. This needs the cooperation not only in multi-disciplinary programs – this also needs transdisciplary understanding of crop protection: Different disciplines need to find together and need to indentify mutual interests and cooperate in innovative ways.

Keywords: innovative techniques, decision support systems, disease diagnosis, precision pathogen control

SESSION 6 (SPECIAL SESSION): 7TH EUROPEAN UNION RESEARCH FRAMEWORK PROGRAMME FUNDING OPPORTUNITIES FOR MEMBER STATES ASSOCIATED COUNTRIES AND THIRD COUNTRIES

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O7.1 Molecular detection of apple scab inoculum

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Apple scab, caused by the ascomycete fungus *Venturia inaequalis*, is of serious concern to the South African apple industry due to the decrease in yield and lowered quality of the fruit. To estimate the efficiency of alternative control strategies such as orchard sanitation in an integrated management approach we have optimised a molecular detection method based on real-time polymerase chain reaction (Q-PCR) of *V. inaequalis* DNA. The spore pressure was assessed in two climatically different apple growing regions in South Africa from bud break to full bloom. In milder climates such as the Koue Bokkefeld in the Western Cape, scab lesions might over-winter in the asexual form and not just as winter-hardy pseudothecial structures. Pseudothecia form in fallen leaves during winter and aerially disperse their sexual spores at bloom in the form of ascospores. Our hypothesis was that the over-wintered (asexual) mycelium or conidia might be the cause for the release of infectious spores before the so-called primary ascospore inoculum is expected. A Q-PCR method was developed using species specific *V. inaequalis* genes (e.g. Cytochrome P450 sterol 14 alpha demethylase, CYP51A1) and Sybrgreen™ to test the increased risk of infection before the start of fungicide spray routines at bloom. Our results show the different levels of spore pressure of conidia and ascospores found in the two regions under analyses.

Keywords: aerial inoculum, over-wintering, quantitative PCR, *Venturia inaequalis*

O7.2 PCR-based detection of pathogens in carrot soil samples to predict development of carrot cavity spot and post harvest carrot diseases

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PCR primers developed to detect five *Pythium* species causing carrot cavity spot and two post harvest pathogens in carrots (*Mycocentrospora acerina* causing liquorice rot and *Fibularhizoctonia carotae* causing crater rot) were tested during 2006-2009. Soil from carrot fields at different locations in Norway was sampled in the autumn before sowing, before sowing in April/May, and at five different times during the growing season from July until harvest in September/October. During the growing season soil adhering to the surface of the carrot roots was sampled. DNA was extracted from the soil samples and PCR reactions were performed. Roots were stored for 4-6 months and assessed for disease incidence. The PCR data were compared to the disease data using regression analyses. Generally the relationship between PCR data on soil samples and disease data was best on sandy soils, less good on organic soils, best for liquorice rot, medium good for cavity spot and less good for crater rot. PCR-data from soil attached to the carrot surface at harvest correlated best to the storage disease data (R^2=76.3 for liquorice rot, and R^2=66.7 for cavity spot). For liquorice rot the soil data from the autumn before sowing also correlated well with the storage disease data (R^2=66.9). PCR of the soil data from the spring correlated to some extent for cavity spot development (R^2=47.5). PCR data on the *Pythium* species tested is complicated to use as predictors for cavity spot development because of fluctuations in levels of detection of the different species during the season.

Keywords: Crater rot, *Fibularhizoctonia carotae*, liquorice rot, *Mycocentrospora acerina*, *Pythium* spp.

O7.3 A new *Phytophthora* species causing root rot in pea and other legumes

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The root rot disease complex is the most important disease problem in pea production. Greenhouse biotests of soil samples is the main strategy for detecting and avoiding infested fields at Findus AB, a company producing frozen green peas in southern Sweden. Around 700 soil samples are tested annually. In addition to the main root rot pathogen *Aphanomyces euteiches*, root rot caused by *Phytophthora* is observed every year in the biotests in approximately 5 % of the samples. The aim of the presented project was to identify the disease-causing organism using molecular tools, to establish the host range with standardised pathogenicity tests, develop diagnostic tools for molecular detection, screen host plant germplasm for disease resistance and conduct field trials in naturally infested fields. Phylogenetic analysis suggests that the pathogen is a novel species (proposed name *Phytophthora pisi* sp. nov) closely related to *P. sojae*. The host range is confined to a group of legumes closely related to pea. This group includes the domesticated leguminous crop species faba bean, vetch, chickpea and lentil, but not soy bean or common bean. A species-specific molecular detection method is under development. Ongoing screening of core collections of the susceptible crop species have not so far revealed the presence of strong resistance genes in the germplasm. The potential for serious disease outbreaks will be assessed in field trials conducted over a period of four years.

Keywords: Oomycetes

O7.4 Development of QOI resistance in Ramularia collo-cygni populations

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Ramularia leaf spot caused by the fungus *Ramularia collo-cygni* is now one of the most damaging diseases to affect barley production in northern Europe. The disease is visible late in the growing season and is characterised by small necrotic spots, usually with a yellow halo. *Ramularia collo-cygni* first appeared as a major pathogen of barley in Scotland during 1998 and was at first effectively controlled by QoI fungicides, including azoxystrobin (Amistar, Syngenta Crop Protection). However, during 2002 it was observed that the control achieved by this group of actives had sharply declined. The target of QoI fungicides is the cytochrome *bc*1 complex and resistance development in plant pathogens is usually linked to the mutations occurring at codons 129, 137 and/or 143 of the cytochrome *b* protein. This study showed that all recent isolates sampled from fields in Scotland were resistant and carried A143 alleles (replacement of glycine (ggt) by alanine (gct) at codon 143). Older isolates (before 2002) from the SAC collection were sensitive and showed no mutations at codon 143. Bioassays also showed a high level of resistance in all recently collected *Ramularia* isolates from France, Denmark and England. We also examined the evolution of QoI fungicide resistance in *Ramularia* populations over a longer time span using infected leaf and grain samples from the long-term Hoosfield spring barley experiment at Rothamsted. The presence of G143A was detected from 2001 onwards using three different test methods, PCR-RFLP, allele-specific real-time PCR and Pyrosequencing.

Keywords: fungicide, resistance, Strobilurin.

P7.1 Molecular detection and species identification of necroviruses

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Taking into account the abundance and diversity of necroviruses affecting olive trees, *Olive latent virus 1*(OLV-1), *Olive mild mosaic virus* (OMMV) and *Tobacco necrosis virus D* (TNV-D), a fast PCR based method was developed for their simultaneous detection and identification and also of *Tobacco necrosis virus A* (TNV-A). A pair of degenerated primers, parRdRp5' and parCoat3', was designed based on conserved regions in the RNA-dependent RNA polymerase (RdRp) and coat protein (CP) of the four viruses. The primers were designed so that the primer selection of sequence variants within these viral species is minimized and so that the amplified region contains partial sequence of both genes. This way the deduced aminoacid sequences can be used for phylogenetic analysis allowing accurate species identification. RT-PCR assays using this pair of primers were performed for seven different viral isolates obtained from olive to test its applicability in detecting those necroviruses. To identify the species and to evaluate the molecular variability within and between viral isolates of the same species, the amplified RT-PCR products of four isolates (V8, SA_6P_5, V6 and V10) were cloned and sequenced. V8 and SA_6P_5 were clearly identified as TNV-D isolates and V6 and V10 as OLV-1 isolates. The described assay showed to be a rapid and useful method to find necrovirus infections. Furthermore, sequencing the single RT-PCR amplicon allows the viral identification without the need of sequencing the complete RdRp and CP genes.

Keywords: olive, OMMV, OLV-1, TNV-D, TNV-A, RT-PCR

P7.2 Development of a reverse-transcription loop-mediated isothermal amplification for the broad spectrum detection of *Grapevine leafroll associated virus 3* (GLRaV-3)

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Loop mediated isothermal amplification (LAMP) is a nucleic acids amplification method developed some years ago which can be used for RNA or DNA templates. It requires a DNA polymerase with strand displacement activity and four or six primers which recognize six or eight regions in the template. The amplification originates a multimeric stem-loop structure. A striking characteristic is the amount of DNA produced, ranging from one to two orders of magnitude higher than PCR. This allows the use of very simple methods for monitoring the amplification like changes in the turbidity and viscosity of the reaction mix, appearance of a precipitate of magnesium pyrophosphate, changes in color in the presence of reagents commonly used for titrating the alkaline earth metals in water, or other methods relying in fluorescent dyes or probes. Together with the fact that a thermocycler is not needed and that the reaction is completed within one hour (including reverse transcription), it appears as a very interesting diagnosis method for non-sophisticated laboratories. The number of papers referring the use of LAMP has dramatically increased in medical fields in the last two years. However, only a few papers report its use in phytopathological fields. In this work, we report the development of an immunocapture RT-LAMP assay designed for the broad detection of GLRaV-3. The primers were based in the consensus sequences of the five phylogenetic groups reported for this virus and, as shown, the assay is able to detect samples from all the groups.

Keywords: RT-LAMP, immunocapture, GLRaV-3

P7.3 Development and implementation of molecular tools for the study of oil palm bud rot disease

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Oil palm bud rot disease (BR) is the main limiting factor for the production of the crop in Colombia and other Latin American cultivation areas. In Colombia, the disease has been expanding quickly and generating devastating effects in some of the production zones. Currently, *Phytophthora palmivora* is recognized as the causal agent of the initial lesions that finally lead to the rotting process typical of BR, although involvement of other microorganisms is recognized. Due to the importance of the disease and to the diversity of microorganisms involved, it became necessary the implementation of molecular tools enabling the identification of the associated species and the development of diagnosis techniques. The molecular identification of the isolates obtained from some production zones was carried out through the amplification and sequencing of the rDNA ITS region. A total of 35 species were identified, including 6 Oomycetes, 1 Zygomycete, 15 Ascomycetes and 13 Basidiomycetes. Species of *Fusarium* represented 38% of the identified isolates, followed by *Thielaviopsis paradoxa* with 7.7% and *Phytophthora palmivora* with 4.3%. New species never reported before associated with BR disease were found, including some endophytes. With the generated sequences, species-specific primers were designed for the most representative microorganisms, and allowed a successful and quick identification of these microorganisms. Additionally, diagnosis techniques have been developed that allow the direct detection of the pathogen and the other associated species from affected tissues, demonstrating the utility of the molecular tools in the diagnosis of the disease.

Keywords: Molecular diagnosis, *Phytophthora palmivora*, Species-specific primers.

P7.4 First report of *Lasiodiplodia theobromae* associated with cankers and dieback of grapevine (*Vitis vinifera*) in Portugal

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Dieback and cankers of wine and table grapevine cultivars are a common problem in several grape-producing regions of Portugal (Estremadura, Alentejo and Algarve). Symptoms include progressive death of shoots associated with brown cankers along the shoots and canes. *Lasiodiplodia theobromae* was consistently isolated from the diseased tissues of affected vines. Morphological, cultural, molecular traits and pathogenicity of isolates were analyzed. Black, ostiolate pycnidia were formed on sterile pine needles on 2% water agar, after 20–30 days of incubation, at 25ºC under NUV light. Conidia were initially hyaline and aseptate. Mature conidia were subovoid to ellipsoid-ovoid, broadly rounded at apex, truncate at base, 16.6–27.7 × 7.5–10.8 µm, dark brown and one-septate with longitudinal striations. Cultures were initially white, becoming dark-grey or olive-green with dense aerial mycelium, not producing a dark pink pigment in PDA at 35ºC. Optimum temperature for growth was 30ºC and at 10ºC no growth was recorded. Sequence data from the ITS regions and EF1-α gene supported the identification. All *L. theobromae* isolates were pathogenic on grapevine of cv. Aragonez. This is the first report of a dieback and cankers of grapevine caused by *L. theobromae* in Portugal.

Keywords: "*Botryosphaeria" rhodina*, Botryosphaeriaceae, *Vitis vinifera,* wood diseases.

P7.5 Phytosanitary Status of Olive Germplasm on Istrian Peninsula (Western Croatia)

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Phytosanitary status of autochthonous and introduced olive varieties in Istrian region (Croatia) were examined during 2009 and 2010 by performing a virological survey on olive trees using one-step reverse transcription-polymerase chain reaction. Sixty trees of ten autochthonous and five introduced olive varieties were examined. Cortical scrapings of olive cuttings, leaves and flowers of the checked trees representing the main varieties in this region were sampled and tested for the presence of eight viruses of major importance. A Rather unespected percentage of infection of the different varieties were found. Specifically, the total percentage of infection was 20% (12/60) and the prevalent virus was *Cherry leaf roll virus* (11.7%), followed by *Strawberry latent ringspot virus* (3.3%) and *Olive latent 1 virus* (5%). The percentage of infection of autochthonous and introduced trees was 20% (ten trees versus two trees). In addition, mechanical inoculation assays in herbaceous hosts were performed and developed symptoms of viral infections were visually inspected.

Keywords: biological assay, *Cherry leaf roll virus*, Croatia, *Olive latent virus 1*, reverse transcription-polymerase chain reaction, *Strawberry latent ringspot virus*.

O8.1 *Citrus tristeza virus* is a slowly evolving virus

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Citrus tristeza virus (CTV) isolates of diverse origin and biological properties were used to study the genetic variability of the 3' end proximal region of the CTV genome. The analysis of this region, comprising the genomic region between p25 and p23 (an extension of about 3000 nucleotides), is of paramount importance since it encodes genes that modulate the interaction with the host. Phylogenetic analysis of the sequences obtained for this region showed the existence of seven well defined phylogenetic groups. Moreover, for two of the phylogenetic groups it was for the first time obtained a complete sequence for this region, since the available sequences in GenBank only correspond to individual genes. The stability of the genetic structure was also inferred from the search for recombination events in the 3' end proximal region. Our results suggest a relative low recombination rate between CTV isolates even in isolates harbouring a mixture of haplotypes and co-habiting the same host for more than 12 years. The recombination events, when detected, occurred always in the same region, the intergenic region between p13 and p20, suggesting that this recombinational hot-spot. The rate of evolution of the p25 gene (coat protein), was also studied, using a bayesian approach. The mean genomic substitution rate was estimated to be 8.42×10^{-5} nucleotide substitutions per site per year. These results suggest that CTV isolates maintain a high level of stability over time in the part of the genome which modulates the interaction with the host.

Keywords: Genetic Stability, Rate of Evolution, Recombination

O8.2 Specific aminoacids of *Olive mild mosaic virus* coat protein are determinant for transmission by *Olpidium brassicae*

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Olive mild mosaic virus (OMMV) is a *Necrovirus* widespread in Portuguese olive orchards reaching infection levels of ca. 30 %. OMMV can infect plant roots freely at a relatively low rate but transmission in the presence of *Olpidium brassicae* in the soil is greatly increased. A variant, OMMV L11, obtained from mechanically passaging OMMV 15 times through *Chenopodium murale*, showed to be deficient in fungal transmissibility. To examine whether the coat protein (CP) had a role in this, a construct, OMMV/OMMV L11, was made. The OMMV L11 CP gene sequence was amplified by RT-PCR and the amplicon cloned to replace that of OMMV. Viral particles derived from this construct were infectious but failed to be efficiently transmitted in the presence of *O. brassicae* thus indicating a clear role of viral CP in transmission. Deduced aminoacid sequence of OMMV L11 CP was found to contain two aminoacid substitutions when compared to that of OMMV. Tyrosine and threonine on positions 3200 nt and 3281 nt in the OMMV L11 isolate, replaced asparagine and alanine present in the OMMV isolate, respectively. To check the biological importance of such alterations, those aminoacids changes were separately introduced into OMMV through *in vitro* site directed mutagenesis and it was found that the substitution of alanine to threonine is largely responsible for lowering transmissibility.

Carla Marisa R. Varanda is recipient of a PhD fellowship from Fundação para a Ciência e a Tecnologia - FCT (SFRH/BD/29398/2006)

O8.2A *Plum pox virus* is present in the green bark tissues of hypersensitive prune cv. Jojo

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Results of eight individual evaluations of prune trees (*Prunus domestica* L.) cv. Jojo infected with *Plum pox virus* (PPV) are presented in this study. Inoculation with mildly pathogenic PPV-D strain resulted in partial hypersensitive reaction of plants of prune cv. Jojo. No symptoms were observed in the year of PPV-D inoculation (2003) by grafting. Stunting and partial death of shoots were observed in the first year after the inoculation of PPV-D. One plant died in 2005, but shoots of St. Julien rootstock with PPV symptoms in leaves appeared after that. PPV-D had therefore been transported from the infectious graft via cv. Jojo into the St. Julien rootstock. Recovery of the three PPV-D inoculated plants started in the same year and continued in 2005. No systemic symptoms of PPV were observed in leaves of two cv. Jojo plants, and the virus was detected in leaves neither by ELISA nor by RT-PCR. Necrotic plates were observed on the trunk of two inoculated trees in 2007-2009. The presence of PPV was proved both by ELISA and RT-PCR in green bark of the trunk close to the necrotic plates, but not in leaves in 2007-2009. PPV is present in plant tissues of prune cv. Jojo. This study was supported by the Ministry of Agriculture of the Czech Republic, Project No. MZe 0002700604.

Keywords: local infection, partial hypersensitivity; PPV-D strain, plum, sharka disease

O8.3 What do bacterial genes tell: symbiont or pathogen?

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Pathogenesis and symbiosis are rare among bacteria and as bacteria emerged before plants, the distribution of plant pathogens and symbionts in divergent clades reflects the repeated and independent acquisition of these lifestyles. What then does it take to become a pathogen or a symbiont? The case of rhizobia and agrobacteria, which are phylogenetically related and yet have quite different lifestyles, is discussed. Bacterial genomes possess two main components: core and accessory genes. The core genome encodes genes that are mostly essential and the accessory genome encodes special ecological adaptations. Thus, strains belonging to the "core" species may show completely different lifestyles. Rhizobia provide a rare example of bacteria spread over large phylogenetic distances yet sharing a specific biological function. Genome integrated comparisons allowed interesting findings, as for example the fact that no gene seems to be common or specific to all rhizobia. Furthermore, coexistence of symbiosis and pathogenicity genes in *Rhizobium rhizogenes* allows the bacterium to be beneficial or pathogenic depending on the host. Moreover, an *Agrobacterium tumefaciens* strain with no *vir* genes has been described as able to form effective nitrogen-fixing symbiosis with *Sesbania* plants. Pathogens and symbionts depend on similar mechanisms for interacting with hosts. For instance, legume roots exudate flavonoids, which were traditionally considered as part of the plant defence mechanisms, were later found to be involved in the establishment of the legume-rhizobia symbiosis. Changes in accessory genome, occurring through gene acquisition and deletion, are the major events underlying the emergence and evolution of bacterial genomes. Thus, genome wide analysis will allow a better understanding of the role of lateral gene transfer (LGT) in bacterial genome organization and evolution of pathogens and symbionts.

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Keywords: accessory genome, core genome, evolution, lateral gene transfer (LGT), pathogenesis, symbiosis.

O8.4 Thaxtomin and non-thaxtomin producing potato pathogenic *Streptomyces* from Iran

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Streptomyces species are soil habitant bacteria a few of which are pathogenic on some plants. Potato scab inducing *Streptomyces* species are the most important plant pathogenic group. The main phytotoxins produced by potato pathogenic strains include thaxtomin, concanamycin and a compound named as FD-981. Potato scab is one of the most important diseases of potato in Iran. Potato tubers showing raised, netted, shallow and deep pitted lesions were collected from many potato fields and the *Streptomyces* strains were isolated. Based on the induced symptoms and phenotypic features isolated *Streptomyces* strains were heterogeneous. They induced symptoms on potato, parsnip, horseradish, carrot and other tested plants. Most of them carried a linear plasmid as revealed by pulsed field gel electrophoresis and they had sequences related to the thaxtomin biosynthetic genes. Raised and netted scab-inducing strains produced thaxtomin, as determined by thin layer chromatography, but thaxtomin could not be detected in the pitted lesion-inducing strains. The latter strains, which did not produced thaxtomin, also did not hybridize to the thaxtomin biosynthesis gene probes. Representative of the deep pitted-inducing strains produced disease inducing toxins other than thaxtomin.

Keywords: Concanamycin, Potato deep lesion, Potato raised lesion, Potato scab disease, Streptomyces scabies, Thaxtomin

P8.1 Discrimination of *Xanthomonas campestris* pv. *campestris* races by means of Rep-PCR

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Xanthomonas campestris pv. *campestris* (Xcc), the bacterial causal agent of black rot, is widely distributed around the world in cultivated *Brassica* species, and is a major constraint on *Brassica* production. Based on avirulence/virulence patterns to six differential host genotypes, 9 races have been identified, which should been taken into account when searching for sources of resistance and to design adequate breeding programs. The established method to discriminate among races using differential series entails growing the host genotypes, inoculating them, and waiting until disease symptoms appear. It is therefore time consuming procedure. Repetitive DNA polymerase chain reaction-based fingerprinting (rep-PCR) is a rapid, low-cost, and reliable diagnostic method that has already been used to study genetic diversity within *Xanthomonas* and can be applied to identify the 9 existing Xcc races. DNA extraction and rep-PCR amplification were performed for type strains representing the nine races using REP, ERIC, and BOX primers. Strains were also classified into races using the differential series of *Brassica* spp. Based on DNA fingerprinting, BOX and ERIC primers discriminated 4 races each, and REP primers discriminated 3 races. Five out of 9 races could be discriminated with rep-PCR method. Race 1 could not be differentiated from race 7 and race 3 could not be differentiated from race 9, therefore more primers need to be added to the proposed method in order to discriminate between these pairs of races. Currently the reliability of the method is being checked by testing Xcc isolates collected in northwestern Spain and comparing the results using the differential series of *Brassica* spp.

Keywords: bacterial disease, black rot, Brassica crops, breeding, genetic fingerprinting, resistance

P8.2 Classification and genetic diversity of *Rhizoctonia solani* populations causing damping-off of cotton in Iran

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Isolates of *Rhizoctonia solani* were obtained from cotton seedlings showing damping-off symptoms in Iran during 2007–2009. Characterization of various taxonomic groups was done using species-specific primers designed for conserved regions of ribosomal DNA internal transcribed spacer (rDNA-ITS) sequence and restriction fragment length polymorphism analysis of PCR-amplified rDNA-ITS region. Results revealed that from 168 isolates, 84 were AG4 HG-I, 35 were AG4 HG-II, and 49 were AG4 HG-III. All of the isolates were pathogenic on cotton and caused damping-off. Amplified fragment length polymorphism (AFLP) analyzes were used to investigate genetic structure of the pathogen populations collected from various geographic regions in different years. Cluster analysis using different methods and principal co-ordinate analysis (PCO), based on the AFLP data from 489 monomorphic and polymorphic bands generated with seven primer combinations, was performed. This revealed four separate AFLP groups among a total of 168 isolates, which typically showed more than 86% fingerprint similarity. Isolates of the three different intraspecific groups of *R. solani* AG4 were clearly separated in the dendrogram obtained from AFLP data. Within each AFLP group, two or more haplotypes were detected with a genetic similarity of 100%. Analysis of Molecular Variance (AMOVA) revealed that geographic region was the dominant factor determining genetic structure of *R. solani* AG4 populations, but year of sampling had no significant effect.

Keywords: AFLP, rDNA-ITS, seedling, taxonomic groups, *Thanatephorus cucumeris*, variability.

P8.3 Survey of *Mycosphaerella* species complex on *Eucalyptus globulus* in Portugal

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Eucalyptus spp. are the foremost global hardwood pulp crop. Since 1999, *Mycosphaerella* leaf disease has been regarded as an important disease in Portugal causing serious defoliation on young *E. globulus* trees. MLD, caused by several species of *Mycosphaerella* and *Teratosphaeria* and several anamorphic form genera, is one of the diseases threatening *Eucalyptus* plantations worldwide. Approximately 78 species of *Mycosphaerella* and about 38 anamorphs have been reported on *Eucalyptus* spp. worldwide. This complex of species reduces the photosynthetic capacity of leaves causing premature defoliation, and decreased growth resulting in significant losses of volume of produced wood in extreme cases. The most efficient means to control the *Mycosphaerella* complex is through the use of disease tolerant or resistant *Eucalyptus* clones. To impliment this control measure it is essential to have complete information of the main species of the pathogen and the way in which populations vary according to season. Therefore the aim of this work was to determine the species of *Mycosphaerella sensu* lato that occur in Portuguese *Eucalyptus* plantations. The species were identified by molecular methods based on the ITS region of the ribosomal DNA, together with morphological characters.

Keywords: Pathology, Dothideomycetes, *Mycosphaerella*, *Teratosphaeria,* Leaf Disease, MLD

P8.4 Morphological and molecular characterization of *Diaporthe/Phomopsis* species on almond in Portugal

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In a survey of dieback of almond, the most frequent fungi detected were *Diaporthe/Phomopsis* species. Although *Phomopsis amygdali* is recognized as the causal agent of almond twig canker and blight, combining morphological, cultural, molecular and pathogenicity data, allowed the recognition of three different species on this host. The isolates were characterized on the basis of their morphology and phylogenetic relationships were inferred from the ITS rDNA region (ITS1-5.8S-ITS2) for representatives of the different groups recognized in MSP-PCR profiles. Pathogenicity tests showed that *Phomopsis amygdali* is the main pathogen on almond. *Diaporthe neotheicola* is reported for the first time on this host. A third species represented by a single isolate could not be unequivocally identified.

Keywords: Almond, *Diaporthe*, ITS, *Phomopsis*, systematics

P8.5 Screening for AFLP Markers Linked to Virulence Profiles in the Coffee Rust *Hemileia vastatrix*

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Coffee leaf rust due to *Hemileia vastatrix* has been a permanent threat to coffee production for more than a century, reaching nowadays a worldwide distribution. Coffee breeding for rust resistance proved to be a successful method of minimizing yield losses and controlling the disease, but the high variability and adaptability of the pathogen with the consequent occurrence of frequent shifts in pathotypes have been compromising long-lasting effectiveness of resistant commercial varieties. The development of molecular markers that can identify and distinguish different pathotypes represents a great improvement to monitor the disease, since the physiologic differentiation of rust races on differential hosts is a laborious and time consuming process, whose dependence on environmental and host-pathogen physiologic conditions makes it sometimes very hard to perform and fallible. Here we report a first approach to ultimately identify AFLP markers associated to race-specific pathotypes as a tool to enable rapid virulence characterization. After a prescreening test using 40 *Pst*I/*Mse*I and *EcoRI*/*Mse* primer combinations with + 2 or + 3 selective nucleotides, an initial strategic sampling of 28 rust isolates comprising representatives of specific virulence profiles and mutant races artificially induced to overcome specific plant resistance genes was analyzed. For this preliminary study, 4 AFLP *Pst*I/*Mse*I primer combinations were selected and applied displaying within-group polymorphisms and specific fragments apparently exclusive to certain isolates. Although screening of a higher number of isolates and AFLP loci is needed, these results show already promising distinctive variations and putative relationships that are worth exploring.

Keywords: virulence markers, rust pathotypes, association studies, *Coffea* sp.

P8.6 Analysis of the molecular evolution of a mating-type gene in species complex of the *Colletotrichum* genus

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In ascomycetes, sexual reproduction and mating choice is genetically controlled by genes on the mating-type locus (MAT). However, the contribution of these sex related genes often goes beyond the mere fitness maintenance of individuals as they may influence virulence and pathogenic potential or be targets of selection to evolve reproductive barriers between emerging pathogens. Aiming at the analysis of the molecular evolution of a MAT gene, we sequenced the full *MAT1-2-1* gene in more than fifty isolates from complex of six species of the *Colletotrichum* genus, mainly from coffee hosts, with focus on the putative asexual and highly virulent species, *C. kahawae*, as well as other mild pathogens or saprophytic species and the cosmopolitan sexual species, *C. gloeosporioides*. So far, our results reveal the presence of a conserved *MAT1-2-1* gene on all studied isolates, irrespective of sexual mode, under a strong purifying selection and without evidence of positive selection acting between species. Interestingly, translated amino acid sequences of this gene are not correlated with the current species status of the isolates. The same inferred protein is found in isolates from the sexual species *C. gloeosporioides* and putative asexual species such as *C. siamense, C. asianum* and *C. fruticola*, and two different proteins were found on isolates of *C. kahawae*, diverging by only one non-synonymous mutation. The implications of the shared sex related gene in sexual and putative asexual species as well as the its divergence in a single species are discussed in light of its impact for plant disease control.

Keywords: Mating-type, *Colletotrichum*, Coffee Berry Disease, Selection.

P8.7 Molecular characterization of Portuguese isolates of *Leptosphaeria maculans* using PCR-ISSR and RAPD markers

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PCR-ISSR and RAPD markers were used to study the genetic relationships of 30 isolates of *Leptosphaeria maculans* including 18 Portuguese isolates from Beja region (south Portugal). Foreign isolates were also included to determine the relationships of Portuguese isolates to other related isolates. Cluster and principal components analyses were conducted using PCR-ISSR and RAPD data from amplification with selected ISSR primers and RAPD markers, detecting 234 polymorphic fragments. The results showed that the 30 isolates clustered into two distinct groups (Tox^+ and Tox^0 isolates) and 4 subgroups: i) a large and compact subgroup containing all the Tox^+ *L. maculans* "brassicae" isolates including all Portuguese isolates; ii) the unique "*Lepidium*" isolate; iii) a relatively heterogeneous subgroup with the Tox^0 NA2 *L. biglobosa* "canadensis" isolates; and iv) a dispersed subgroup with the other Tox^0 NA1 and NA3 *L. biglobosa* isolates and NA2 *L. biglobosa* "erysimii" isolate. There is low similarity between these three isolates. The Portuguese and foreign Tox^+ *L. maculans* "brassicae" isolates could be further divided into phenetic groupings. These groupings did not corresponded to their pathogenicity groups as revealed by inoculation of plant differentials.

Keywords: *Phoma lingam, L. biglobosa,* isolate grouping, numerical taxonomy, molecular markers.

KN9.1 Detecting exotic forest pathogens, and predicting their impact in Mediterranean ecosystems

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In light of increased movement of goods and people at the global scale, the likelihood of introduction of exotic forest pathogens is now higher than ever.
Although the introduction of exotic plant and animal pathogen is a well-known phenomenon, the severity of such introductions in forest ecosystems is compounded by the fact that not a single successful eradication of an exotic forest pathogen is known. While most governments currently regulate individual commodities, further introductions can be halted only if general pathways and not individual commodities are regulated. This effort needs to include innovative and state-of-the-art approaches to enhance our diagnostic power. Once introduced, early detection and mitigation efforts (including stopping repeated introductions of the same organism) are required in order to significantly alter the course of potential epidemics caused by exotic pathogens. In order to design appropriate mitigation efforts, an understanding of the biology of the pathogen and of the epidemiology of the disease is required. This task is not necessarily easy because, inevitably, introduced pathogens normally give rise to completely novel pathosystems. Predictive models can greatly assist in focusing and properly directing mitigation efforts: these models need to take into account the pathogenic potential of the causal agent (host range, life cycle, etc), the variability in response among host populations and individuals, and the effect of environmental heterogeneity on infection and disease progression. Generalist pathogens represent a more significant threat to native ecosystems than specialized ones, and the lack of disease tolerance or resistance among host populations will hasten disease severity. Nonetheless, mounting evidence is indicating that environmental and ecological factors are equally important. In this talk, I will present information on introduction pathways for *Fusarium circinatum*, *Phytophthora cinnamomi*, *P. ramorum* and *Heterobasidion irregulare.* For each pathogen I will present a summary of factors favoring higher disease levels, and for *Fusarium circinatum* and *P. ramorum* I will summarize available mitigation strategies. I will conclude with a brief description of the massive hybridization ongoing in Italy

between the European *Heterobasidion annosum* and the introduced North American *H. irregulare*, a discovery that highlights that introductions do not only alter the ecology of native ecosystems, but also the evolutionary trajectories of native microorganisms

O9.1 Pine wilt disease: a threat to European forestry

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Bursaphelenchus xylophilus, the pinewood nematode (PWN), and causal agent of pine wilt disease (PWD), was detected for the first time in 1999, in Portugal and Europe. The PWN has been detected in new forest areas in the center of the country, in 2008, despite efforts developed by the national forestry and quarantine authorities to control the nematode and its insect vector (*Monochamus galloprovincialis*). The nematode has also recently been reported to be present from Spain. Circulation of non-treated wood and wood products may explain the spread of the nematode. Control strategies have been focused on the vector by using chemical traps, by cutting down symptomatic trees, heat-treatment of lumber, and monitoring of main roads and ports through which lumber and wood products are transported, by the Portuguese authorities. The nematode constitutes a threat to the rest of Europe, if proper measures are not taken by European governments. A significant body of scientific knowledge has been generated including nematode and insect bioecology, pathogenicity, use of molecular biology in diagnostics and detection, etc. Many gaps in the knowledge of this complex biological system persist. The involvement of bacteria, associated with the PWN in causing pine wilt, has been claimed. New quick detection methods and the understanding of the nematode population dynamics are being developed. Nematode genomics may provide some insight to better understand the pathogenic effects caused inside the plant. Pathogenicity testing of susceptible pine species is imperative. A review of the progress is hereby presented.

Acknowledgments: current research on the pinewood nematode in the NemaLab/ICAAM, is partially supported by the Portuguese government, through a national AFN project ("A doença do nemátode da madeira do pinheiro").

Keywords: *Bursaphelenchus xylophilus*, *Monochamus galloprovincialis*, *Pinus*, Portugal.

O9.2 PathoPlant-assisted prediction of two kinases simultaneously involved in the *Arabidopsis* response to fungal and bacterial pathogens

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PathoPlant is a database on plant-pathogen interactions. It displays signal transduction components from different plant species and contains *Arabidopsis thaliana* microarray gene expression data, obtained with pathogen-related stimuli. We used the database to determine which *Arabidopsis* genes are strongly (>4fold) and simultaneously upregulated by diverse pathovars of *Pseudomonas syringae*, by *Erysiphe orontii*, *Botrytis cinerea*, and *Phytophthora infestans*. A cell wall-associated kinase and a membrane-associated receptor like kinase gene are found to be upregulated by all stimuli. To analyse if these genes are required for the defense response to *Pseudomonas syringae* and *Botrytis cinerea,* the corresponding *Arabidopsis* mutants are studied. The current state of the PathoPlant database, the database-assisted analyses, and results of infection experiments as well as expression studies will be presented.

Keywords: *Arabidopsis thaliana, Botrytis cinerea*, gene expression analyses, plant-pathogen interaction, *Pseudomonas syringae*, signal transduction

O9.3 Powdery mildew-induced transcription factors HvWRKY1/2 mediate compatibility and repress the defense related gene HvGER4c in barley

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WRKY transcription factors are crucial components in the plant defense systems. In barley, the expression of HvWRKY1 and HvWRKY2 is rapidly and strongly induced during the infection of powdery mildew fungus (*Blumeria graminis* f.sp*. hordei, Bgh*). Transient over-expression of HvWRKY1 and HvWRKY2 enhanced susceptibility of epidermal leaf cells to powdery mildew indicating a role as negative regulators of plant basal defense. HvGER4c, a member of the germin-like GER4 gene cluster, has high transcript level in powdery mildew infected barley leaf epidermis and functions positively in plant immunity. The promoter of HvGER4c contains several W-boxes thereof at least 4 positively regulate promoter activity after pathogen attack. To examine the possible interaction of HvWRKY2 with HvGER4c promoter, transient expression of the HvWRKY2 protein was performed using particle bombardment assay. Co-expression of HvWRKY2 with HvGER4c promoter-β-glucuronidase fusions reveals a repression effect on pathogen-induced promoter activity, which suggests that HvWRKY2 competitively binds to the W-boxes in HvGER4c promoter. This indicates a mechanism in which the HvGER4c expression is negatively regulated by the transcription repressor HvWRKY1/2, which might be deployed by the powdery mildew fungus to suppress barley defense responses.

Keywords: transcription repressor, W-box, germin-like protein

O9.4 Matrix metalloproteinase as a factor in plant innate immunity

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In mammals, extracellular matrix metalloproteinases (MMPs) play fundamental roles in various biological processes, such as tissue remodeling, embryogenesis and tumor invasion. Despite their wide distribution in plants, little is known about their function in green tissues. We aimed to elucidate a possible function of MMPs in plant defense responses. Five matrix metalloproteinases of *Arabidopsis thaliana* were investigated (*At1-MMP* to *At5-MMP*). Using RT-PCR analysis, we found expression of both *At2-MMP* and *At3-MMP* upregulated in Arabidopsis leaves after *Pseudomonas syringae* or *Botrytis cinerea* infection. Because *At2-MMP* showed the strongest pathogen-responsiveness, we tested the Arabidopsis *at2-mmp* mutant for disease resistance. We found that *at2-mmp* was 33% more susceptible to the necrotrophic fungus *Botrytis cinerea* than wild-type plants. Consistently, ectopic over-expression of *At2-MMP* in Arabidopsis increased 32% resistance to *Botrytis cinerea* compared to the empty vector control. We addressed the question whether resistance to *B. cinerea* depended on the salicylate(SA) or jasmonate(JA)/ethylene(ET) defense pathway. *B. cinerea* induced the expression of *At2-MMP* in the signaling-defective mutants including *NahG, jar1.1, ein2-1, npr1-1* as strong as in the wild-type plants. This data suggests that the pathogen-induced expression of *At2-MMP* is independent of SA, JA and ET signaling. *At2-MMP* has typical characteristics of other plant and animal MMPs, i.e. the recombinant protein exhibited myelin basic protein proteolytic activity, and was inhibited by the zinc-chelator EDTA in a dose-dependent manner. Together, these results suggest that pathogen-responsive metalloproteinase may be involved in plant defense.

Keywords: *Botrytis cinerea*, matrix metalloproteinase, resistance

O9.5 Defence response of oilseed rape against a hemibiotrophic pathogen *Leptosphaeria maculans*

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The ascomycete fungus *Leptosphaeria maculans* is a hemibiotrophic pathogen that causes stem canker of oilseed rape (*Brassica napus*). Despite the importance of this disease, signalling pathways participating in defence response against this serious pathogen have not yet been fully elucidated. Our work was aimed at activation of defence mechanisms in an incompatible interaction of *B. napus* with *L. maculans*. Two near isogenic lines with active or mutated allele of the avirulence gene *AvrLm1* and *B. napus* cultivar containing the resistance gene *RLm1* enabled us to study triggering of specific defence response after pathogen recognition. Both salicylic acid- and ethylene-dependent signalling was activated to a similar extent regardless of compatible or incompatible interaction in infected tissues. Since the content of mycelia in tissues infected with virulent isolate was 17 times higher than in the incompatible interaction we can assume that the activation of defence gene expression is much stronger after *AvrLm1* recognition. These results indicate quantitative differences in the defence response of *B. napus* against *L.maculans* in compatible and incompatible interactions. Treatment of the plants with benzothiadiazole (BTH), a synthetic inducer of resistance, resulted in a significant decrease in disease symptoms on cotyledons, which resembled lesions caused by avirulent isolates. Detection of hydrogen peroxide *in situ* revealed significantly higher accumulation of hydrogen peroxide in cotyledons infected with an avirulent isolate.

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Keywords: salicylic acid, jasmonic acid, ethylene, signalling

O9.6 Transcriptomic analysis of *Hemileia vastatrix* in pre- and post-penetration stages

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Leaf rust (*Hemileia vastatrix*) is the main limiting factor for coffee production worldwide. The recent emergence of new rust races, able to overcome resistant coffee varieties, reinforces the necessity for a deep knowledge of the rust mechanisms of pathogenicity, in order to better understand plant resistance and devise durable resistance breeding strategies.

In this work, 454 RNA pyrosequencing was applied for *H. vastatrix* gene discovery of transcripts produced in three key differentiation/infection stages *in vitro* (germinated uredospores and appressoria) and *in vivo* (leaves densely colonised by intercellular hyphae and haustoria). For selected genes, RT-qPCR was performed on a detailed time course of infection.

Among ca. 11000 predicted genes, 10% were expressed in the three libraries. Comparisons among these transcriptomes indicated a shift in the putative function of transcripts detected, suggesting higher levels of biogenesis, lipid transport and energy production in appressoria, and higher levels of signalling, intracellular trafficking and secretory activity both in germinated uredospores and in hyphae/haustoria. Genes involved in DNA replication and cell division are more frequently found in the *in vivo* sample, announcing the onset of the production of sporogenic hyphae.

One fourth of predicted secreted proteins are shared among the three libraries, but 20% are specific to germinated uredospores, frequently with high expression levels.

Sixteen percent of predicted secreted proteins are homologues to those from other rusts, with three Rust Transferred Protein-homologues presenting marked differences in their expression profiles. Thirty-seven percent had no database homology and could be specific to *H. vastatrix*.

Keywords: 454 RNA pyrosequencing; RT-qPCR; fungal effectors; secreted proteins; biotrophy

O9.7 Characterization of the activity of phylogenetically distinct silencing suppressors of *Citrus tristeza virus*

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Plant viral infection by an RNA virus triggers the RNA silencing process by formation of sequence specific dsRNAs precursors that are processed to 21-24 nt small interfering RNAs (siRNAs), which can then guide the degradation of similar RNAs. However, viruses have also developed a mechanism to overcome this RNA silencing process, based on the existence of the suppressor genes. Three distinct suppressors of the RNA silencing are encoded by the 20-kb plus-strand RNA genome of *Citrus tristeza virus* (CTV), namely the p20, the p23 and the p25 gene. Here we report the characterization of the activity of the p23 gene obtained from phylogenetically distinct isolates. Assays were done in the *Nicotiana benthamiana* line 16C system through the transient expression of GFP, alone or co-infiltrated with p23. Differences in the suppression activity were monitored visually under UV light and by northern analysis. The best suppressing activity was found in the suppressor obtained from the severe quick decline isolate Q3 (Group 1), followed by the severe stem pitting isolate 11 (Group 3a), which were able to suppress the local but not the systemic silencing. The suppressors from other phylogenetic groups, including from the Mild group M, showed similar activity, although not so intense as Q3 and 11. These results point to the existence of a relationship between strain severity and the suppressing ability of the p23 protein.

Keywords: Post Transcriptional Gene Silencing; RNA interference

O9.8 Identification of a RNA silencing suppressor in the genome of *Grapevine leafroll associated virus 3* (GLRaV-3)

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Post-transcriptional gene silencing (PTGS) is a mechanism involved in plant defense against viruses. As part of a counter defense strategy numerous viruses evolved or acquired functions for suppressing PTGS. GLRaV-3 is involved in the leafroll disease of the grapevine and is the type member of the genus *Ampelovirus*, (family *Closteroviridae*). Although in this family diverse suppressors have been described, until now there is no published evidence of suppressors in the genus *Ampelovirus*. By analogy with the genomic location of silencing suppressors in the sister genus *Closterovirus* and molecular signatures of suppressors previously described for that genus, we decided to screen the 3' genes p21, p19.6 and p19.7 of GLRaV-3 for proteins with RNA silencing suppressor activity. Clones of these genes were obtained from isolates of Portuguese varieties that were previously found to have single infections with each one of the five phylogenetic groups that exist based in the CP gene. *Agrobacterium*-mediated transient co-expression of the candidate gene and GFP in the *Nicotiana benthamiana* 16C line, (which constitutively expresses GFP) was used in the assays. Suppressor activity was found for the p19.7 but not for the other genes assayed. The nucleotide sequence of p19.7 is more similar than the others assayed to the family of p21-like family of suppressors of the genus *Closterovirus*, which are able to suppress intercellular silencing. The characterization of the suppressor properties of p19.7 is underway.

Keywords: *Agrobacterium*-mediated transient expression, p19.7 protein, RNA silencing, viral suppressors

P9.1 High environment temperatures are suitable for corn stunt spiroplasma

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Thirty two maize genetic materials (including a susceptible control) were inoculated with *Spiroplasma kukelii* and cultivated under two different environmental conditions. The plants were cultivated in a screen house with a plastic roof, during April-June, and September-November, under day/night average temperatures of 27.8±1.8/ 15.6±2.5 °C (average 20.2±1.7 °C) and 30.4±2.4/ 18.9±1.4 ^{0}C (average 23.7±1.2 °C), conditions 1 and 2, respectively. Each treatment was replicated five times, with an experimental unit of one plant per pot with 5 Kg of soil (320 pots for each experiment). For inoculation, two spiroplasma infective leafhoppers *Dalbulus maidis*, obtained under controlled conditions, were confined per seedling, eight days after sowing during four days. Negative control were plants exposed to healthy, non-infective leafhoppers. At maize flowering, the disease symptoms were evaluated on a 1 to 5 scale. The plants were harvested, and dry weights determined. Percentage dry weight reduction caused by the disease in relation to healthy plants was calculated for each material. In conditions 1 and 2, respectively, 18 and 20 genetic maize materials showed symptoms of corn stunt and 22 and 54% of total diseased plants showed symptom severity of 3 or more. Average reduction of dry weight was 7 and 12%. Five materials showed a higher incidence and symptom severity at higher temperatures with dry weight reductions of 2, 0, 0, 8, 34%, and 31, 16, 22, 31, 51%, for conditions 1 and 2, respectively. Results showed that average of temperatures around 30/19 °C (day/night) can intensify symptoms and damage by corn stunt spiroplasma.

Keywords: *Dalbulus maidis*, *Spiroplasma kunkelii*, *Zea mays*

P9.2 *Xanthomonas campestris* pv. *campestris* affecting *Brassica oleracea* in northwestern Spain: race identification and search for resistance

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Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), is an important bacterial disease affecting *Brassica* crops worldwide. The seed-borne pathogen is especially damaging and destructive in warm and humid conditions. Nine races of the pathogen have been described, with races 1 and 4 being the most aggressive and widespread. In Spain no previous works concerning either the pathogen or resistance to the disease have been carried out, even though this country is an important *Brassica* consumer and producer. The objectives of this work were to identify Xcc races present in cabbage and kale crops in this area and to evaluate a *Brassica oleracea* collection for the most important races. In this study, 161 isolates from black rot infected fields were typed using an established differential series. Race 4 was the most frequent, although races 6 and 1 were also present. A collection of 256 *B. oleracea* accessions including cabbage (*capitata* group), kale (*acephala* group) and tronchuda (*costata* group) was evaluated for resistance to races 1 and 4 using a 1 (resistant) to 9 (susceptible) subjective rating scale. From the analysis of variance, statistical differences among groups were found for race 1 but, in general, the accessions showed a high level of susceptibility to both races. Two commercial cabbage accessions ('Quintal de Alsacia' and 'Balon') showed some degree of resistance to both races and one kale landrace (MBG-BRS0070) and two commercial cabbage ('Corazón de Buey' and 'Golden Acre') included plants with resistance to race 4. These are promising results since resistance to black rot in *B. oleracea* is scarce.

Keywords: black rot, cabbage, differential series, kale, sources of resistance, tronchuda

P9.3 Virulence of a *Meloidogyne hispanica* isolate on pepper cultivars

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The root-knot nematode (RKN) *Meloidogyne hispanica* is a highly virulent pathogen of many cultivated plants and presents a great concern due to its ability to reproduce on *Mi*-gene resistant tomato, which may confer resistance to *M. arenaria*, *M. incognita* and *M. javanica*. The ability of some *Meloidogyne* isolates to overcome plant resistance can be induced by a selection pressure through repeated exposure to plant resistance genes. The main goal of this work was to evaluate the influence of two inoculum levels, 2500 or 5000 eggs+second-stage juveniles (J2)/per plant, on the reproduction of a *M. hispanica* isolate on three pepper cultivars ('Aurelio', 'Solero' and 'Zafiro') at four temperatures (24.4±8.2ºC, 25.0±2.7ºC, 29.3±1.8ºC and 30.8±3.0ºC). Sixty days after inoculation, roots were assessed for gall index (GI) and reproductive factor (Rf). All cultivars were considered resistant hosts ($0.00 \leq GI \leq 1.80$ and $0.00 \leq Rf \leq 0.40$) at all temperatures. However, in cultivar 'Aurelio', a limited number of J2 developed into females and produced eggs. These eggs were inoculated in cultivar 'Aurelio' and the process was repeated twice to increase nematode population density. Host suitability assessment was repeated using 5000 eggs+J2/plant at 25ºC. The three cultivars were classified as susceptible (GI=5 and $11.57 \leq Rf \leq 21.42$). PCR-specific markers are being used to screen these cultivars for the presence of *Me* resistance genes which confer resistance to RKN. Pepper plants with *Me* gene can be an alternative to the chemical nematicides but the repeated exposure to resistance genes may lead to a selection of virulent lines.

Keywords: host status, reproduction, root-knot nematodes

P9.4 Fungal endophytes associated with Olea europaea leaves in Portugal

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The importance of *Olea europaea L.* in the Portuguese economy justifies the main objective of this work, namely the study of endophytic fungi of *Olea europaea* leaves. *Olea europaea* subsp. *europaea* and *O. europaea* subsp. *sylvestris* occurring in three locations within Portugal (Alentejo - Ourique; Estremadura - Serra da Arrábida and Beira Litoral - Alvados) were screened for the presence of fungal endophytes. The endophytes were isolated in summer and autumn from living symptomless leaves of olive-trees and oliasters. Identification of individual fungi was established subsequently on the basis of cultural characteristics. Fungal endophytes are common and diverse in woody plants. The filamentous fungi from the genera *Alternaria, Chaetomium, Nodulisporium* and *Trichothecium* were the most frequent ones. Other endophytic taxa that are reported belong to fungal groups composed of morphologically similar endophytes and pathogens (weakly) as *Fusicoccum* sp., *Phomopsis* sp., and *Pestalotiopsis* sp. Others are typically saprophytes, e.g. *Chaetomium* sp., *Cladosporium* spp., *Sordaria* sp., *Ulocladium* and *Spormoniella* sp. or belong to genera in which some species are reported as antagonists, such as *Gliocladium* and *Trichoderma* sp. The results were analyzed as a function of the geographical position of the site, host subspecies and season. The diversity of the endophytic assemblage was low. However the frequencies of colonization of endophytic species recovered were high and comparable to those reported for temperate zones.

Keywords: Endophyte, Fungi, *Nodulisporium,* olive-tree, oleaster, *Phomopsis.*

P9.5 The Induction and Growth of Potato Microtuberization (*Solanum tuberosum*.) Variety Santa in Response to Various Concentrations of BAP and Sucrose in Histological Tissue Culture Conditions

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In histological tissue culture conditions, the impact of different concentrations of BAP and sucrose as inducing compounds on microtuberization and also on some parameters such as time, number, dry and fresh weights of microtubers was investigated in the present study. In order to induce the microtubers in MS liquid media, different concentrations of sucrose (30, 40, 60, 80 gl^{-1}) and BAP (1, 2, 5, 10 mgl^{-1}) and also perpetual darkness were applied. In induced media containing low densities of sucrose (30 gl^{-1}), the increment of BAP concentration was of no inducing effect on microtuberization. Following the increase of sucrose concentration up to (40 gl-1) and only in high concentration of BAP, the microtubers were induced at the end of 4^{th} week with a short delay. Howsoever, the microtubers grew emanating from the alteration in meristem growth pattern and biomass of stolon sub-apical area. Moreover, the microtubers became larger and began to emerge as attached to rhizome following the enhancement in BAP concentration. Whilst the sucrose level was augmented to (60 gl^{-1}) and even in low levels of BAP, the induction of microtubers occurred with a short delay during the first two weeks until the sixth week. The aforementioned microtubers didn't survive in media culture in vitro. In high concentrations of sucrose and BAP, the average numbers of microtubers were influenced along with the induction of microtubers until the second week. In induction media comprising high concentrations of sucrose (80 gl^{-1}) and BAP (10 mgl^{-1}), the microtubers dormancy and health were more likely. The topmost proportion of arid weight of microtubers to arid weight of branches was attained in high concentrations of BAP (10 mgl^{-1}) and sucrose (80 gl^{-1}%). The media having high concentrations of BAP (5 mgl^{-1}) and sucrose (80 gl^{-1}), were of the utmost number of microtubers whereas the maximum fresh weight of microtubers appeared in media containing BAP (5 mgl^{-1}) and sucrose (60 gl^{-1}). Howsoever, high concentrations of Sucrose along with high concentrations of BAP reduced the induction period and also decreased the

microtubers formation to two weeks. Both Sucrose and BAP were of a paramount role on decrease and increase in microtuber fresh weights. An increase in Sucrose concentration had a significant impact on rise of dry weight and microtubers' biomass. To sum up, in order to select the most suitable induction media, not only the number and fresh weight of induced microtubers are to be considered but also other parameters such as health, dormancy period and the proportion of dry weight of microtubers to dry weight of branches should be fully taken into consideration.

Keywords: potato, microtuberization, benzylaminopourine, sucrose

P9.6 The Study of Potato's Microtuberization Responses (*Solanum tuberosum* L.) in Histological Tissue Culture Conditions to the Various Levels of Benzylaminopourine

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The potato (*Solanum tuberosum* L.) is one of the most important agricultural plants in the world which is propagated predominantly by asexual method (tubers and minitubers). This crop comprises more than half of the world's yearly cultivation of microtuberization. It is also considered as the primary portion of about one billion people diet in developing countries. Potato production during last thirty years was approximately 260-370 million tons, while the area of potato under cultivation in the same period has declined from 22 to 18 million hectares. Since this plant is of an exceedingly importance, one of the ways to for accessing normal plants is microtuberization in induction media in tissue culture condition. Therefore, the plants cultivated through mono-nodule explanation, were moved from shoot-formation induction media to MS induction media or the purpose of microtuberization of which the concentration/density of BAP (1, 2, 5 & 10 mg-^{1}) and sucrose (30, 40, 60 & 80 mg-1) were shifted in darkness. Notwithstanding no microtuberization was observed in the induction media containing 3% sucrose, the number of white branches sprouted from peripheral buds were enhanced. It is also observed that increase in BAP concentration on one hand led to decrease in the number of the aforementioned branches. In media containing 4% sucrose and with low BAP concentration (1 mgl-^{1}, 2 mgl-^{1}) shoot-formation (branching) was not perceived. While BAP in the levels (5 mgl-^{1} to 10 mgl-^{1}) was augmented in the induction media containing 4% sucrose, the growth of lateral buds accompanied by delayed microtuberization was four weeks after induction. In such media, the microtubers were created from the alteration to the growth pattern in sub-apical area with positive geotropism. In BAP 10 mgl-^{1} concentration, 50% of tubers were attached to the branches. In induction media containing 6% sucrose and BAP 1 & 2 mgl-^{1}, the microtubers were grown on peripheral branches until the end of 2nd week. In these groups, no tubers bigger than 7 mm, was seen. In sucrose with 8% concentration, the highest percentage of microtuberization was perceived in BAP

different concentrations (5mgl^{-1}, 2 mgl^{-1}, 10mg^{-1} &1mg^{-1}). In BAP 10 mgl^{-1} concentration joined by 8% sucrose, the maximal number of normal tubers attached to the pedicle/rhizome with larger dimension was formed. In such induction medium, the formation of microtubers in the 1st week was commenced and was completed in the 2nd week. The growth of microtubers continued up to the 7th week. The latency of microtubers was protracted for about 30 months.

Keywords: potato-microtuberization – benzylaminopourine - sucrose

P9.7 Expression analysis by RT-qPCR of *NPP1* gene from *Phytophthora cinnamomi*

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In recent years, a novel class of necrosis-inducing proteins, known as Nep1-like proteins (NLPs) has been identified in bacteria, fungi and oomycetes. Species of the genus *Phytophthora* are causal agents of serious plant diseases infecting *Castanea sativa*. *Phytophthora cinnamomi* secrete necrosis-inducing protein (NPP1) causing necrosis on leaf and roots of the plant, leading to death. The *NPP1* ORF contains 770 bp and encodes a 256 aa protein. The study of factors that affect *NPP1* gene expression is extremely important to better evaluate the mechanism of plant necrosis induced by *Phytophthora cinnamomi*. In order to understand its function, we proceeded to the heterologous expression in *Pichia pastoris* and we evaluated the expression in three different inducers media by RT-qPCR and SDS– PAGE. High levels of expression were obtained in a medium rich in glucose as carbon source.

Keywords: *Castanea sativa* Mill, Nep1-like proteins.

P9.8 Cloning and expression analysis of glucanase genes from *Phytophthora cinnamomi*

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Phytophthora cinnamomi is one among the most destructive species of *Phytophthora* associated to the decline of forestry, ornamental and fruit species. Associated with this oomycete is the ink disease of *Castanea sativa*. Glucan endo-1,3-β-D-glucosidase catalyzes the hydrolysis of 1,3-β-D-glucoside linkages in callose, laminarin and several carbohydrates found in the cell wall of plants and fungi. It is generally thought that glucanases play a role in plant defence by digesting wall components of the fungal pathogen. In oomycetes, glucanases have been studied at biochemical level for their possible role in hyphal tip growth and branching, where there is thought to be a delicate balance between the cell wall synthesis and hydrolysis. Fungal cell wall degrading enzyme production is influenced by a number of factors including the type of strain, the culture conditions and substrate type. The aim of this work was the analysis of homologous expression, in *P. cinnamomi,* and heterologous expression, in *Pichia pastoris*, of the endo-1,3-β-D-glucosidase encoding gene *ENDO1* produced by *P. cinnamomi*. The expression was studied during growth in different carbon sources and was also performed a time course of endo-1,3-β-D-glucosidase production. Different plasmids were used to clone the gene on each organism and we used RT-PCR analysis to examine its expression. The major expression levels occurred at the medium with glucose as carbon source. These and other results will be presented.

Keywords: *Castanea sativa* Mill, *ENDO1*, heterologous expression, homologous expression.

P9.9 Expression analysis by RT-PCR of *GIP* gene from *Phytophthora cinnamomi*

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Species of the genus *Phytophthora* secrete glucanase inhibitor proteins (*GIPs*) to inhibit the activity of enzymes involved in plant defense responses, including during plant infection process of *Castanea sativa* by *Phytophthora cinnamomi.* GIPs show structural homology to the chymotrypsin class of serine proteases (SP) but lack proteolytic activity due to the absence of an intact catalytic triad and, thus, belong to a broader class of proteins called serine protease homologs (SPH), nonfunctional because one or more residues of the essential catalytic triad is absent (His-Asp-Ser). GIPs show high homology to the S1A subfamily of SP, however questions remain about the expression patterns and potential roles of different GIPs during pathogenesis and their possible interaction with host EGases in the plant apoplast. ORF of *GIP* gene from *P. cinnamomi* encodes a 269 aa protein. In order to understand its function, we proceeded to the heterologous expression in *Pichia pastoris*. The expression was studied during growth in different carbon sources and a time course of glucanase inhibitor protein production by RT-PCR was also performed. The major expression levels occurred at the medium with glucose as carbon source.

Keywords: *Castanea sativa* Mill, glucanase inhibitor proteins.

P9.10 Production of the phytohormone indole-3-acetic acid (IAA) and ethanol by the plant trypanosomatid *Phytomonas serpens*

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Phytomonas serpens, a trypanosomatid infecting tomatoes, presents a gene encoding a pyruvate/indolepyruvate decarboxylase (*PDC/IPDC*), which shares high similarity with genes from phytobacteria. Phylogenetic analyses confirm this evidence. IPDCs convert indolepyruvate into indole-3-acetic acid (IAA), whereas PDCs convert pyruvate to the ethanol precursor acetaldehyde. The goal of this study was to investigate the functionality of *P. serpens PDC/IPDC* gene. HPLC and GC-FID analyses disclosed the presence of IAA (4µg/10^8cells) and ethanol (40mg/10^8cells) in *P. serpens* conditioned media. IAA produced by the trypanosomatid is functional *in vitro* since it promoted curvature responses and elongation of tomato hypocotyls (~15% size increase) with an effect analogous to that obtained with the synthetic auxin (9 µM). Infection of tomato fruits with *P. serpens* induced 90% and 51% enhancement of the amount of auxin conjugated with amino acids and sugar, respectively. This indicates that the *PDC/IPDC* gene is active *in vivo* and suggests that after conjugation IAA is targeted to a degradation pathway, probably to avoid compromising the fruit viability. Because PDC and IPDC display high sequence and structural similarities, we investigated the possibility that the PDC/IPDC is a bifunctional enzyme. PDC activity evaluated in semi-purified parasite lysates showed a K_M of 1.1 mM for the pyruvate substrate. Addition of 2 mM of the IPDC indolepyruvate substrate determined ~10-fold K_M increase, with a behavior typical of a competitive inhibitor. These data support the assumption that PDC/IPDC is involved in the production of IAA or ethanol according to the substrate availability.

Support: FAPESP; CNPq.

Keywords: phytoprotozoan, tomato, auxin, indolepyruvate decarboxylase, pyruvate decarboxylase.

P9.11 Assessing the suppressor activity of p20 protein from phylogenetically distinct isolates of *Citrus tristeza virus*

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One of the functions of the RNA silencing mechanism is the protection of the cell against invasion by viruses. The RNA silencing mechanism involves the cleavage of double-stranded RNA (dsRNA) by the Dicer enzymes and formation of 21-24 nucleotide small interfering RNAs (siRNAs), which then prime the degradation of homologous viral RNAs. Viruses encode silencing suppressor proteins that interfere with this response. The three distinct supressors of Citrus tristeza virus (CTV), namely p20, p25 and p23, were previously reported to target distinctly the single-cell and/or systemic levels of the RNA silencing mechanism (see a similar paper regarding p23 in these abstracts). In this study the suppressing ability of the p20 protein obtained from phylogenetically distinct isolates of CTV was assayed. Transgenic *Nicotiana benthamiana* line 16C expressing GFP was used to analyse the GFP related siRNA formation in the presence of the transient expression of GFP alone or co-infiltrated with p20. Differences of the suppressor activity were monitored visually under UV light, by fluorometry and by northern analysis. The p20 suppressor activity was higher for the stem pitting isolate 199.7 (Group 5), followed by the quick decline isolate Q3 (Group 1). Results show that, at the intracellular level, the p20 protein from severe isolates causing stem pitting and quick decline symptoms have higher suppressor ability then the mild isolates.

Keywords: Post Transcriptional Gene Silencing; RNA interference

P9.12 *Aspergillus* section *flavi* populations in cornfields of Jalisco, Mexico, and their potential for aflatoxin contamination

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Maize is a worldwide staple food for human consumption. The state of Jalisco is the main corn producer in Mexico where corn is the main agricultural product used for food and feed. It is known that aflatoxin contamination begins in the field and increases during storage. For this reason, a study was undertaken to determine the toxigenic *Aspergillus* population in maize fields and grain samples. Monitoring the levels of contamination can help suggest strategies to prevent the risk of contamination. The objectives of this research were to quantify the toxigenic populations of *Aspergillus* section *flavi* from soil and grain samples of 17 municipalities of Jalisco and determine the distribution of aflatoxin contamination in Jalisco. Both *A. flavus* and *A. parasiticus* isolates were recovered from all the sampled municipalities of Jalisco with *A. flavus* isolated more frequently than *A. parasiticus*. The greatest *Aspergillus* populations were encountered in the North Coast and South Jalisco. The S strain (small sclerotia < 400 µm in diameter) of *A. flavus* was only found in Puerto Vallarta region while 36% of the *Aspergillus* species could not be identified to species level. A total of 27% of isolates of *A. flavus* were capable of producing aspergillic acid on AFPA medium. Aflatoxin analyses (using a rapid test of AflaCheck™ by VICAM) showed only four municipalities (Tequila, Puerto Vallarta, Atotonilco El Alto y La Barca) with ≥20 ppb aflatoxin levels. To our knowledge, this is the first report of *Aspergillus* section *flavi* populations (including *A. flavus* S, T, or L morphotypes) in Mexico and its relationships with aflatoxin contamination of maize.

Keywords: aflatoxins, maize, *Aspergillus* strains

P9.13 The ambrosia fungus *Acremonium crotocinigenum* causes canker on cork oak

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The insect *Platypus cylindrus* (Coleoptera: Platypodidae) is an ambrosia beetle that transports and cultivates its symbiotic fungi in hosts trees, including the cork oak (*Quercus suber*),. The ambrosia fungi, besides being essential for the insect's survival, might play an important role by weakening the tree host, which may favour the development of pests and pathogens thus contributing to cork oak decline. *Acremonium crotocinigenum* was isolated from the insect body and its galleries in the tree. This species was identified based on its morphological and cultural characters, and from ITS analysis. Pathogenicity tests were conducted on *Q. suber* seedlings grown under greenhouse conditions and tobacco leaves were used to evaluate the phytotoxicity of fungus extracts. The inoculated plants exhibited conspicuous cankers from which the fungus was re-isolated and evident chlorotic and necrotic lesions were observed in tobacco leaves treated with fungal extracts. *Acremonium crotocinigenum* has been considered to be an ubiquitous species and its production of several compounds with antibacterial and mycotoxic activities are already known. Nevertheless, its status should be reviewed taking into consideration its pathogenicity towards *Q. suber*.

Keywords: pathogenicity, phytotoxicity, *Platypus cylindrus*, *Quercus suber*

P9.14 Evaluation of methyl-salicylate and methyl-jasmonate as inducers of priming for enhanced resistance of tomato plants against an infection by *Meloidogyne javanica*

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Plant-parasitic nematodes such as the root-knot nematode *Meloidogyne javanica* are responsible for significant agricultural losses amounting to over 100 billion dollars per year. It is very important to identify new methods of controling plant-parasitic nematodes to replace the presently used nematicides which are highly toxic and pose an environmental problem. It is possible to induce a "primed" state in plants, that is to enhance their natural defenses, by treatments with various natural and synthetic compounds. Both methyl-salicylate and methyl-jasmonate play a part in the natural immunological system of plants and as such are good candidates to function as "priming agents". Here we investigate the effect of applying (by volatilization) different concentrations of each of these phytohormones on susceptible tomato plants before infection with *Meloidogyne javanica*. The use of methyl-salicylate increased in a significant manner the number of root galls and so increased the susceptibility of tomato to the nematode instead of reducing it. The use of methyl-jasmonate did not produce such clear results thus more data must be obtained before any conclusion be taken.

Keywords: Root-knot, *Lycopersicon esculentum*, plant-nematode interaction

P9.15 The diseases of roots, stems and branches of coniferous introducents in Mardakan Arboretum, NAS of Azerbaijan

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Inspections of conifers (*Picea excelsa*, *Picea pungens*, *Pinus eldarica*, *Pinus nigra*, *Pinus pithusunda*) introduced into the Mardakan Arboretum of ANAS has established that diseases of roots, stems and branches cause damage and death of the trees. The fungal pathogens causing root rot, stem rot, twig blight, and bark canker were identified as *Heterobasidion annosum*, *Armillaria mellea*, *Cronartium ribicola*, *Ascocalyx abietina*.

P9.16 Physiological study of *Mauginiella scaettae* isolated from inflorescence rot of date palm in the region of Ouargla, Southeast of Algeria

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The anamorphic fungus *Mauginiella scaettae* causes inflorescence rot of date palms. Isolates from Algerian date palms were identified and studied under different growth conditions. Our findings show that malt extract agar (MEA) is the most appropriate medium for its growth, with the following conditions: temperature range between 25°C and 30°C, pH ranging from 4 to 8, and concentration of NaCl between 5 and 50g/L.

Keywords: Ouargla, date palm, inflorescence rot, physiology.

P9.17 Determination of deoxynivalenol in corn crop at Ardabil province (Moghan) and related Fusarium species in 2008-2009

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Ear rot of corn caused by *Fusarium* species can result in several problems. The *Fusarium* species can produce mycotoxins, such as trichothecenes that threaten the human health. Mycotoxins are the secondary metabolites of certain fungi and are produced in several agricultural products in the farm and post harvest. The amount of mycotoxin contamination is related to geographical and environmental factors, agricultural activities and the sensitivity of plants to fungal infection in all stages of growth, storage or processing. Forty (5-10 kg) samples each including 10 subsamples of corn ears collected at harvest time, kernels separated from the ears, dried and divided into two parts: one part for mycological studies and another part for toxicology studies. Nash-Snyder Agar, PDA and Czapeck's media were used for isolation of *Fusarium* species, PDA and the CLA media for identification. Detection and quantification of deoxynivalenol (DON) in the corn samples were carried out using IAC+HPLC methods. To study the potential DON production of *Fusarium* species isolates, ten isolates (two isolates of each species) were cultured on rice powder media. DON production of each isolate was measured using HPLC+IAC method after two weeks of incubation at 27–25ºC and 12ºC alternatively. To ensure the DON production of the two *F. proliferatum* isolates, these isolates were inoculated into corn ears and after ten days the amount of DON was detected in the grains. The results showed that *Fusarium* species and the frequency of isolation were *Fusarium verticillioides*, 42.3 %; *F. proliferatum* , 33.9 %; *F. moniliform*, 15.3 %; *F. nygamai*, 4.9 %; *F. oxysporum*, 3 .1 %. DON was detected in 45% of the samples. The range of DON contamination was 59.4–542 ng/g and the total mean contamination was 95.30 ng/g, which is less than the advised maximum amount of DON for corn (1 ppm) in the world. The two representative *Fusarium proliferatum* isolates were the only *Fusarium* isolates that produced DON while none of the *F. moniliforme*, *F. nygamai*, *F. oxysporum* and *F. verticillioides* isolates produced DON. The two *F. proliferatum* isolates produced DON in inoculated artificially maize grain.

Keywords: deoxynivalenol, Fusarium species, corn

P9.18 Cellular and transcriptomic analysis of host and non-host resistance of coffee to rusts (*Hemileia vastatrix* and *Uromyces vignae*)

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Timor hybrid (HDT), a natural hybrid between *Coffea arabica* and *Coffea canephora*, has been successfully used as a resistance source to leaf rust (*Hemileia vastatrix*) in coffee breeding programmes worldwide. However, recent loss of resistance of some improved commercial varieties raises the need to better characterise the resistance of some HDT genotypes, as opposed to the durable nature of non-host resistance. This work aimed to characterize the resistance of HDT 832/2 to *H. vastatrix* comparatively to its non-host resistance to the cowpea rust fungus *(Uromyces vignae)*, combining light microscopy with 454 pyrosequencing data.

Non-host resistance was typically pre-haustorial (the fungus growth ceased before the formation of haustorial mother cells – HMC and haustoria). The host resistance was closer to non-host resistance, as the fungus stopped growth more frequently at the penetration hyphae stage forming HMC with haustoria only in 6% of infection sites. The first plant responses were also similar and characterized by the hypersensitive death of stomatal cells and phenol accumulation, but they were detected earlier in the non-host (6hpi) than in the host interaction (12hpi). Time points corresponding to 50% of infection sites showing plant responses were selected for transcriptomic analyses.

cDNA libraries representing healthy and coffee leaves infected either with *H. vastatrix* and with *U. vignae* were pyrosequenced, generating ca. 293000 reads per library (8.8 Mbp), assembled into ca. 8500 contigs (average length 633bp), >15% of which are potentially non-described genes. Several contigs with homology to genes

involved in response to fungi were identified, particularly in the libraries representing infected leaves.

Keywords: 454 RNA pyrosequencing; RT-qPCR; cytology; Híbrido de Timor; durable disease resistance; hypersensitive reaction

P9.19 Expression profile of superoxide dismutase in *Coffea arabica* – *Hemileia vastatrix* interactions

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The activity of the antioxidant enzymes superoxide dismutases (SODs) was investigated in *Coffea arabica* leaves inoculated with a virulent and an avirulent race of *Hemileia vastatrix* (compatible and incompatible interaction). Biochemical studies have shown that in the incompatible interaction SODs activity increases by 17-24 hpi, prior to cell death observation (hypersensitive reaction-HR). On the contrary, in the compatible interaction no significant changes in SODs activity were observed at this early stage of the infection process. However by 48 hpi an increase in SODs activity was observed for both interactions, which was significantly different from healthy leaves. Transcriptional expression of coffee *Cu/Zn-sod*, *Fe-sod* and *Mn-sod* genes were studied using RT-qPCR analysis. No significant activation was recorded for *Cu/Zn-sod* and *Fe-sod* genes. In contrast, an activation of *Mn-sod* gene was obtained in the incompatible interaction by 17 hpi, which was delayed in the compatible interaction. Transcriptional activation of *Mn-sod* suggests a contribution of this gene for the increase of SODs activity observed 17-24 hpi, playing a role in the early apoptotic events of the HR in *C. arabica–H. vastatrix* incompatible interaction.

Keywords: hypersensitive reaction, enzymatic activity, RT-qPCR, coffee leaf rust

SESSION 10 (SPECIAL SESSION): INTERNATIONAL COOPERATION ON COFFEE RUST RESEARCH - A LEGACY OF PROFESSOR BRANQUINHO D'OLIVEIRA

SS10.1 Coffee Rusts Research Centre: Fifty Five Years Devoted to International Cooperation

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Coffee Rusts Research Centre (CIFC), a unit of the Tropical Research Institute (IICT), was founded in 1955 by the prominent plant pathologist Prof. Branquinho D'Oliveira. Since then, the main purpose of CIFC is to internationally centralise research on coffee leaf rust – CLR (*Hemileia vastatrix*). CIFC has played a central role in developing an International Research Network of more than 40 coffee growing countries (CGC) on CLR, and more recently also on coffee berry disease - CBD (*Colletotrichum kahawae*). Combining applied and fundamental research, the main goal of CIFC research activities has been to obtain coffee cultivars resistant to both diseases.

The main activities are to: discover sources of resistance on *Coffea* spp. to CLR and CBD; characterise the genetic nature of such resistances; identify pathotypes; supply coffee resistant material to coffee breeding institutions; provide consultancy and training of technicians and researchers from CGC; study cytological, biochemical and molecular mechanisms of coffee resistance and pathogenicity of *H. vastatrix* and *C. kahawae*; analyse molecular variability of these pathogens. Other pathosystems studied include coffee - *M*eloidogyne *exigua* and olive - *Colletotrichum acutatum*.

The research enabled to establish unique world germplasm collections (*Coffea* spp., *H. vastatrix* and *C. kahawae*), to characterise over 45 *H. vastatrix* races, to identify nine resistance genes in coffee, to determine *C. kahawae* pathotypes and to select coffee varieties with resistance to both diseases. Over 90% of rust resistant coffee varieties cultivated in the world were created from these studies.

CIFC has also a strong collaboration with European Research Institutions through EU and national projects.

Keywords: CIFC, Coffee, Rust, CBD, Resistance

P11.1 Impact of the pinewood nematode, *Bursaphelenchus xylophilus*, on gross calorific value of *Pinus pinaster* biomass

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease, has been detected in several regions of Portugal affecting *Pinus pinaster*, a coniferous species of a great economic value for the country. The nematodes, migrating through resin canals and feeding on parenchyma cells, cause the development of cavitation, plugging of tracheids, and decrease of xylem water contents. They also induce quick metabolic changes in ray parenchyma cells in cavitation areas, denaturation and necrosis of parenchyma and cambial cells. The objective of this research is to understand how anatomic changes of wood and biochemical incidences of tree defense reaction affect the gross calorific value (GCV) and chemical composition of wood. This interdisciplinary study stresses important technological and economic aspects, namely suitability of use of infested pinewood by woodworking industries, and interrelationship between economic criteria and disease control activities. In order to evaluate technological aptitude of infested pinewood biomass as a raw material for pellet production, a comparative analysis of GCV of seventeen infested wood samples, with different values of PWN/100g, and non-infested samples was performed using a Parr 6300 automatic isoperibol calorimeter. Comparative analysis of chemical composition of infested and non-infested wood was also performed. The results revealed statistically significant differences between the GCV of infested and non-infested wood. The carbon, oxygen, sulfur and ash contents did not differ significantly, while hydrogen and nitrogen contents were significantly different. The overall conclusion is that PWN-infested *P. pinaster* wood is suitable for use as a raw material for energy industries.

Keywords: disease control activities, economic criteria, heating value, pine wilt disease, technological aptitude of infested pinewood.

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