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ABSTRACTS

Abstracts of invited talks, oral and poster presentations given at the 15th Congress of the Mediterranean Phytopathological Union, June 20–23, 2017, in Córdoba, Spain

The 15th Congress of the Mediterranean Phytopathological Union entitled "Plant health sustaining Mediterranean Ecosystems", was held in Córdoba, Spain on June 20–23, 2017. The mission of the meeting was to promote dissemination of the latest scientific advances and encourage dialogue, interaction and collaboration between researchers from different disciplines interested in all aspects of Phytopathology. More than 200 participants from 26 countries attended the congress, making this an outstanding scientific event. The presentations covered a broad range of aspects related to plant diseases including Genome Analysis, Invasive Emerging Pathogens, Integrated Disease Management, Food Safety, New Tools In Diagnostics and Management, Molecular Pathogen-Host Interactions, Biocontrol, Epidemiology and Modelling, and Microbiomes and their Role in Plant Health. Abstracts of the invited talks, and the oral and poster presentations are given in this issue.

Key note lectures

Olive quick decline and *Xylella fastidiosa* in Southern Italy: the state of the art. D. BOSCIA, M. SAPONARI. *CNR – Institute for Sustainable Plant Protection, University of Bari, Via Amendola 122/D, 70126, Bari, Italy. E-mail: donato.boscia@ipsp.cnr.it*

The identification in 2013 of an outbreak of *Xylella fastidiosa* (Xf) in olive groves in the Salento peninsula (southern Italy) resulted in a plant health emergency of unprecedented proportions for the EU. Infected olive trees show extensive canopy desiccation and severe quick decline symptoms. In the outbreak area, the bacterium was found to be efficiently spread by the meadow spittlebug *Philaenus spumarius*, abundant on the olive canopies during the dry season. The initial demarcated foci rapidly expanded over the following 4 years, establishing a new demarcation line 80 km from the first reported outbreak; while few species were found infected in 2013 the currently known susceptible hosts reached approx. 30 different plant species. Phytosanitary measures to combat the spread and mitigate the impact of the bacterial infections, included restrictions for new plantations and movement of propagating materials, and removal of infected trees. The severe damage and the imposed phytosanitary restrictions caused severe economic and social impacts in the local community, raising concerns against the containment measures and failure to implement timely, effective and coordinated preventive measures. Due to the novelty of the *Xylella*-associated disease in olives and the new outbreak in the EU, the EU Commission mobilized dedicated resources to build research activity to address research gaps for this emerging pathogen. Between 2015 and 2016, two projects in the H2020 framework have been funded. These are: "Pest Organisms Threatening Europe" (POnTE) and "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy" (XF-ACTORS), the latter exclusively targeting *Xf*. From the increased research activity developed in the past 3 years, new knowledge is providing data on the genetic and biological properties of the Xf population, the host range, vector identification and biology, and identification of olive cultivars with promising resistance traits.

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Keeping up with the plant destroyers in the post-genomics era. S. KAMOUN. *The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom. http://www.KamounLab.net*

Infectious plant diseases cause severe losses in world agriculture, and threaten to slow laudable efforts to launch a second green revolution to meet the food security needs of a rapidly increasing world population. Pathogens such as the rice blast fungus, wheat stripe and stem rusts, the Irish potato famine pathogen, and many others continue to trigger recurrent epidemics with far-reaching consequences. When faced with these threats, knowledge of the pathogens is essential. The genome sequence of a plant pathogen is a deep look into its soul. From important and often unexpected insights into the biology of the pathogen to the tools for surveillance and diagnosis, the genome is an invaluable resource that accelerates research and output delivery. The costs of genome sequencing are rapidly decreasing, and genome sequences can rapidly provide knowledge to develop DNA markers, outline population structures, and indicate pathogen origin. These This paper discussed ways in which genome biology impacts plant pathology, particularly, how pathogen genomics drive basic and applied plant pathology, and how new findings on pathogen biology can be exploited for development of new approaches to breeding disease-resistant crops. Detailed knowledge of pathogen genomes coupled with novel methods of plant genome editing is ushering the era of next-generation disease resistance breeding in plants.

***Fusarium* pathogenomics: understanding fungal pathogenicity through genomics.** L.-J. MA. *Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst MA, USA. E-mail: lijun@biochem.umass.edu*

The root-infecting fungal pathogen *Fusarium oxysporum* causes vascular wilt in over 100 different plant species. These pathogens produce thick-walled resting structures that remain viable for long periods, making disease control challenging. This study takes a systems biology approach to dissect the molecular mechanisms underlying fungal pathogenesis and host defense using the *F. oxysporum-Arabidopsis* pathosystem. Comparative genomics and comparative meta-transcriptomics were employed to study compatible (inoculation of a *F. oxysporum* strain results in diseased plants) and incompatible (*F. oxysporum* inoculation has no negative effect on plant health) interactions by inoculating the same plant host (Col-0) with different *F. oxysporum* isolates. The study focused on the "*primary determinative phase*", including fungal penetration and colonization from the cortex to xylem. Comparative study enables identification of genes and pathways that contribute to the *co-evolutionary arms race* between wilt pathogens and their hosts. Distinct sets of genes from two *F. oxysporum* strains contribute to the different disease phenotypes. Plant genes involved in pathogen-associated molecular patterns triggered immunity (PTI) were induced in compatible and incompatible interactions, while there were more distinct expression profiles for genes involved in effector-triggered immunity (ETI) in two different interactions.

This research was supported by a National Science Foundation CAREER award (1652641), a Burroughs Wellcome Fund Investigator award (1014893), a National Institute of Food and Agriculture National Research Initiative Hatch Grants Program grant (MAS00441), competitive Grants (2008-35604-18800 and MASR-2009-04374) and a seed grant from Massachusetts Green-energy High Performance Computing Center.

Understanding host specificity via comparative genomics. D.S. GUTTMAN. *Department of Cell & Systems Biology. University of Toronto, Toronto, Ontario, Canada. E-mail: david.guttman@utoronto.ca*

The *Pseudomonas syringae* species complex includes diverse lineages that can infect numerous agricultural and wild plant species. The recent widespread application of next-generation sequencing technology has greatly expanded understanding of the genetic structure of this species complex, although there is still limited understanding of the specific genetic factors underlying niche specificity. We present a comparative and evolutionary genomic analysis of >400 strains of *P. syringae*, including 62 type and pathotype strains. We specifically focussed on the compositional dynamics of the genome, the impact of selection and recombination on genes associated with host adaption, and the use of association tests to identify previously unrecognized genes with host-specific associations. We found that virulence-associ-

ated genes are more likely to be recombinogenic and under selection, and that there is a strong signal for inter-pathogroup recombination. This inter-pathogroup recombination of niche-associated genes is likely to be critical for maintaining genetic cohesion, and thereby delimiting the species complex.

Will climate change affect IPM in the Mediterranean environment? I. PERTOT[1,2], E. ECCEL[2], A. ALIKADIC[3], C. DOLCI[3], C. ZARBO[3], A. CAFFARRA[2], R. DE FILIPPI[3], C. FURLANELLO[3]. [1]*Center Agriculture Food Environment, University of Trento via E. Mach 1, 38010 TN, Italy.* [2]*Predictive Models for Biomedicine and Environment, Fondazione Bruno Kessler, via Sommarive 18, 38123 Povo, Trento, Italy.* [3]*Research and innovation Centre, Fondazione Edmund Mach, via E. Mach 1, S. Michele all'Adige, 38010 Tn, Italy. E-mail: ilaria.pertot@fmach.it*

In the nearfuture, climate change is expected to have significant influences on the agricultural sector, and particularly on plant protection, due to temperature increase and variation in precipitation. Temperature, rain and relative humidity are the main environmental factors influencing disease epidemiology. Several studies have assessed, global and regional scales, the effects of temperature increase in the last century, and predicted future trends of climate change. Although there is uncertainty in climate-modeling, especially at the regional scale, there is general consensus that global average surface temperature will increase and precipitation will vary. However, translation of climate prediction into accurate quantification of the impacts on biological systems, particularly forpathogens and related diseases, has not been attained. This is for several reasons including: i) incomplete understanding of short and long term effects of climate on the complex interactions among plants, pathogens and their biocontrol agents, and the lack of accurate models that capture this complexity; ii) the natural seasonal variability of weather; iii) inability to accurately predict temperature and relative humidity changes at local and microenvironment levels; and iv) unpredictable or unrecognized factors that may affect disease epidemiology (e.g. variation in pathogen virulence). The uncertainty of predictions and the impacts of climate change on the efficient implementation of IPM in the Mediterranean region will be discussed.

Forest pathogen invasion: pathways, surveillance and early detection. A. SANTINI[1], L. GHELARDINI[1,2] D. MIGLIORINI[1,3] A.L. PEPORI[1], N. LUCHI[1]. [1]*Institute for Sustainable Plant Protection, CNR, Via Madonna del Piano, 10 50019 Sesto Fiorentino, Italy.* [2]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente DiSPAA, Università di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy.* [3] *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. E-mail: alberto.santini@cnr.it*

Human-driven species expansion has greatly increased during the last century, as a consequence of the unprecedented growth of international travel and trade, resulting in disturbance to ecosystems and severe socio-economic impacts. In plants, emerging infectious diseases (EIDs) are tightly linked to biological invasions. More than half of the world plant EIDs in the last few decades have resulted from the arrival of previously unrecognized pathogens. Many studies confirm that the main pathway of entrance of pathogens was the trade of living plants, and the trade of ornamental woody plants plays a role of primary concern. These observations should focus attention on the risk inherent in the trade of ornamental plants for planting in soil, which also constitutes the main pathway of introduction of pests. This pathway is particularly insidious because invasive harmful organisms are not easily detectable in soil, and they are, in addition, almost unknown and neglected in their native ranges. Several unexpected introduction pathways are becoming of increasing importance. Eradication is likely to to be impossible, so increasing surveillance and prevention by early detection of new introductions are among the few reliable prevention measures, although detection is difficult in the face of global mobility and climate change.

Emerging pathogens as a consequence of globalization and climate change. M.L. GULLINO[1,2], G. GILARDI[1], A. GARIBALDI[1]. [1]*Centre of Competence for Agro-Environmental Innovation (AGROINNOVA) and* [2]*Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Torino, Italy. E-mail: marialodovica.gullino@unito.it*

Many new diseases caused by soil-borne and foliar pathogens have been recorded for the first time worldwide, on a number of crops, resulting from globalization and climate change. The horticultural sector is one of the most affected by the spread of new diseases. Production of leafy vegetables (including lettuce, rocket, spinach, basil and lamb's lettuce, grown as ready-to-eat sector products, is a good case study. Italy is the second largest producer of fresh-cut leafy vegetables in Europe. During recent years many new pathogens have been recorded, causing severe losses. Among foliar diseases, those caused by *Fusarium equiseti* on wild and cultivated rocket and lettuce, *Allophoma tropica* on lettuce, *Colletotrichum kahawae* on cultivated rocket, *Myrothecium roridum* on lamb's lettuce and *M. verrucaria* on spinach and wild rocket were observed. *Fusarium oxysporum* f. sp. *lactucae*, present in Europe since 2002, is spreading in new countries. A new race has been recently isolated from lettuce for the first time in the Netherlands, while race 1 of this pathogen has been found very recently in France. Some of these new pathogens are seed transmitted. The globalization of the seed market is a major cause of the rapid spread of such pathogens. Some of the newly introduced pathogens, tytical of warmer areas, are spreading due to the increased in temperatures. The possible influences of globalization and climate change on the appearance and spread of new pathogens will be discussed.

This research was supported by the European Union's Horizon 2020 research and innovation programme "Effective Management of Pests and Harmful Alien Species - Integrated Solutions"(EMPHASIS).

Integrated management strategies for prevention and control of mycotoxins. M.D. KAMINIARIS, M.C. ILIADI, C.S. LAGOGIANNI, O. RAFTOPOULOU, I. DANELI, M. ANDRIOLATOU, A.A. GKATZOUNI, E.F-N. VARVOUNI, A.S. ARSENI, A. BENAKIS, M.G. DIMAKOPOULOU, D.I. TSITSIGIANNIS. *Laboratory of Phytopathology, Department of Crop Science, School of Agricultural Production, Infrastructure and Environment, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece. E-mail: dimtsi@aua.gr*

Mycotoxin contamination of agricultural commodities poses one of the greatest threats for safety and quality of food and feed. There is a continuous risk of mycotoxins "from the farm to the fork", and climate conditions and/or production practices have impacts by favouring growth of mycotoxigenic fungi and mycotoxin production. The economically effective solutions are those that, with assistance from agricultural precision technology, will contribute to the exclusion of fungi from the plant hosts. Restriction of mycotoxin production in plants, or downstream in production lines, with Integrated Pest Management (IPM) systems, will provide effective, durable and environmentally sustainable mycotoxin control. IPM strategies of mycotoxigenic fungi and mycotoxins in pistachios (aflatoxins), grapes (ochratoxins), corn (aflatoxins, fumonisins) and barley are outlined, that are based on epidemiology, breeding of less susceptible plant genotypes and evaluation of resistant/tolerant varieties, evaluation of biocontrol products and fungicides. For biocontrol, a large number of endemic isolates of yeasts, bacteria and non-toxigenic *Aspergillus flavus* were tested in laboratory and field studies. Isolates were found to inhibit production of mycotoxins. Additional experiments to evaluate several fungicides of different chemical groups led to effective commercial formulations that can reduce the aflatoxin contamination by up to 100%. Epidemiological data and identification of critical pre- and post-harvest control points points that influence the mycotoxin production is crucial for developing predictive systems to reduce the mycotoxin biosynthesis.

Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi. F. BI[1,2], S. BARAD[1], A. DUBEY[1] , D. KUMAR[1], V. CASADO[3] , J. DIAZ MÍNGUEZ[3], E. ESPESO[4], D. PRUSKY[1]. *[1]Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel. [2]Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, and Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, Guangzhou 510640, China. [3]Department of Microbiology and Genetics, CIALE, Universidad de Salamanca, Salamanca, Spain. [4]Department of Molecular and Cellular Biology, Centro de Investigaciones Biológicas (C.I.B.), Madrid, Spain. E-mail: dovprusk@volcani.agri.gov.il*

Fruit pathogens can contribute to acidification or alkalization of host environments. This capability has

been used to categorize fungal pathogens into acidifying and/or alkalizing classes. We have shown that diverse classes of fungal pathogens, including *Colletotrichum gloeosporioides*, *Penicillium expansum*, *Aspergillus nidulans,* and *Fusarium oxysporum*, secrete small pH-affecting molecules. These molecules modify environmental pH that dictates acidic or alkaline colonizing strategies, and induce expression of PACC-dependent genes. In many organisms, acidification is induced under carbon excess, i.e. 175mM of sucrose (the most abundant sugar in fruits). In contrast, alkalization occurs under conditions of carbon deprivation, i.e., less than 15mM sucrose. The carbon source is metabolized by glucose oxidase (*gox2*) to gluconic acid, contributing to medium acidification, whereas catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (*gdh2*) results in the secretion of ammonia. Sucrose concentration also affects secondary metabolite accumulation, suggesting the importance of fruit sugar content for fungal metabolism. Our results have indicate that differential pH modulation by fruit fungal pathogens is a host-dependent mechanism, affected by host sugar content, which modulates environmental pH to enhance fruit colonization.

Using population genomics to identify genes affecting pathogen adaptation. B.A. McDONALD[1]. [1]*Plant Pathology Group, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland. E-mail: bruce.mcdonald@usys.ethz.ch*

Zymoseptoria tritici causes Septoria tritici blotch, the most damaging wheat disease in Europe. This fungus is globally distributed and is well adapted to a wide range of climatic conditions. *Zymoseptoria tritici* populations in Europe and North America evolved rapidly to become resistant to fungicides and virulent on resistant wheat cultivars. Little is known about the genetic basis of pathogen adaptive traits such as melanization, thermal sensitivity, host specialization and fungicide resistance. We used QTL mapping, based on 700 progeny from two crosses among four Swiss parental strains, and analyses of genome-wide associations (GWAS) based on 150 strains drawn from four global field populations, to elucidate the genetic architecture of these and other adaptive traits. Finished genome sequences and extensive RNA-seq profiles from across the infection cycle were obtained for all four parents used to make the mapping populations. RADseq generated more than 17,000 segregating SNP markers for the QTL mapping analyses, while comparisons of entire genome sequences provided over 700,000 informative SNPs for the GWAS. While genotyping has become easy, phenotyping remains difficult and limits progress in most marker-trait association studies. We developed high-throughput phenotyping methods to accelerate progress. Automated analyses of digital images generated approx. 2.7 million phenotypic measurements associated with melanization, virulence, host specialization, fungicide sensitivity and thermal adaptation. Significant QTLs and marker-trait associations were found for every trait, and several candidate genes were identified. Several of the candidate genes have been functionally validated, including three encoding small secreted proteins acting as pathogen effectors.

This research was supported by the Swiss National Science Foundation and ETH Zurich.

***Pseudomonas syringae* pathogenesis of plants: effectors and immunity.** J.R. ALFANO. *Center for Plant Science Innovation, University of Nebraska, Lincoln, Nebraska, 68588 U.S.A. E-mail: jalfano2@unl.edu*

The bacterial pathogen *Pseudomonas syringae* uses a type III secretion system to inject type III effector proteins into plant cells to favour pathogenicity. When plants are infected by pathogens, two types of plant immunity can be triggered. Conserved molecules, known as pathogen-associated molecular patterns (PAMPs), can be recognized by surface-localized receptors known as pattern recognition receptors (PRRs), which induce pattern-triggered immunity (PTI). Pathogen effectors can be recognized by specific NOD-like receptors (NLRs) leading to effector-triggered immunity (ETI). The majority of type III effectors in *P. syringae* pv. tomato DC3000 can suppress PTI and/or ETI. An update is presented on type III effectors currently being investigated. One of these is HopE1, which was recently shown to use the calcium sensor calmodulin as a co-factor, and to target MAP65-1, a microtubule-associated protein 65, which functions in the *Arabidopsis* microtubule network. A recently commenced project is focussing on

Arabidopsis orosomucoid (ORM) proteins and their involvement in plant immunity. ORMs are known regulators of sphingolipid biosynthesis. *Arabidopsis* plants over-expressing ORM1 or ORM2 lack signaling from the FLS2 PRR, and are of increased susceptibility to *P. syringae*. Moreover, plants over-expressing ORM1/2 are greatly reduced in their FLS2 levels; ORM1/2 mutants have enhanced levels of FLS2. The molecular explanation behind these phenotypes is presented.

New bio-inspired treatments derived from microbiome and metabolome studies. M. LORITO[1], S.L. WOO[2]. [1]*Department of Agricultural Sciences, and* [2] *Department of Pharmacy, University of Naples Federico II, Via Università, 100, 80055 Portici (Naples) Italy. E-mail: lorito@unina.it*

A new generation of bio-inspired products, useful for disease control and bio-fertilization, is reaching market development phases. Some are derived from formulations and technologies, mainly based on microbes and bioactive molecules, applied for a long time in biological control. However, the new products have been optimized for efficacy, reliability and cost, also using data generated by recent -omics and plant-microbe interaction studies. Other products are based on novel concepts, which may be designed and constructed by assembling small beneficial microbiomes, where microbe combinations are defined by using new knowledge on factors and molecular mechanisms positively affecting crop vigour, yield and resistance to biotic and abiotic stresses. Two new formulations have been recently marketed in about 70 countries for specific application on wheat and corn. More products are in the pipeline, particularly for application on Mediterranean crops. Their development will be supported by the recently established initiative Partnership for Research and Innovation in the Mediterranean Area (PRIMA), a large (approx. 500M Euro) research programme involving about 20 countries. PRIMA is devoted to the use of "innovative solutions" and to"promote their adoption for improving the efficiency and sustainability of food production and water provision" in Mediterranean agriculture. The programme has a major focus on novel and sustainable approaches to reduce the impacts of pests and pathogens.

Can we breed for durable disease resistance in pea and faba bean? The cases of broomrapes and powdery mildews. D. RUBIALES, M. FERNANDEZ-APARICIO, A.M. VILLEGAS-FERNANDEZ, S. FONDEVILLA. *Institute for Sustainable Agriculture, CSIC, Avda. Menendez Pidal s/n, 14004, Córdoba, Spain. E-mail: diego.rubiales@ias.csic.es*

Legume crops, such as pea and faba bean, can be damaged by a number of diseases, but insufficient levels of host resistance are available in most instances. As a result, only cultivars with moderate levels of resistance are available to farmers. Two important groups of diseases are caused by distinct groups of biotrophic pathogens: parasitic weeds (the broomrapes) and airborne fungi (the powdery mildews). Several clearly distinct species of these pathogens are known (the broomrapes *Orobanche crenata*, *O. foetida* and *O. aegyptiaca*; or the powdery mildews *Erysiphe pisi* and *E. trifolii*). These pathogens can infect the same legume crops. Historic and recent achievements are reviewed for pea and faba bean, and compared with those experienced in other non-legume crops. Implications in resistance breeding will be critically discussed, with a special focus on potential durability of host resistance.

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Plant disease epidemics and food security. S. SAVARY. *AGIR, INRA, Université de Toulouse, INPT, INP-EI PURPAN, Castanet-Tolosan, Centre Inra Occitanie-Toulouse, France. E-mail: serge.savary@inra.fr*

There is increasing interest in linking plant disease epidemics and crop health with global food security. Recent studies have revisited the possible linkages between the occurrences of plant disease epidemics and major historical events. Aside from their dramatic effects on food systems, plant disease epidemics may also cause regular, progressive attrition in agrosystem performance. A useful way to address the impacts of plant diseases on food security is through the different components of food security, including: (1) primary food production; (2) imports and stockpiles; (3) physical access to food; (4) economic access to food; (5) stability of food availability; and (6) quality and nutritive value of food. All six components

can be affected by plant diseases, to varying degrees, depending of individual cases. Another approach considers different spatio-temporal types of plant disease epidemics, including: (1) chronic (occurring regularly over very large areas, usually leading to moderate losses); (2) acute (occurring occasionally over limited areas, leading to important losses); or (3) emerging (occurring over expanding areas, leading to variable losses). Epidemiological modelling can contribute to assessment and comparison of plant disease impacts on food security. This assessment can then contribute to informing decisions in policy-making and research prioritization and planning. Examples of epidemiological analyses are provided for some major world food crops.

Role of epidemiological models in decision making for crop protection. V. ROSSI[1]. [1]*Department of Sustainable Crop Production, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84 29122 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it*

Traditionally, growers strongly rely on calendar applications of chemical pesticides. Directive 128/2009/EC makes integrated pest management (IPM) mandatory across Europe, to reduce the negative impacts of pesticides on human health and the environment. A key principle of IPM is to protect crops only when it is necessary, i.e., when there is risk for pathogens to develop, attack plants and cause damage. Weather is one of the main drivers for disease development. Relationships between weather conditions and pests and diseases have long been studied. In recent years, however, new approaches have increased our ability to investigate and understand these complex relationships. Similarly, advanced modeling techniques have made it possible to incorporate this knowledge in a new generation of mechanistic models, able to produce accurate and robust disease predictions. Advances in information and communication technologies have allowed incorporation of models into decision support systems (DSSs), and to effectively deliver these to growers. DSSs are now characterized by: (i) holistic vision of crop management problems and their interactions; (ii) incorporation of mathematical prediction models for plant growth and development, disease development, and fungicide modes of action of fungicides; (iii) provision of information on the focus of decisions as easy-to-understand decision supports; (iv) easy and fast access through the Internet; and (v) two-way communication between users and providers. These characteristics make it possible to consider context-specific information, such as crops and varieties, and soil characteristics, in addition to weather data.

The plant microbiota: mycorrhizal fungi and all the others. P. BONFANTE. *Department of Life Science and Systems biology, University of Torino, Viale Mattioli 25, 1025, Torino, Italy. E-mail: paola.bonfante@unito.it*

Plants have specific microbiotae, which may exert powerful effects on their health. Several studies of plant microbiota have focused on identification of microbial biodiversity on roots or epigeous organs, and have detected influences of plant genotype on the microbiota composition. Bacteria and fungi with beneficial functions, such as root symbionts and plant growth-promoting rhizobacteria, coexist with endophytes, saprotrophic microbes, and also with some pathogens. However, studies seeking to understand how plants build up their microbiota, or whether there are relationships between the microbiota and plant genotypes, are rare. Arbuscular mycorrhizal (AM) fungi are common members of root microbiota in wild and agricultural ecosystems, where they improve mineral plant nutrition, and in turn receive reduced carbon. They offer good tools for unravelling how plants respond to beneficial microbes. Using a combination of cellular, genetic and molecular approaches, we have demonstrated how phosphate is uptaken by the AM *Gigaspora margarita*, and is released to host plants through activity of fungal and plant phosphate transporters active in different rhizospheric and root compartments. A transcriptomic data set developed for tomato fruit led to the characterization of an additional phosphate transporter, which well responds to phosphate availability and mycorrhization. We conclude that improving the nutritional status and by affecting the source-sink relationships of whole plants, mycorrhizal fungi, as plant microbiota members, have strong impacts on plant nutrition and health.

This research was supported by the Project Mycoplant (CSP and Unito), Mycoceres, Green-Rice and 60% UNITO funds.

The plant microbiome: beyond collecting stamps. J.M. RAAIJMAKERS[1,2], M. MEDEMA[3], V. TRACCANA[3], M. DE HOLLANDER[1], J. PEREZ-JARAMILLO[1], V. CARRION BRAVO[1]. [1]*Netherlands Institute of Ecology (NIOO-KNAW), Netherlands.* [2]*Institute of Biology, Leiden University, Netherlands.* [3]*Wageningen University, Department of Bioinformatics, Netherlands. E-mail: J.Raaijmakers@nioo.knaw.nl*

Plant roots are colonized by many microorganisms, poulations of which can reach cell densities much greater than the number of plant cells. Various studies have shown that members of the plant microbiome contribute to plant tolerance to abiotic (e.g. drought) and biotic (e.g. pathogens) stress factors, but also to plant nutrition, growth and development. For the majority of plant-associated microorganisms, however, there is limited knowledge on their support functions and the mechanisms involved. Novel -omics technologies have provided in-depth knowledge of the diversity and functioning of plant microbiomes and significant advances are being made to uncover mechanisms, genes and metabolites involved in the multi-trophic interactions in these microbiomes. To better understand this complexity, reductionist and systems approaches are needed to identify the biotic and abiotic factors involved in microbiome assembly and activity. New results are presented on the role of rhizosphere and endosphere bacteria in protection of plants against soil-borne pathogens. For rhizosphere bacteria, we have shown that representatives of the Proteobacteria protect plants from pathogen infection by the production of chlorinated peptides and alteration of root architecture and plant growth via modulation of sulfur assimilation. In-depth metagenomic sequencing of the endosphere allowed *de novo* assembly of high quality bacterial genomes, and revealed various yet unknown biosynthetic genes and pathways with potential for plant protection and antibiotic discovery. An overview is presented on the wealth of genes and functions of the plant microbiome.

Innovative remote sensing and species distribution modeling to detect and predict the potential spread of *Xylella fastidiosa*. J.A. NAVAS-CORTÉS. *Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Menéndez Pidal s/n, 14004 Córdoba, Spain. E-mail: j.navas@csic.es*

Control of *Xylella fastidiosa*, once established in a territory, is difficult to attain, so efforts should focus on development of preventive measures. Remote sensing has been shown to be a useful decision support tool for crop management, through early detection and implementation of surveillance programmes that assist limitation of pathogen spread to new areas. A collaboration between JRC-European Commission and the POnTE consortium was established to develop a robust and accurate method for the automatic classification of *X. fastidiosa* infection and disease severity at large scales. Remote sensing information can be combined with species distribution models (SDMs), that determine relationships between sampled locations for a species and associated environmental variables, and these are used to estimate the ecological requirements of the species. SDMs provide realistic scenarios to explain the influence of bioclimatic variables on the epidemiology of plant diseases, particularly those caused by "new" plant pathogens. We used correlative niche models to quantify and map the global patterns of the potential geographic distribution of *Xylella fastidiosa*. Overall, projected potential distribution from estimated models conformed well with the current known distribution of *X. fastidiosa*. The application of SDMs to the most prevalent *X. fastidiosa* subspecies will be discussed.

This study was supported by the European Union's Horizon 2020 research and innovation programme, under grants agreement No. 635646 Pest Organisms Threatening Europe (POnTE) and No. 727987 *Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy (XF-ACTORS).

Grapevine trunk diseases: a need for clarity in concepts and definitions. G. SURICO, L. MUGNAI. *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: giuseppe.surico@unifi.it*

Grapevine wood diseases (more than 20 of these can be listed), usually called Grapevine Trunk Diseases (GTD), became a major and increasing problem for vinegrowers in the last decades. Besides the economic impacts of GTDs, there is in recognition, description, attribution and nomenclature for some of them. At the beginning of the 20th century, several authors

paid considerable attention to various grape diseases, but there was confusion in their attempts to explain symptoms and development over time. Examples include "mal nero", folletage and California disease. Symptoms of what are now known to be virus diseases were frequently attributed to the actions of fungal pathogens. In the case of esca, all symptoms recorded on the leaves (i.e., the well-known "leaf stripes" and others) were thought to be the effects of white rot of vine wood. Something similar is happening today with the introduction of some "new" GTDs. Different reports and lack of knowledge has led to the introduction and use of disease names that should now be revised and updated in the light of new knowledge for the esca complex, and for grapevine trunk diseases in general. It is important to clarify what was traditionally linked to wrong interpretations, and to use disease names that can be useful to share knowledge. These disease names should be based on official parameters applied in the naming of plant diseases.

Communications

Invasive pathogens and new emerging plant diseases

Establishment potential of citrus black spot, caused by *Phyllosticta citricarpa*, in Mediterranean environments. J. MARTÍNEZ-MINAYA[2], D. CONESA[2], A. LÓPEZ-QUÍLEZ[2], A. VICENT[1]. [1]*Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada 46113, Valencia, Spain.* [2]*Departament d'Estadística i Investigació Operativa, Universitat de València. Burjassot 46100, Valencia, Spain.*

Citrus black spot (CBS), caused by *Phyllosticta citricarpa*, is the main fungal disease of citrus worldwide, causing external fruit blemishes and yield losses. The Mediterranean Basin is free of the disease, so phytosanitary measures are in place to avoid the entry of *P. citricarpa* in the EU. However, the suitability of Mediterranean climates for CBS establishment is debated. As a case study, an analysis of climate types and environmental variables in South Africa was conducted to identify potential associations with CBS distribution. In 1950, CBS was confined to climates with summer rainfall (Cw, Cf). The disease later spread to drier regions, and the hot arid steppe (Bsh) is the main climate region where CBS now develops. The disease was not detected in the Mediterranean-type climates (Csa, Csb). Arid steppe (Bs) climatesare common in important citrus areas in the Mediterranean Basin. Hierarchical Bayesian analyses were also conducted by considering latent Gaussian models, which allowed the use of the integrated nested Laplace approximation (INLA) methodology. The spatial effects were implemented with the stochastic partial differential equation (SPDE) approach. Spatial models outperformed non-spatial models in the 1950 dataset. Problems of model convergence were detected in 2014 due to the strong spatial structure of CBS. Spatial models with principal components for 1950 had better classification accuracy of CBS distribution in 2014 than non-spatial ones. Therefore, previous models based solely on climate may underestimate the potential geographical distribution of this disease.

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Advances on the study of emerging *Southern tomato virus* infecting tomato crops in the Mediterranean basin. L. ELVIRA-GONZÁLEZ[1], C. CARPINO[1,2], A.V. PUCHADES[1], A. ALFARO-FERNÁNDEZ[3], M.I. FONT-SAN AMBROSIO[3], L. RUBIO[1,4], L. GALIPIENSO[1,4,5]. [1]*Instituto Valenciano de Investigaciones Agrarias (IVIA), Ctra. CV-315, 46113 Moncada, Valencia, Spain.* [2]*Department of Agricultural and Forestry Science, University of Palermo, Piazza Marina 61, 90133 Palermo, Italy.* [3]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.* [4]*Euro-Mediterranean Institute of Science and Technology (IEMEST), Vía Michele Miraglia 20, 90139 Palermo, Italy.* [5]*Departamento de Biotecnología, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.*

Southern tomato virus (STV; genus *Amalgavirus*, family *Amalgaviridae*), has a double stranded RNA genome. STV has been detected in different tomato (*Solanum lycopersicum)* varieties showing symptoms of stunting, and fruit discoloration and reduced size. This virus was first detected in North America, and recently

in Asia and the Mediterranean basin (Italy, France and Spain). The role of STV in symptom development remains unclear, since the virus is frequently detected in mixed infections with other viruses, and it has been found in some asymptomatic tomato plants. STV is seed transmitted at high rates, but "horizontal" transmission by vectors is unknown. We developed sensitive methods for STV detection and quantification, to study the role played by STV in symptom development, to test horizontal transmission by insect vectors, and to implement sanitation programmes. Molecular hybridization and nucleic acid isothermal amplification (RT-LAMP) enabled sensitive detection of STV from different tomato plant tissues. The virus was detected in field samples collected from different production regions of Spain and Italy. A real time PCR assay after reverse transcription (RT-qPCR) was also developed for STV detection and quantification. STV titre remained constant over time, as for other persistent viruses. The virus was detected in individual tomato seeds, and in seed coats and embryos, making seed disinfection difficult. Nucleotide sequencing of different STV isolates showed very low genetic variation, which may be related to a strong symbiotic-mutualistic interaction between STV and the tomato host.

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Development of new potato varieties with late blight and potato cyst nematode resistance, reduced bruising and improved processing quality. M. PAIS[1], K. WITEK[1], L. TOMLINSON[1], A. WITEK[1], F. JUPE[1], M. SMOKER[1], S. PERKINS[1], S. MARILLONNET[2], P.E. URWIN[3], N. CHAMPOURET[4], C. RICHAEL[4], J.D.G. JONES[1]. [1]*The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.* [2]*Department of Cell and Metabolic Biology, Leibniz Institute of Plant Biochemistry, Halle, Germany.* [3]*Centre for Plant Sciences, University of Leeds, Leeds, United Kingdom.* [4]*Simplot Plant Sciences, J.R. Simplot Company, Boise, Idaho, USA. E-mail: marina.pais@tsl.ac.uk.*

Solutions were sought for several important problems faced by potato industries, including: late blight (LB), potato cyst nematodes (PCN), potato browning, acrylamide formation and blackening upon cooking. These are linked to significant crop losses, increased production costs, extensive use of agrochemicals and food wastage. We followed a genetic modification approach, based on: the use of a 3-resistance (*R*)-gene stack that combines genes from *Solanum venturii* (*Rpi-vnt1*) and *Solanum americanum* (*Rpi-amr3i* and *Rpi-amr1e*) to control LB; the use of genes encoding a rice cystatin and a synthetic repellent peptide that confer resistance to PCN by two different mechanisms; and the use of silencing constructs to reduce browning, the cold-induced accumulation of reducing sugars and the levels of asparagine (therefore reducing the potential for acrylamide formation and blackening). The Golden Gate cloning technique is used to generate constructs for potato transformation, and the binary vector was used to decrease chances of backbone integration. Several constructs are in the transformation pipeline. The three LB-resistance genes have been cloned individually or as a 3-*R*-gene stack, and the two genes conferring resistance to PCN as a single module or in combination with the 3-*R*-gene stack, with or without the silencing modules for improved tuber quality. The first transgenic lines obtained have been evaluated for resistance to different isolates of the LB pathogen in detached leaf assays, and some are being tested with PCN in glasshouse conditions. A field trial is planned to assess resistance against LB strains in field conditions.

This research is supported by the British Biotechnology and Biological Sciences Research Council (BBSRC) and by The Gatsby Charitable Foundation.

Screening of European potato varieties for resistance to pathotype 18(T1) of *Synchytrium endobioticum* in Greece. I. VLOUTOGLOU[1], K.B. SIMOGLOU[2], H. ELEFTHERIADIS[2], D. TSIROGIANNIS[1], C. KRITIKOS[1], I. SARIGKOLI[2], N. NIKOLAIDIS[2], A. KOTZAMPIGIKIS[1], A. THEOCHARIS[1], M. KONSTADINIDOU[2], C. ARAMPATZIS[3], I. KAGIAS[3], D. GKILPATHI[4]. [1]*Benaki Phytopathological Institute, Department of Plant Pathology, Laboratory of Mycology, 8 St. Delta Street, 145 61 Kifissia, Athens, Greece.* [2] *Region of Eastern Macedonia & Thrace, Regional Unit of Drama, Rural Economy & Veterinary Directorate, Department of Quality and Phytosanitary Control, Dioikitirion, 661 00 Drama, Greece.* [3]*Hellenic Ministry of Rural Development and Food, Directorate General of Plant Produce,*

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The quarantine pathogen *Synchytrium endobioticum* (which causes potato wart) was detected for the first time in Greece in two commercial potato (*Solanum tuberosum* L.) fields, in Kato Nevrokopi (Regional Unit of Drama, Northern Greece), during the 2011 official surveys. The pathotype in both fields was identified as 18(T1), an aggressive and rare pathotype. In compliance with the EU and National legislation, phytosanitary measures were implemented in the area, including demarcation of a buffer zone (c. 200 ha) around the infested fields. In addition, field trials and bioassays (pot tests) were carried out according to the EPPO Standard PM 07/28(1) for identifying resistant potato varieties to be used in the buffer zone. A total of 50 commercial potato varieties, eight of which were reported by other EU Member States and/or European potato breeding companies to be resistant to pathotype 18(T1), were evaluated in field trials over four consecutive years (2013-2016). Results showed that most of the commercial potato varieties tested were very susceptible to this pathotype. Only four varieties, out of the eight varieties reported elsewhere as resistant, constantly exhibited field resistance to pathotype 18(T1). Based on the results of the bioassays conducted under controlled environment conditions using very high *S. endobioticum* inoculum pressure, only two of the varieties constantly showing field resistance to pathotype 18(T1) could be potentially used in the buffer zone, as, in line with Council Directive 69/464/EEC, they provided adequate protection against secondary infections by *S. endobioticum*.

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Is *Calonectria pauciramosa* established in Portugal? Occurrence in ornamental nurseries and in public gardens. A.P. RAMOS[1], T. VALADA[2], F. MAIA[2], B. FERREIRA[2], M.F. CAETANO[2], A. LIMA[1]. [1]*LEAF Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [2]*Laboratório de Patologia Vegetal "Veríssimo de Almeida", Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: pramos@isa.ulisboa.pt*

Myrtus communis plants, from an historical garden and from an ornamental nursery, showing severe symptoms of chlorosis and wilting of growing tips, root and crown rot, were received for diagnosis in 2016. *Cylindrocladium*-like isolates were obtained from roots, crowns, branches and leaves of diseased plants. Eight representative isolates were selected to confirm their identity using morphological, cultural and molecular methods. Sporulating cultures on synthetic nutrient-poor agar, incubated in darkness at 25°C, had macroconidiophores with stipe extensions terminating in broadly ellipsoidal to obpyriform vesicles, with the widest dimension below the mid-points and a penicillate arrangement of fertile branches each terminating in 2-6 phialides. Phialides produced clusters of cylindrical conidia (38.1-64.4 × 3.5-5.4 μm), rounded at both ends, 1-sepate (thirty structures measured). On malt extract agar, the isolates grew faster at 25°C (growth rate 6.3 mm d^{-1}) than at 15°C (3.0 mm d^{-1}). None of the isolates grew at 35°C. These features agree with descriptions of *Calonetria pauciramosa*. To further confirm the identity of the fungus, the rDNA-ITS and the β-tubulin gene regions were amplified. Comparison of the sequences with other sequences available in the GenBank database showed they were identical to the *Ca. pauciramosa* 1031-ITS6 isolate from *M. communis* in Italy (AM749819), and to the *Ca. pauciramosa* CYL1/04 isolate from *Polygala myrtifolia* in Spain (AY923867). After the first record of *Ca. pauciramosa* in Portugal in 2003, our results indicate that the disease may now be spread in ornamental nurseries as well as in historical gardens.

Spread of *Tomato leaf curl New Delhi virus* in Italy: a new challenge for the cultivation of zucchini squash. S. DAVINO[1], M. LUIGI[2], S. BERTIN[2], A. MANGLLI[2], S. PANNO[1], A. CARUSO[1], E. TROIANO[3], L. OTGIANU[4], M. NANNINI[4], G. PARRELLA[3], L. TOMASSOLI[2]. [1]*Università degli Studi di Palermo, Dipartimento Scienze Agrarie e Forestali, Viale delle Scienze ED. 5 - 90128 Palermo, Italy.* [2]*Consiglio per*

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Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (family *Geminiviridae*) that was first identified in Asia in tomato plants, and then spread to several countries worldwide. The virus is responsible for economic damage to cucurbitaceous and solanaceous crops. After the virus was first recorded in Spain (2012) and Tunisia (early 2015), ToLCNDV was detected in Italy in October 2015. The rapid spread of the virus through the Mediterranean region was likely mediated by the vector, *Bemisia tabaci*. In Italy, the first ToLCNDV outbreak occurred in Sicily on zucchini squash, and immediately prompted intensified monitoring in the southern and central regions where *B. tabaci* occurs. In summer 2016, ToLCNDV-infected zucchini plants were found in Sardinia, Campania and Lazio regions. Phylogenetic analyses of the coat protein (CP) sequences showed that Italian ToLCNDV isolates split were in two well-supported groups. A cluster grouped all isolates from Sicily together with the reference isolates from Tunisia and Spain and a few isolates from Campania and Lazio. This cluster included a subgroup represented by the Sardinian isolates. The second cluster only included isolates from Campania and Lazio. These results suggest that in Sicily at least two independent introductions of ToLCNDV occurred; the first in 2015 from Spain and the second in 2016 from Tunisia. The pathogen spread from Sicily to Sardinia and to Campania and Lazio, but divergent isolates have been introduced in these regions through a different route, likely from Spain.

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Deciphering copper resistance in *Xanthomonas citri* pv. *citri*. D. RICHARD[1,2,3], V. RAVIGNÉ[1], A. RIEUX[1], B. FACON[4,5], C. BOYER[1], K. BOYER[1], P. GRYGIEL[1], S. JAVEGNY[1], M. TERVILLE[1], B. I. CANTEROS[6], I. ROBÈNE[1], C. VERNIÈRE[7], A. CHABIRAND[2], O. PRUVOST[1], P. LEFEUVRE[1]. [1] *CIRAD, UMR PVBMT, F-97410 St Pierre, Réunion, France.* [2] *ANSES, Plant Health Laboratory, F-97410 St Pierre, Réunion, France.* [3] *Université de la Réunion, UMR PVBMT, F-97490 St Denis, Réunion, France.* [4] *INRA, UMR PVBMT, F-97410 St Pierre, Réunion, France.* [5] *INRA, UMR CBGP, F-34090 Montpellier, France.* [6] *INTA, Estación Experimental Agropecuaria Bella Vista, Argentina.* [7] *CIRAD, UMR BGPI, F-34398 Montpellier, France. E-mail: olivier.pruvost@cirad.fr*

Copper-based compounds are widely used in integrated pest management (IPM) programmes aiming to control important plant bacterial pathogens, which have adapted in response to this selective pressure. Copper resistance of *Xanthomonas citri* pv. *citri* (*Xcc*), a major citrus pathogen worldwide causing Asiatic citrus canker, was first observed in Argentina two decades ago, and was subsequently reported as a *copLAB*-based, plasmid-encoded system. The emergence of resistant strains has since been reported in Réunion (South West Indian Ocean) and Martinique (Eastern Caribbean Sea). Disease severity was markedly increased in groves established with susceptible cultivars and infected with copper-resistant *Xcc*. Using tandem repeat-based genotyping and *copLAB* PCR, we demonstrated that the genetic structure of the copper-resistant strains from these three regions included two distant clusters, and varied for the detection of *copLAB* amplicons. We sequenced six copper-resistant *Xcc* strains from Argentina, Martinique and Réunion, together with reference copper-resistant *Xanthomonas* and *Stenotrophomonas* strains, using long-read sequencing technology. Genes involved in copper resistance were found to be strain-dependent, with the novel identification in *Xcc* of *copABCD* and a *cus* heavy metal efflux resistance-nodulation-division system. The genes providing the adaptive trait were part of a mobile genetic element similar to Tn3-like transposons, and included in a conjugative plasmid. The mining of all bacterial genomes available from public databases suggested that the mobile elements containing copper resistance genes and their plasmid environments were primarily detected in the Xanthomonadaceae family.

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1731-0006632) and European Agricultural Fund for Rural Development (EAFRD), Conseil Départemental de la Réunion, Région Réunion, État Français, the French Agropolis Foundation (Labex Agro – Montpellier, E-SPACE project number 1504-004), ANSES and CIRAD.

Is *Xanthomonas citri* subsp. *citri* (*Xcc*) knocking at the doors of the Mediterranean region? P. CARUSO[1], R. PAVONE[1], C. LICCIARDELLO[1], M.P. RUSSO[1], V. CATARA[2], G. LICCIARDELLO[2], O. PRUVOST[3], I. ROBENE[3], J. CUBERO[4], C. REDONDO[4], Y. AYSAN[5], R. CETINKAYA-YILDIZ[6], S. HORUZ[7], A. URSO[2], G. TIMPANARO[2]. [1]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale, Italy.* [2]*Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania.* [3]*CIRAD, UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT), 7 chemin de l'irat - 97410 Saint Pierre, La Réunion, France.* [4] *INIA, Departamento de Protección Vegetal, Ctra De La Coruna Km 7.5, 28040 Madrid, Spain.* [5]*PPD-CU, 01330 Balcali, Saricam - Adana, Turkey.* [6]*BCRI, Kısla Mah. Yuregir - 01321 Adana-Turkey.* [7]*Erciyes university Köşk Mahallesi, Talas Blv., 38030 Melikgazi/Kayseri, Turkey. E-mail: paola.caruso@crea.gov.it*

The Mediterranean region is free of citrus bacterial canker (CBC), a disease caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) and *X. fuscans* subsp. *auratifolii* (*Xfa*). In 2014, EFSA highlighted ornamental rutaceous species (ORS) as a possible pathway for CBC entry. The ORPRAMed research project is underway to evaluate the risk of introduction of *Xcc* and *Xfa* through ORS in Europe. ORPRAMed partners are focusing on ORS not covered by 2000/29EC Directive through phytopatological, molecular, genetic, detection and economic approaches. We have analysed the trade flows in vegetal material for non-food uses. We have considered the import flows from countries where *Xcc* is classified as present. From the UN-ComTrade dataset (2015), with Code 06, Mediterranean countries reported imports of over 8.7 million kg from areas where *Xcc* is present, for an overall value of $46.0 million. The re-exportation from these countries of plant material must also be included, which is estimated at 156,384 kg for a value of $0.276 million. A field survey was also conducted in Turkey, to exclude the presence of CBC, due to its vicinity to infected areas. In the survey carried out in Adana, Mersin and Hatay provinces, 61 commercial nurseries and approx. 8500 ha of citrus orchards were screened, and disease symptoms were not observed. Growers and the nurserymen were also informed about the possible introduction risk of this severe citrus disease.

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***Phytophthora capsici* emerging simultaneously in different greenhouse crops in Southeast Spain.** M. DE CARA-GARCÍA[1], A.M. AGUILERA-LIROLA[2], A. PÉREZ-HERNÁNDEZ[1], I. ESPITIA-VÁZQUEZ[1], J.M. GÓMEZ-VÁZQUEZ[1]. [1]*IFAPA Centro La Mojonera, Camino de San Nicolás, 1, 04745, La Mojonera, Spain.* [2]*S.C.A. Campoadra, Avda. de la legión española, 2, 04779, Adra, Spain. E-mail: franciscom.cara@juntadeandalucia.es*

From 2014 to 2017, a general survey of greenhouse crops in western Almería province (Spain) was performed to detect soil-borne pathogens associated with wilting and/or root and crown rot. Fifty-three farms with diseased plants were surveyed. Four symptomatic plants and rhizospheric soils per greenhouse were sampled and analyzed. Symptomatic plants were only observed in sweet peppers for the first season, but extended to other crops (melon, watermelon, cucumber, tomato and zucchini) in the following three years. The prevalent pathogen isolated was *Phytophthora* sp., showing distinguishing features of *P. capsici*. *Phytophthora* sp. was isolated from 44.1% of pepper greenhouses, 90% of melon greenhouses and 100% of greenhouses with the other crops. One fourth of the surveyed greenhouses had been partially or completely flooded before the occurrence of symptoms and subsequent sampling, and greater association was noted between symptoms and the presence of *Phytophthora* sp. in these greenhouses. Wilted plants and *Phytophthora* sp. were present in 75% of flooded pepper greenhouses, 80% for melon, and 100% for flooded greenhouses containing watermelon or zucchini. Mating type was checked for 57 *Phytophthora* sp. isolates (obtained from all the host species). All isolates belonged to the A1 group. Molecular identification as *P. capsici* was

confirmed for 24 isolates by sequencing ITS-rDNA region. These isolates were inoculated on pepper, and four on the other plant species. All of the isolates were pathogenic. These results demonstrate simultaneous emergence of *P. capsici* causing soil-borne diseases in different economically important greenhouse crops in Almería.

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Assessment of the host status of ornamental rutaceous species to *Xanthomonas citri* pathovars causing citrus bacterial canker. G. LICCIARDELLO[1], O. PROUVOST[2], I. ROUBENE[2], J. CUBERO[3], C. REDONDO[3], A. CARUSO[1], C. LICCIARDELLO[4], P. CARUSO[4], V. CATARA[1]. [1]*Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy.* [2]*CIRAD, UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT), 7 chemin de l'irat - 97410 Saint Pierre, La Réunion, France.* [3]*INIA, Departamento de Protección Vegetal, Ctra De La Coruna Km 7.5, 28040 Madrid, Spain.* [4]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale, Italy. E-mail: vcatara@unict.it*

Xanthomonas citri pv. *citri* (*Xcc*) and *X. citri* pv. *aurantifolii* (*Xca*) cause citrus bacterial canker (CBC), a severe disease responsible for defoliation and fruit blemish and drop, requirings costly control measures. *Xcc* and *Xca* are quarantine pathogens for the UE, and are not recorded in the Mediterranean region. The probability of their entry, via import of ornamental rutaceous plants, through the commercial trade and passenger pathways, is rated as likely by EFSA (2014). To provide useful information for pest risk assessment, 25 ornamental rutaceous plants in the genera *Atalantia, Balsamocitrus*, *Clausena*, *Eremocitrus*, *Glycosmis*, *Melicope*, *Microcitrus*, *Murraya* and *Vespris*, not covered by Directive 2000/29EC, as well as *Citrus* and *Fortunella*, were tested for resistance to strains of *Xcc* (pathotypes A, A* and A^w) and *Xca* (pathotypes B and C), in controlled environment detached leaf assays. Nine plant species were presumptively classified as non-hosts, among them *Murraya paniculata.* Only *M. ovatifoliolata* and *Eremocitrus glauca* were susceptible to all pathotypes. The remaining species were susceptible at least to one of the pathotype A strains. Bacterial population densities ranged from 10^3 to 10^6 cfu mL^{-1} in plants showing HR or no response, and 10^7 to 10^9 cfu mL^{-1} in plants showing typical CBC lesions. Crystal violet staining showed aggregation of citrus canker strains on *M. paniculata* leaves similar to that on citrus species but different to that found for a non-citrus *Xanthomonas*. A *de novo* sequencing of the *M. paniculata* genome, already completed, will serve for RNAseq studies on both *Murraya* species.

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Evaluation of the presence of *Gnomoniopsis smithogilvyi* (syn. *castanea*) in chestnuts, rootstocks and grafts of six varieties of chestnut trees. M. CONTI[1], J. CROVADORE[1], B. COCHARD[1], R. CHABLAIS[1], M. JERMINI[2], F. LEFORT[1]. [1]*Plants and Pathogens Group, Institute Land Nature Environment, hepia, University of Applied Sciences and Arts Western Switzerland (HES-SO), 150 route de Presinge, 1254 Jussy, Switzerland.* [2]*Agroscope, Cadenazzo Research Centre, A Ramél 18, 6593 Cadenazzo, Switzerland. E-mail: francois.lefort@hesge.ch.*

Gnomoniopsis smithogilvyi is an endophytic fungus, recently identified in Europe and Switzerland as the main cause of chestnut brown rot and as a cause of chestnut canker. The pathogen causes high plant mortality in chestnut nurseries and orchards. The presence of this fungus and of the chestnut canker agent *Cryphonectria parasitica* was assessed in the propagation material of six chestnut varieties, used by the Ticino Cantonal Nursery to restore fruit orchards. Sixty root samples, 41 shoot samples from germinated chestnuts and 17 chestnut rootstock samples were analysed, along with 112 samples from 56 rootstock/graft pairs, to determine whether the pathogen was transmitted by rootstocks or grafts. DNA extraction was followed by specific amplification primers for *G. smithogilvyi* and *C. parasitica. Gnomoiopsis smithogilvyi* was detected as an endophyte, but *C. parasitica* was never detected. Six of the 60 roots analysed from seed chestnuts were contaminated with *G. smithogil-*

vyi (in varieties *Lüina*, *Torcione Nero*, *Marrone Michelangelo*, *Marrone Lattecaldo* and *Bouche de Bétizac)*, as well as two of 41 shoots from seed chestnuts (*Lüina* and *Bouche de Bétizac*), and two of 17 rootstocks (*Lüina* and *Torcione Nero* varieties). For 112 samples from 56 rootstock/graft pairs, *G. smithogilvyi* was found in 12% of the rootstocks and 60% of the grafts. These results showed low incidence of *G. smithogilvyi* in rootstock propagation material, and high contamination of grafting material in all varieties, and confirm that *G. smithogilvyi* is an endophyte.

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Characterization of *Elsinoë ampelina*, the causal agent of grapevine anthracnose in Brazil. R.F. SANTOS, M. CIAMPI-GUILLARDI, L. AMORIM, N. S. MASSOLA JÚNIOR, M. B SPÓSITO. *Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 13418-900 Piracicaba, SP, Brazil. E-mail: ricardofeliciano@usp.br*

Anthracnose, caused by *Elsinoë ampelina*, is an important disease in vineyards in South and Southeast Brazil, the main grape-producing regions in the country. This study characterized *E. ampelina* isolates associated with grapevine anthracnose in Brazil through molecular analysis, morphological characterization and pathogenicity tests. Thirty-nine *E. ampelina* isolates were obtained from leaves, stems and berries with anthracnose symptoms collected in the Rio Grande do Sul and São Paulo States. Fungus characterization was carried out using molecular analysis based on ITS, TEF 1-α and HIS3 regions, in combination with cultural and conidial morphology. For pathogenicity tests, ten isolates were inoculated onto *Vitis labrusca* cv. Niagara Rosada. ITS sequences showed only two polymorphic sites within the 602 bp sequenced and TEF 1-α sequences were monomorphic. However, HIS3 was the most informative region showing 55 polymorphic sites. Haplotype network analysis based on multilocus alignment (ITS, TEF 1-α and HIS3) grouped the isolates into seven haplotypes. Colonies of *E. ampelina* isolates showed slow growth (23 to 28 mm diam. at 30 d), variable colouration and wrinkled texture on PDA medium. Conidia were cylindrical to oblong with rounded ends, hyaline, aseptate, 3.6 to 7.0 µm long and 2.0 to 3.4 µm wide. Inoculations on 'Niagara Rosada' confirmed the pathogenicity of all isolates inoculated. These caused reductions of shoot dry weight by up to 80%, and severity of leaf disease reached a maximum of 72%.

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Genetic and phenotypic diversity of *Verticillium dahliae* populations from sunflower in Europe. A. MARTÍN-SANZ[1], A. GARCÍA-CARNEROS[2], S. RUEDA[1], P. MIRANDA-FUENTES[2], L. MOLINERO-RUIZ[2]. *[1]Pioneer Hi-Bred International, Inc., Campus Dupont – Pioneer, Ctra. Sevilla-Cazalla (C-433) km 4.6, 41309 La Rinconada, Spain. [2]Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain. E-mail: lmolinero@ias.csic.es*

The incidence of *Verticillium dahliae* (*Vd*) affecting sunflower in France, Italy, Spain and countries around Black Sea has greatly increased in the last five years, becoming a major constraint for sunflower production in some regions. Twenty Isolates of *Vd* collected in these countries, and one from Argentina, were characterized under a multidisciplinary study. The isolates were inoculated, by root immersion in suspensions of conidia, to seven sunflower genotypes with different phenotypic responses according to previous experiments. Some of the isolates were also inoculated onto different hosts (artichoke, eggplant, cotton, tomato and lettuce) to determine the host pathogenicity spectrum of *Vd* from sunflower. The vegetative compatibility groups (VCGs) were determined through complementation between nit mutants of the fungal isolates and VCG reference strains. Phenotypic and genetic data indicated that the isolates from Black Sea countries were distinguishable from those from West Europe and Argentina, which could be due to the presence of at least two different races. Artichoke was very susceptible to all the isolates and significant crop × *Vd* isolate interactions were found for disease variables. Ongoing experiments using SSR reference markers for *Vd* will provide extensive information about the molecular structure of populations from sunflower and the re-

lationships with populations from other crops. This study is the first attempt to increase understanding of the genetics, virulence and phenotypic characteristics of the *Vd* isolates affecting sunflower in Europe.

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Identification of two species belonging of *Polerovirus* in hot pepper (*Capsicum* spp.) in Italy: a new phytosanitary risk. A. TIBERINI[1], I. ADAMS[2], A. FOX[2], A. FOWKES[2], S. DAVINO[3], L. TOMASSOLI[4]. [1]*Università degli Studi "Mediterranea" di Reggio Calabria, Feo di Vito, 89121 Reggio Calabria (RC) Italy.* [2]*Food and Environmental Research Agency (FERA), Sand Hutton, York, UK.* [3]*Università degli Studi di Palermo, Dipartimento Scienze Agrarie e Forestali, Viale delle Scienze ED. 5, 90128 Palermo, Italy.* [4]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: antonio.tiberini@unirc.it*

Diseases caused by aphid-borne poleroviruses (genus *Polerovirus*, family *Luteoviridae*) are emerging threats to the production of important crops. During the current decade, several first outbreak and survey studies have been reported for poleroviruses in *Capsicum annuum*. In Italy, two simultaneous detections of *Pepper vein yellows virus* (PeVYV) occurred in 2015 in central Italy (Lazio) in hot pepper in open fields, and southern Italy (Sicily) in greenhouse-grown sweet pepper. During recent investigations, hot pepper has been found affected by multiple viruses, causing a range of symptoms including leaf yellowing, brittleness, crinkling, mosaic and necrosis. Most of the viruses were endemic (*Tomato spotted wilt virus*, *Alfalfa mosaic virus*, *Broad bean wilt virus 2*, *Potato virus Y*, *Pepper mild mottle virus*) but *Chilli veinal mottle virus* (ChiVMV) and PeVYV were new for Italy. The concern that other alien viruses could be introduced through intensive but free exchange and trade of foreign germplasm has led to the use the NGS technique to analyse the whole *viroma* of some severely symptomatic chilli plants of other *Capsicum* spp. A new isolate has been identified sharing high nucleotide sequence similarity with the putative species Pepper yellow leaf curl virus (PYCV) in the genus *Polerovirus*, for which taxonomy is under debate to be considered as new species or a PeVYV strain. On the basis of preliminary partial genome analysis, this isolate showed a mosaic sequence related to PeVYV and *Tobacco vein distorting virus* as previously reported in the first PYCV outbreak in Israel. This study aims to clarify the taxonomic position of this putative Polerovirus.

Range of expansion and genetic diversity of *Bemisia tabaci* populations in Italy, under the recent threat of *Tomato leaf curl New Delhi virus* spread. S. BERTIN[1], G. PARRELLA[2], M. GIORGINI[2], M. NANNINI[3], S. DAVINO[4], M. LUIGI[1] and L. TOMASSOLI[1]. [1]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca Difesa e Certificazione (CREA-DC), Via C.G. Bertero 22 - 00156 Roma, Italy.* [2]*Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), Sede di Portici, via Università 133, Portici, Napoli, Italy.* [3]*Agris Sardegna, Servizio Ricerca Studi ambientali, Difesa delle colture e Qualità delle produzioni, Viale Trieste 111 - 09123 Cagliari, Italy.* [4]*Università degli Studi di Palermo, Dipartimento Scienze Agrarie e Forestali, Viale delle Scienze ED. 5 - 90128 Palermo, Italy. E-mail: sabrina.bertin@crea.gov.it*

After the introduction of *Tomato leaf curl New Delhi virus* (ToLCNDV; *Begomovirus*: *Geminiviridae*), the insect vector *Bemisia tabaci* (Hemiptera: Aleyrodidae) strengthens its harmfulness to the horticultural crops in the Mediterranean basin. Within the *B. tabaci* complex, Mediterranean (Med) and Middle East–Asia Minor 1 (MEAM1) species (formerly referred to as biotypes Q and B) are widespread in the endangered areas, and are regarded as the main vectors responsible for ToLCNDV transmission. In Italy, *B. tabaci* has raised the status of the virus in warm areas, including the southern regions, Sicily and Sardinia, and the north-western coast (Liguria). During the last decade, the level of Med populations has progressively increased, and Med displaced MEAM1 in those areas where intensive farming occurs. Following ToLCNDV outbreaks in Italy, surveys were carried out to investigate the infestations of *B. tabaci* in the affected areas. ToLCNDV-associated Med populations of *B. tabaci* were found to be established in the Lazio region (central Italy), where begomovirus epidemics had never occurred and vector presence was thought to be only occasional. Nevertheless, single and mixed

populations of Med and MEAM1 species were found in other ToLCNDV-free locations, suggesting that agro-ecological factors still limit Med outbreaks in this central region. Further south, in the Campania region, MEAM1 has been displaced for a long time and only Med was found. The spreading pattern and the genetic diversity of *B. tabaci* populations are also under investigation in other regions (Sardinia and Sicily), in view of their effects on ToLCNDV epidemiology and disease management.

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New emerging viruses in pepper crops in Turkey. N. BUZKAN[1], B.B. ARPACI[2]. [1]*Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaraş Sütçü Imam, 46060 Kahramanmaraş, Turkey*. [2]*Kilis Yedi Aralık University, Department of Horticulture, Kilis, Turkey. E-mail: nbuzkan@gmail.com*

In Turkey, pepper (*Capsicum annuum*) is economically cultivated in 81,500 ha producing approx. 2-2.5 million tons per year. High incidence of yellow dwarfed pepper plants was observed in major pepper growing areas from the summer of 2013 onwards. Infected plants were mostly found in open-field crops, with symptoms of leaf interveinal yellowing and narrowing, suggestive of polerovirus infections. The fruits of diseased plants were smaller than normal and discoloured, resulting in reduced commercial value. Total RNA was extracted from the infected samples using Trizol. Two-step reverse transcription polymerase chain reaction (RT-PCR) was then performed with primers Pol-G-F/Pol-G-R designed for universal detection of poleroviruses. PCR amplicons were directly sequenced with both primers and subjected to a BLASTn search to identify which virus species each presented. Some sequences were similar to that of *Pepper vein yellows virus* (PVYV) (9%) and others that of *Beet western yellows virus* (BWYV) (10%) in the genus *Polerovirus*. However, some sequence chromatograms showed double peaks, suggesting mixed infections with *Paprika mild mottle virus* (PaMMV) (*Tobamovirus*) and *Broad bean wilt virus* -2 (BBWV-2) (*Fabavirus*). cDNAs from all samples were then subjected to RT-PCR using a primer pair specific to PaMMV and a universal primer pair to detect fabaviruses. BLASTn analysis of the sequenced PCR amplicons proved the presence of PaMMV (4%) and BBWV-2 (6%) for the first time in pepper plants in Turkey.

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Races of *Fusarium oxysporum* f. sp. *niveum* in the Aydın Province, Turkey. B. GEÇİOĞLU ERİNCİK[1], M.T. DÖKEN[2]. [1]*Adnan Menderes University, Koçarlı Vocational School, 09100, Aydın, Turkey.* [2]*Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09100, Aydın, Turkey. E-mail:bgerincik@adu.edu.tr*

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum (Fon),* is a common soil-borne disease in the watermelon production areas of the Aydın Province in Turkey. A total of 73 pathogenic *Fon* isolates were sampled from that Province in 2010 and 2011. Races of *Fon* isolates were determined using the differential watermelon cvs 'Sugar Baby', 'Charleston Gray', 'Calhoun Gray' and 'PI-296341-FR'. Two-week-old seedlings of the cultivars were root-dipped in spore suspensions (1×10^6 microconidia mL^{-1} of each isolate. Plants were incubated in a growth chamber and evaluated for the presence of disease symptoms (yellowing, vascular discolouration, and wilting) at 14 d after inoculation. Three races of *Fon* were detected from the Aydın Province. Among of 73 isolates, 21 were designated as race 0, 27 as race 1 and 25 and as race 2. No race 3 isolates were identified.

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Polyphasic characterization of *Ralstonia solanacearum* strains isolated in Spain from different geographical origins. P. CARUSO[1], E.G. BIOSCA[2], E. BERTOLINI[3], E. MARCO-NOALES[4], M.T. GORRIS[4], C. LICCIARDELLO[1], M.M. LÓPEZ[4]. [1]*CREA-Centro di ricerca per l'agrumicoltura e le colture mediterranee (CREA-ACM), Corso Savoia, 190 – 95024 Acireale (Catania) Italy.* [2]*Departamento de Microbiología y Ecología, Universitat de València, Av. Dr. Moliner 50, 46100-Burjassot, Valencia, Spain.* [3]*Departamento de Fitossanidade,*

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Potato brown rot and bacterial wilt are caused by the bacterium *Ralstonia solanacearum*, that at global level is one of the world's most important phytopathogenic bacteria. An extensive survey revealed presence of this quarantine pathogen in some Spanish regions. We report the characterization and intraspecific diversity of a selection of 48 *R. solanacearum* strains isolated in Spain, from different sources and geographical origins. Phenotypic and genotypic analyses were performed by a polyphasic approach, to evaluate the influence of site and host on strain diversity. All the strains were compared using biochemical and metabolic profiles, and serological relationships were evaluated by Indirect-ELISA using polyclonal and monoclonal antibodies. Molecular analyses included partial sequence analysis of *hrpB* and *egl* genes, repetitive sequences (rep-PCR), amplified fragment length polymorphism (AFLP) profiles and macrorestriction with *Xba*I and *Spe*I followed by pulsed field gel electrophoresis (PFGE). Biochemical and metabolic characterization showed that all analysed strains belonged to phylotype II sequevar 1, and shared homogeneous profiles. Strain homogeneity was confirmed by serological tests, rep-PCR typing and phylogenetic analysis. However, differences among strains were found by AFLP and PFGE techniques, some profiles being related to the geographical origins of the strains. Our results support the hypothesis that several clones of the pathogen have been introduced into Spain.

First report of cobweb disease on shiitake and oyster mushrooms in Spain caused by *Cladobotryum dendroides* and *C. mycophilum*. F.J. GEA[1], M.J. NAVARRO[1], L.M. SUZ[2]. [1]*Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintanar del Rey, Cuenca, Spain.* [2]*Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Richmond Surrey TW9 3DS, UK. E-mail: fjgea.cies@dipucuenca.es*

Several species of *Cladobotryum* cause cobweb disease in mushroom-growing countries worldwide. Recently, *C. mycophilum* was detected in *Agaricus bisporus* (white button mushroom) and *Pleurotus eryngii* (king oyster mushroom) crops from Castilla-La Mancha (Spain). In 2016, symptoms of cobweb were also observed on *Lentinula edodes* (shiitake) and *Pleurotus ostreatus* (oyster mushroom) crops. The disease appeared at the end of the shiitake crop cycle, first on the substrate before spreading to the nearest fruit bodies by means of a fine grey-white mycelium. In oyster mushroom crops, cobweb appeared on fruit bodies at the end of the crop cycle. Eight isolates of *Cladobotryum* recovered from the substrate and diseased fruit bodies of shiitake, and two isolates from diseased oyster fruit bodies, were used to identify the cobweb causal agent. Genomic DNA from the fungal cultures was isolated, and the ITS DNA barcode region was amplified and sequenced. The obtained sequences were combined with sequences from *Cladobotryum* spp. isolated from different edible mushroom crops for phylogenetic analysis. *Cladobotryum dendroides* was identified as the cause of cobweb in shitake, and *C. mycophilum* in oyster mushroom. Pathogenicity tests on fruit bodies of shiitake were performed using conidial suspensions of two *C. dendroides*, and two *C. mycophilum* isolates on oyster mushroom. *Cladobotryum dendroides* and *C. mycophilum* were re-isolated from the inoculated fruit bodies, while the control mushrooms remained symptomless. This is the first report of *C. dendroides* and *C. mycophilum* causing cobweb in shiitake and oyster mushroom in Spain.

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***Phytophthora mekongensis* and *P. prodigiosa*, two new species associated with citrus in Vietnam.** M. EVOLI[1,2], F. LA SPADA[1], F. ALOI[1], B. SCANU[3], D. RUANO-ROSA[4], M. HORTA JUNG[5,6], S. WRIGHT[7], A. PANE[1], G.E. AGOSTEO[2], L. SCHENA[2], G. MAGNANO DI SAN LIO[2], T. JUNG[5,6], S.O. CACCIOLA[1]. [1]*Department of Agriculture, Food and Environment, University of Catania, Catania, Italy.* [2]*Dipartimento di Agraria, Università Mediterranea di Reggio Calabria, Località Feo di Vito, 89122 Reggio Calabria, Italy.* [3]*Dipartimento di Agraria, University of Sassari, Viale Italia 39, 07100 Sas-*

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Two new *Phytophthora* species were found to be associated with brown rot of pomelo (*Citrus grandis*) and root rot of trees of 'King' mandarin (*Citrus nobilis*) and pomelo in the Mekong River Delta area od Vietnam. The two species were characterized from morphological traits, and using the ITS1-5.8S-ITS2 region of the rDNA and the cytochrome oxidase subunit 1 (COI) as barcode genes. One of the two species clustered in the Phytophthora Clade 2 and was designated as *P. mekongensis*. It was closely related to, but distinct from, *P. meadii* and produced papillate, often bi- and tri-papillate, caducous sporangia. The second species resided in Clade 9 and was designated as *P. prodigiosa*. It was closely related to, but distinct from, *P. insolita*, and like *P. insolita* produced non-papillate, internally and externally proliferating, persistent sporangia, chlamydospores and hyphal swellings with bizarre shapes. In pathogenicity tests, both species induced fruit brown rot on various citrus species. In contrast, only *P. mekongensis* induced typical symptoms of Phytophthora gummosis on artificially inoculated citrus trees. *Phytophthora mekongensis* can be regarded as an aggressive pathogen of citrus, while *P. prodigiosa*, although quite common as a soil inhabitant in citrus groves of the Mekong River Delta area, is likely to be an opportunistic pathogen. This is the first report of a *Phytophthora* species from Clade 2 other than *P. citricola* and *P. citrophthora,* as causal agents of citrus diseases worldwide and the first report of a species in Clade 9 in Vietnam.

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First Report of '*Candidatus* Liberibacter solanacearum' in carrot in Italy. V. CATARA, G. LICCIARDELLO, M. LINGUAGLOSSA, F. SALONIA, C. RAPISARDA, R. LA ROSA, , G.E. COCUZZA MASSIMINO. *Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy. E-mail: vcatara@unict.it*

In Europe, '*Candidatus* Liberibacter solanacearum' has been found, with its various psyllid vectors, associated to members of the family Apiaceae, including carrot, celery and parsnip. Symptoms on carrot plants include leaf yellowing or purpling, stunting and hairy growth of secondary roots. No such problems were reported by carrot growers to date in a large area of cultivation in Sicily (Southern Italy). Nevertheless, a survey was undertaken in spring 2017, due to the report of *'Ca.* Liberibacter solanacearum' associated to carrot in countries bordering the Mediterranean Sea, such as France, Greece, Spain, Israel and Morocco. Leaves showing yellowing and purple discoloration were observed in three out of five carrot fields visited, although with a very low incidence. Total DNA was extracted from petiole tissues of symptomatic and asymptomatic plants using the DNeasy Plant Mini Kit-Qiagen. DNA extracts positive for '*Ca.* L. solanacearum' by real-time PCR with the Lso-HLBp-HLBr primer-probe set, and with cycle threshold values between 21.75 and 36.59, were obtained from three field carrot samples. Positive amplifications for '*Ca.* L. solanacearum' were also obtained by conventional PCR using primer pairs LsoF/OI2c targeting a portion of the 16S rDNA. Amplicons obtained from the PCR assays were directly sequenced (BMR, Italy). BLAST analysis of the 16S rDNA sequences (approx. 1000 bp) showed 99% nucleotide identity with '*Ca.* L. solanacearum' strains amplified from carrot in Finland (GenBank: GU373048.1). To our knowledge, this is the first report of '*Ca*. L. solanacearum' in Italy. Numerous psyllids (Hemiptera), presently under identification, have been also collected in the investigated fields.

Exploring the potential invasiveness of *Hymenoscyphus fraxineus* in Mediterranean mountains. C. AGLIETTI[1], F. CANTINI[1], P. CAPRETTI[1], N. LUCHI[2], S. PAPINI[1], A. SANTINI[2], L. GHELARDINI[1,2]. *[1]Department of Agrifood Production and Enviromental sciences (DiSPAA), University of Florence, Piazzale delle Cascine 18, 50144, Firenze, Italy. [2]Institute for Sustain-*

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Hymenoscyphus fraxineus is an aggressive Ascomycete that quickly invaded Central and Northern Europe, causing dieback in *Fraxinus excelsior* and *F. angustifolia*. The fungus was recently discovered in the Northern Apennines, currently the southernmost recorded disease focus. The pathogen's potential to adapt and spread in Mediterranean mountains is unknown. Tests were carried out to ecologically and genetically characterize the population in the Italian Apennines. *In vitro* growth tests were performed to understand the population's reaction to warm summer temperatures that might limit fungal growth or survival in the Mediterranean mountains. These tests on leaf enriched media were performed to understand how leaf age influences mycelial growth, providing circumstantial evidence about the relationship between host phenology and fungal infection, which might change with climate either impairing or increasing disease development. Vegetative compatibility between local and non-local isolates of the fungus was also assessed, to determine the population's genetic variability. A number of isolates had optimum growth temperatures above 20°C, the most common value also in Central-European populations. All isolates resumed growth with no apparent damage after prolonged thermal stress. Large genetic variability was found in the population that, with tolerance to warm temperature, may increase the population's potential to adapt to the Mediterranean environment. The competence of host leaves to support fungal growth was maximum in late spring declining during summer. Synchronization between optimal sporulation pressure and leaf receptivity in this climate will affect the capacity of the pathogen to become invasive in this area.

New emerging viruses in the genus *Polerovirus* in vegetable growing areas in Turkey. N. BUZKAN[1], B.B. ARPACI[2], F. YARALI[2], M. KOÇ[2], A. APALAK[3]. [1]*Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaraş Sütçü Imam, 46060 Kahramanmaraş, Turkey*. [2]*Kilis Yedi Aralık University, Department of Horticulture, Kilis, Turkey.* [3]*Directorate of Provincial Food Agriculture and Livestock, Kilis, Turkey. E-mail: nbuzkan@gmail.com*

There are 17 formally accepted virus species in the genus *Polerovirus* (*Luteoviridae*) (ICTV released in 2014). Two poleroviruses, *Pepper vein yellows virus* (PVYV) and *Beet western yellows virus* (BWYV) were recently detected in field-grown pepper plants in Turkey. During an extensive survey in June 2015, symptoms including chlorosis of young leaves and bright yellow colour of older leaves, suggestive of polerovirus infections, were observed in plants of spinach (*Spinacia oleracea*), muskmelon (*Cucumis melo*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), field bean (*Phaesolus vulgaris*), summer squash (*Cucurbita pepo* var. *pepo*) and broad bean (*Vicia faba*) in some provinces of the eastern Mediterranean and Southeast Anatolia regions. Leaf tissues from symptomatic plants were first tested in DAS-ELISA for the presence of poleroviruses, then in RT-PCR to amplify a 1.1-kb portion of the polerovirus genome with the general polerovirus primer pairs. PCR amplicons were subsequently sequenced and subject to a BLASTn search to identify the polerovirus species. According to multiple alignment of the obtained nucleotide sequences from RdRp region, some broad bean isolates showed nucleotide identity to *Cucurbit aphid-borne yellows virus* (CABYV) and *Chickpea chlorotic stunt virus* (CpCSV) isolates. Broad bean and squash isolates also had nucleotide identity with BWYV isolates from *Capsicum annuum* plants from Turkey (HE978259.1). To our knowledge, this is the first report in Turkey of CpCSV and CABYV in broad bean, and BWYV in broad bean and squash plants.. CABYV infection in broad bean is also the first global record of this association.

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Daylily rust (*Puccinia hemerocallidis*), a new disease of *Hemerocallis* spp. in Europe that entered through the West. P. TALHINHAS, R. CARVALHO, E. SILVA, J.P. MELO E ABREU, A. MONTEIRO, A.P. RAMOS. *LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: ptalhinhas@isa.ulisboa.pt*

Daylilies (*Hemerocallis* spp.) are garden plants appreciated for their dense and bright green foliage and their long flowering periods. While originating from

East Asia, they are adapted to diverse climates, from the tropics to high latitudes, and very many cultivars are available in catalogues. Daylily rust (*Puccinia hemerocallidis*) also originated in East Asia, and was reported in Oceania, Africa and the Americas in the early years of the 21st century. The European Plant Protection Organisation has listed this fungus in the "EPPO A1 List of pests recommended for regulation as quarantine pests", and recognised the existence of routes for its potential introduction into Europe. Starting in November 2015, rust symptoms were observed on daylily plants in several gardens in Portugal, in the Lisbon area and in the Algarve region, as well as in Madeira island, attaining high prevalence, incidence and severity. In cool climates in Europe the disease cycle is naturally broken in the absence of the aecial host (*Patrinia* spp.), because daylilies lose their leaves and urediniospores are not able to survive. However, in Mediterranean conditions, most *Hemerocallis* spp. genotypes retain their leaves, providing conditions for the maintenance and multiplication of inoculum. The relevance of this disease for European ornamental horticulture industry and its potential spread according to agroecological conditions will be discussed.

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Integrated management of almond witches' broom in endemic areas: does grafting represent a promising control measure? P. TAWIDIAN, Y. ABOU-JAWDAH. *Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut 1107 2020,* P.O.Box 11-0236 / (AGSC), Riad El-Solh, Lebanon. *E-mail: abujawyf@aub.edu.lb*

Almond witches' broom (AlmWB), caused by "*Candidatus* Phytoplasma phoenicium", is an invasive disease infecting almond peach and nectarine. In Lebanon, the disease spread rapidly leading to death or complete yield loss of over 200,000 trees. Severe yield losses were also reported in Iran. Planting resistant varieties would be the best approach for environmentally sound management of the disease. Since resistant or tolerant almond cultivars have not yet been identified, grafting on resistant non-host stone fruit rootstocks was assessed. Preliminary field results showed that no symptoms develop on the growth emerging from apricot or plum scions grafted on severely infected almond trees, for more than 2 years post-grafting with one exception: one of the apricot cultivars, initially developed symptoms but recovered 2 months later and remained symptomless thereafter. Preliminary results from greenhouse trials showed that when AlmWB-infected scions were grafted on plum and apricot seedlings, they developed symptomless shoots, except in one case which recovered after 3 months. One year post-grafting the phytoplasma was not detected by PCR in almond grafted on two out of three plum cultivars, and in one apricot cultivar out of the two tested. Results of real time PCR (qPCR) showed significant reductions of phytoplasma titre in the recovered tissue as compared to titres before recovery. These preliminary grafting results look promising, and long term field trials are planned in phytoplasma infested regions. Further studies are recommended to unveil the physiological/molecular mechanisms underlying the recovery phenomenon, which may pave the way for effective curative control measures.

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A deep characterization of *Grapevine Pinot Gris Virus* by molecular and ultrastructural approaches. G. TARQUINI[1], G.L. BIANCHI[2], F. DE AMICIS[2], M. MARTINI[1], A. LOSCHI[1], G. FIRRAO[1], N. LOI[1], R. MUSETTI[1], P. ERMACORA[1]. *[1]Department of Agricultural, Food, Environmental and Animal Sciences. University of Udine. via delle Scienze, 206, I-33100 Udine, Italy. [2]ERSA, Plant Protection Service. via Sabbatini, 5, I-33050 Pozzuolo del Friuli, Udine, Italy. E-mail: giulia.tarquini@spes.uniud.it*

In 2003, an emergent disease characterized by symptoms of stunting and chlorotic mottling and deformation of leaves, has been reported in several grapevine varieties in different regions of Northern Italy. A new *Trichovirus*, named *Grapevine Pinot gris virus* (GPGV), was discovered in 2012 using an NGS approach. Despite increasing reports worldwide, the aetiology of GPG-disease is still unclear, since the virus was detected both in symptomatic and asymptomatic samples. The GPGV morphological and genetic charac-

teristics were investigated, to allow differentiation of virus isolates associated with symptomatic plants from those associated with asymptomatic plants. Ultrastructural observations and immunogold labelling detected filamentous flexuous viruses in phloem parenchyma cells, which also contained enlarged mitochondria, and vesicles hypothetically associated with endoplasmic reticulum alterations. No cytological differences were observed between symptomatic and asymptomatic tissues. Genome sequences of nine GPGV isolates from Friuli Venezia Giulia were obtained by Sanger sequencing and subjected to phylogenetic analysis, together with those available in *GenBank*. Results showed that GPGV isolates from asymptomatic plants clustered in a distinct clade, whereas isolates associated with symptomatic grapevines showed greater diversity. Further analysis of the sequence dataset highlighted features such as recombination breakpoints in the RdRp gene and a positively selected codon site in the CP gene. These analyses may assist understanding the population structure of the virus and the temporal dynamics of its interaction with hosts, although further studies are required to clarify the significance of these evolutionary events in the expression of disease symptoms.

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A new race of F*usarium oxysporum* f. sp. *lactucae* that causes Fusarium wilt of lettuce. S. FRANCO ORTEGA[1], G. GILARDI[1], P.C.J VAN RIJSWICK[3], G.ORTU[1], M.L. GULLINO[1,2], A. GARIBALDI[1]. [1]*AGROINNOVA, Centre of Competence for Agro-Environmental Innovation, University of Torino, Largo Paolo Braccini 2, 10095, Grugliasco, Torino, Italy.* [2]*DISAFA, University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Torino, Italy.* [3]*National Plant Protection Organization, National Reference Centre, P.O. Box 9102, 6700 HC Wageningen, The Netherlands. E-mail: sfrancoo@unito.it*

Fusarium oxysporum f. sp. *lactucae*, the causal agent of Fusarium wilt of lettuce (*Lactuca sativa* L.) occurs in most countries in which lettuce is grown, and causes serious economic losses. Three races (1, 2, and 3) of the pathogen have been previously described, based on their ability to cause disease on differential lettuce cultivars as well as by molecular tools developed to characterize different races of this pathogen. Only race 1 has been detected in Europe. Two isolates of *Fusarium oxysporum*, obtained from lettuce plants grown in the Netherlands showing symptoms of wilt, were characterized in this study, by combining pathogenicity tests using differential cultivars and molecular assays. Phylogenetic analysis of elongation factor 1-alpha (EF1-α) gene and intergenic spacer region (IGS region), IGS-Restriction Fragment Length Polymorphism (RFLP) and Inter-Retrotransposons Amplified Polymorphisms (IRAP) using primers designed within the LTRs of the *Skippy* element and LTRs of *Han* solo-LTR retrotransposons, were carried out to determine whether the isolates were different from the known races of *F.oxysporum* f. sp. *lactucae.* This is the first report of *F. oxysporum* f. sp. *lactucae* in Netherlands, and identifies a new race of *F. oxysporum* f. sp. *lactucae* using the IRAP technique. The primers FPUF and FPUR were designed based on a polymorphic band of the IRAP specific for the two Dutch isolates determined as a new race the pathogen.

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***Plectosphaerella* species as new pathogens of basil and parsley cultivation.** M.L. RAIMONDO, A. CARLUCCI. *Department of Science of Agriculture, Food and the Environment, University of Foggia, Via Napoli 25, 71122 Foggia, Italy. E-mail: antonia.carlucci@unifg.it*

Since 2012, several basil and parsley samples collected from local markets in Foggia province (South Italy) were subjected to laboratory analyses to ascertain the main fungal pathogens occurring. The sampling consisted of young parsley and basil plants showing leaf yellowing, necrotic lesions on stems, collars and roots, and in some cases stunting of the entire plants. Mycological analyses revealed mainly a common presence of fungal isolates belonging to *Plectosphaerella* genus. Morphological and molecular studies identified four different species of *Plectosphaerella* including *P. cucumerina*, *P. pauciseptata*, *P. plurivora* and *P. ramiseptata*. To understand the pathogenic roles of these *Plectosphaerella* species, and another five reference species (*P. alismatis*, *P. citrulli*, *P. delsorboi*, *P. melonis* and *P. oratosquillae*), pathogenicity tests were

performed *in vitro* and *in vivo*, using basil cv. 'Napoletano' and parsley cv. 'Gigante di Napoli'. Pathogenicity tests were carried out on detached leaves (*in vitro* conditions), and on 30-d-old basil and parsley plants grown in plots in a greenhouse (*in vivo* conditions). All four species isolated during this study caused symptoms both on basil and parsley leaves and on young plants, although producing different symptoms (necrotic spots, parenchimatic patches, hydropic areas, collar and root disclorations) and at disease severities. *Plectosphaerella ramiseptata* was the most aggressive species. To our knowledge, this is the first report of *P. cucumerina*, *P. pauciseptata* and *P. ramiseptata* on parsley, and the first report of *P. pauciseptata*, *P. plurivora* and *P. ramiseptata* on basil.

Occurrence of *Xanthomonas campestris* pv. *campestris* isolates on wheat in Algeria. This pathosystem has never been seen. B. KHENFOUS-DJEBARI[1], M. KERKOUD[2], C. BRAGARD[3], Z. BOUZNAD[1]. [1]*Laboratoire de Phytopathologie et Biologie Moléculaire, département de Botanique, ENSA El Harrach, Alger, Algérie.* [2]*DiagGene, 8, Rue le Nôtre 49066 Angers, France.* [3]*Appliedmicrobiology-Phytopathology, Earth&Life Institute, Université catholique de Louvain. E-mail: b.djebari@ensa.dz*

In a study of diseases caused *by Xanthomonas translucens* in Algerian wheat plots, two isolates were obtained, X7 in 2010 and X14 in 2011. Although they were morphologically identical on yeast dextrose chalk agar and nutrient agar,they were shown to differ rom other isolates when sequencing was performed on the housekeeping genes gyrase subunitB (*gyrB*), RNA polymerase sigma factor(*rpoD*), Chaperone protein DnaK (*DnaK*) and ATP synthase subunit beta (*atpD*). The sequences obtained for the different genes closely link the isolates to *Xanthomonas campestris* pv. *campestris* and pv. *Raphaani*, with similarity indices of 98 to 99%. A pathogenicity test performed by inoculation onto the sensitive wheat cv. Acsad 885 (Ramada) at the three-leaf seedling stage, and by dip-inoculation, in order to fulfill all Koch's postulates, was completed by inoculation of three cabbage cultivars (Chou de Milan de pontoise, Chou Milan gros de vertus and Lucien Clause chou rouge tête noire) and one cauliflower cultivar boule de neige). Similar to that described for *X. c. pv. campestris* on *Brassicaceae*, characteristic symptoms of black rot and V-shaped necrotic leaf lesions and blackening of vascular tissues were obtained on the four cultivars, while inoculated wheat seedlings showed water soaked lesions and necrotic to black lesions.

Genome analysis: applications to plant health

Experimental evolution in the fungal pathogen *Fusarium oxysporum* to study mechanisms of genome plasticity and host adaptation. C. LÓPEZ-DÍAZ[1], D. HAZAL AYHAN[2], J.J. GINÉS-RIVAS[1], I. OKEKE-INFANTE[1], LI-JUN MA[2], A. DI PIETRO[1] .[1]*Department of Genetics, University of Córdoba, Spain.* [2]*University of Massachusetts, Amherst, USA. E-mail: g02lodic@uco.es*

Filamentous plant pathogens undergo rapid evolution, leading to shifts or expansions in host ranges. The *Fusarium oxysporum* species complex collectively causes vascular wilt in more than a hundred different crops, provoking severe losses in global agriculture. The evolutionary mechanisms underlying host adaptation and host range dynamics in this pathogen remain poorly understood. We followed an experimental evolution approach, involving serial passages of the tomato pathogenic isolate *Fol* 42-87, either through plants or on artificial media plates. Independently evolved populations obtained after ten consecutive passages displayed notable phenotypic differences with respect to the initial clonal isolate. These included alterations in growth, sporulation and virulence. Four of the five plate-passaged populations had reduced virulence on tomato plants. Resequencing of the evolved populations revealed the presence of segmental duplications and deletions, particularly in the transposon-rich lineage-specific regions of the genome. In addition, single nucleotide changes and small Indels were detected in the evolved lines, some of which affected genes with known functions in fungal development and virulence. Collectively, our findings suggest that chromosome plasticity acts as a major evolutionary driver in *F. oxysporum*, and provide new insights into the genetic mechanisms underlying host adaptation in this important fungal pathogen.

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Genome size variability across fungi and the occurrence of very large genomes among rust fungi. P. TALHINHAS[1,2], A.P. RAMOS[1], S. TAVARES[1,2,3], M.C. SILVA[1,2], J. LOUREIRO[4], M. COELHO[5]. *[1]LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. [2]Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa, Quinta do Marquês, 2780-505 Oeiras, Portugal. [3]Section for Plant and Soil Science, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Frederiksberg Copenhagen, Denmark. [4]CFE, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3001-401 Coimbra, Portugal. [5]UCIBIO-REQUIMTE, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal. E-mail: ptalhinhas@isa.ulisboa.pt*

The completion of genome sequencing for some rust fungi indicates a link between biotrophic specialisation and genome size expansion. The measurement of genome sizes for 60 rust fungi, using flow cytometry, has revealed some of the largest genomes among fungi, with nine rust species with haploid genomes between 300 and 893 Mbp. The genome of *Uromyces appendiculatus* was 652 Mbp, *Phakopsora pachyrhizi* 716 Mbp, *U. transversalis*746 Mbp, *Hemileia vastatrix* 772 Mbp, and *Gymnosporangium confusum* 893 Mbp. The genome of *U. bidentis*, was 2489 Mbp. The overall average of the 60 rust fungi was ca. 380 Mbp. Genome size information is available for over 1800 fungal species, either arising from flow cytometry, genome sequencing, or other methods. This reveals an overall average of 44 Mbp. Departing from our genome size measurements of Pucciniales fungi, in this work we analyse genome size variability across representatives of the entire fungal phylogeny and attempt to relate such variations with relevant biological and genomic traits (e.g. life style, sexuality, nutrient use, composition in transposable elements). The analysis of genome size variation can unveil clues suggesting polyploidisation events or transposable elements activity of evolutionary/adaptive relevance. Such traits can be associated with reproduction strategies (sexual, asexual, parasexual and/or rare sexual) and substrate utilization (saprobic, mutualistic, obligate/facultative pathogenic, biotrophic/necrotrophic, and combinations of these).

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UV damage repair in the *Fusarium* species complex. S. MILO-COCHAVI, S. COVO. *Department of Plant Pathology and Microbiology, Robert H. Smith Faculty of Agriculture, Food and Environment. Hebrew University, Israel. E-mail: shay.covo@mail.huji.ac.il*

Fungal plant pathogens are exposed to various sources of DNA damage that can cause cell death and genome instability. Recent genome resequencing projects demonstrated high degrees of genome instability in fungal plant pathogens, suggesting poor DNA repair capacity. However, we found that the major fungal plant pathogens are equipped with various redundant DNA repair genes. We have focused on the *Fusarium* species complex that causes severe diseases in crops throughout the Mediterranean region. This complex has a diverse host range, and causes wilt, fruit deformation and head blight diseases. Preliminary results showed that while the soil-borne pathogen *F. oxysporum* survived sunlight exposure to a great extent, the foliar pathogen *F. mangiferae* was sensitive. We recapitulated the sun exposure sensitivity results by UVC irradiating both species. Sequence comparison of the most important UV damage repair genes between *F. oxysporum* and *F. mangiferae* failed to reveal a major difference. In contrast, *F. mangiferae* is more resistant to the methyl methanesulphonate (MMS), indicating that its base excision repair capacity is greater. RNA sequencing of both species revealed strong transcriptional response to DNA damage. Unlike *Saccharomyces cere-visiae*, *Fusarium* species show over expression of the nedd8 pathway in the context of nucleotide excision repair, but lack activation of the ribonucleotide reductase pathway. There is no evidence for low capacity of DNA repair in the *Fusarium* species complex, suggesting that the high degree of genome instability stems from cis DNA elements.

Characterization of mating type genes of *Alternaria alternata* isolated from onion. W. BIHON[1], L. KENYON[2]. *[1]World Vegetable Center, West and Central Africa, Samanko Research Station, BP 320, Bamako, Mali.*

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Alternaria is a cosmopolitan fungal genus comprising many saprophytic, endophytic and pathogenic species. Pathogenic *Alternaria* species cause major pre- and post-harvest losses on diverse agricultural crops including vegetables. Understanding the mode of reproduction in a plant pathogenic fungus is essential because it affects the population genetic structure, evolution and epidemiology, and so will influence effective disease management. Plant pathogenic fungi in general have different means of reproduction, including sexual, asexual and parasexual mechanisms. Sexual reproduction in ascomycetes is controlled by a mating type (*MAT*) locus. In this study, the complete genomes of two randomly selected strains of *Alternaria alternata* from onion were sequenced, and two genes (*MAT1-1-1* and *MAT1-2-1*) at the MAT locus were identified and characterized. The high mobility group (HMG) and alpha-1 (α-1) box are highly conserved and the genes are, respectively, 1083 and 1217 base pairs in length. The flanking region of both idiomorphs contained DNA lyase. These results suggest that this fungus is heterothallic, since the two opposite mating type genes were found from two different strains. Sexual structures have not been observed in *A. alternata* and the presence of both mating types indicates the existence of cryptic sexual process. This would result in an increase in the genetic diversity of the pathogen and complicate the management practices used. A more detailed study of the frequency and distribution of the two genes in major onion growing locations is necessary to determine how frequent recombination occurs in these populations.

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PRATYTECH: Biotechnological approaches towards control of the root lesion nematode *Pratylenchus penetrans*. C.S.L. VICENTE[1], J. BRANCO[2], J. FIGUEIREDO[3], I. ESTEVES[3], J. CARDOSO[3], A.C. FIGUEIREDO[2], J. BARROSO[2], I.L. CONCEIÇÃO[3], I. ABRANTES[3], M. MOTA[1], P. VIEIRA[1]*. [1]*ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.* [2]*CESAM Lisboa, Faculdade de Ciências da Universidade de Lisboa, CBV, C2, Campo Grande, 1749-016 Lisboa, Portugal.* [3]*Centre for Functional Ecology, Universidade de Coimbra, 3004-517 Coimbra, Portugal.* [*]*E-mail: pvieira@uevora.pt*

Root lesion nematodes (RLN) are ranked third worldwide as plant parasitic nematodes with economic impacts. *Pratylenchus penetrans*, one of the most damaging species of this group, affects more than 400 hosts, and is considered a limiting factor in production of important crops (e.g. corn, potato), ornamentals (e.g. lily, roses) and fruit trees (e.g. apple, cherry orchards). Surveys conducted in Portugal have revealed different RLN species associated with important crops, with *P. penetrans as* one of the most abundant species found in potato fields. Host resistance to RLN is very limited, as only a few genetic loci have been linked to resistance/tolerance to some species. Effective and long-lasting control strategies based on current pesticide compounds are hampered by increasing regulations, due to their non-specificity and potential toxic effects to ecosystems and human health. A promising research area is the identification of critical metabolic and parasitism-related genes of these plant pathogens, in which silencing through RNA interference (RNAi) can promote lethal or inhibitory effects on nematode development or parasitism strategy. The main goal of the project PratyTech is to identify protein-coding genes in *P. penetrans* which may be established as new nematode targets for the development of specific and efficient crop resistance strategies. Another relevant aspect of this project is the study of the host gene expression profile and cellular changes upon *P. penetrans* infection in potato, which should provide important insights into the molecular mechanisms involved in RLN parasitism.

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Tolerance of olive (*Oleae europaea*) cv. Frantoio to *Verticillium dahliae* relies on differential basal and pathogen-induced transcriptomic responses.

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Verticillium wilt (VW) is one of the most serious biotic constraints for olive trees. Knowledge of the genetics of tolerance/resistance to this disease is very limited. To analyze the susceptibility/tolerance of olive cultivars Frantoio (tolerant) and Picual (susceptible) to *Verticillium dahliae,* a comparative transcriptomic analysis (RNA-seq) was carried out in host root tissues. Results showed that a large number (27,312 unigenes) of differentially-expressed genes (DEGs) were found between 'Frantoio' and 'Picual' non-manipulated control roots. Dissimilar root system architecture was also observed between the two cultivars. Upon infection with *V. dahliae*, 'Picual' and 'Frantoio' plants also responded in completely different ways. Genes induced in 'Picual' roots were basically different to the DEGs observed in 'Frantoio' non-manipulated/uninoculated roots. Transcriptome changes occurring in each cultivar at early stages of *V. dahliae* infection were also very dissimilar. When targeting for tolerance/resistance-related genes, the most noticeable expression differences between the cultivars were: i) a pathogenesis-related protein of the Bet v I family, likely encoding a major latex protein; ii) a dirigent-like protein involved in lignification; iii) several *BAK1* (Brassinosteroid insensitive 1-Associated receptor Kinase) and *NHL1* (Harpin-INduced protein-like) unigenes; iv) six unigenes involved in ROS stress response (stronger in 'Picual' but no expression in 'Frantoio'); and v) an overall induction of *BAM* unigenes (involved in starch degradation) in 'Picual' in contrast to 'Frantoio'. These results show that tolerance of 'Frantoio' plants to VW is a complex polygenic plant trait.

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Expressional and positional candidate genes for resistance to *Didymella pinodes* in pea. S. FONDEVILLA, M.D FERNANDEZ-ROMERO, D. RUBIALES. *Institute for Sustainable Agriculture, CSIC, Alameda del Obispo s/n, 14004, Córdoba, Spain. E-mail: sfondevilla@ias.csic.es*

Didymella pinodes, causing Ascochyta blight, is the most destructive foliar pathogen of dry peas. Resistance identified so far is incomplete, and more frequent in wild pea relatives than in cultivated pea. One of these wild relative resistant accessions is the *P. sativum* ssp. *syriacum* accession P665. Quantitative Trait Loci (QTLs) associated with resistance to Ascochyta blight have been identified in the recombinant inbred lines (RIL) population P665 × Messire and in other crosses, but the resistance genes underlying these QTLs are unknown. expressional and positional candidate genes for resistance to *D. Pinodes* were identified by selecting 15 candidate genes to be mapped in the P665 × Messire RIL population. They were differentially expressed in resistant reactions in previous transcriptomic studies, or putatively located into QTLs associated with resistance to this disease according to other pea maps. Thirteen QTLs were successfully amplified in the parental lines. Two were monomorphic, direct polymorphism was found for another, and CAP markers were developed for the remaining ten genes. Therefore, eleven genes could be analysed and mapped in the available P665 × Messire map. Four genes were located within the confidence interval of previously described resistance QTLs or highly associated with resistance parameters. These are therefore suggested as putative candidate genes for resistance to Ascochyta blight.

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Genomic analysis of nontoxigenic strains of *Pseudomonas syringae* pv. phaseolicola. P. LLOP[1], L. BARDAJI[1], M. ECHEVERRÍA[1], P. RODRÍGUEZ-PALENZUELA[2], J. SÁNCHEZ-COLMENERO[2], C. RAMOS[3], J. MURILLO[1]. [1]*Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Universidad*

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Pseudomonas syringae pv. *phaseolicola* (Pph) is an economically important pathogen of bean (*Phaseolus vulgaris*), causing halo blight, and is a relevant research model. Efficient control of halo blight is difficult because the pathogen is transmitted by seed and has very high epidemic potential, and is primarily based on the use of pathogen-free seed and resistant cultivars. However, the efficacy of these methods can be compromised by the variability of local pathogen populations. At least 16 Pph races have been identified, facilitating breakdown of resistance. Likewise, most field isolates of the pathogen from Spain are nontoxigenic and cannot be detected using commercial ELISA antibodies or by PCR targeting the phaseolotoxin cluster. We therefore undertook a comparative genomics approach to better characterize the Spanish pathogen Pph populations, and to develop appropriate detection methods, for a better control of halo blight. Representative Pph isolates were sequenced using Illumina MiSeq with paired-end technology, and their genomes are being compared to the closed genome of the race 6 model strain *P. syringae* pv. *phaseolicola* 1448A. As expected for this highly clonal pathovar, the genomes are highly conserved, with very high sequence identity, which hampers epidemiological studies. The Type III effector repertoire is highly conserved, with variations in only five effectors between strain CYL314 (nontoxigenic) and the reference strain 1448A, including *avrPphF* (*hopF1*). We confirmed that strain CYL314 completely lacks the phaseolotoxin cluster, containing an alternative genomic island. The conservation of other virulence genes will be presented and discussed.

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High-frequency rearrangements of virulence plasmids from *Pseudomonas syringae* are mediated by MITEs and IS*801*. L. BARDAJI[1], M. AÑORGA[1], M. ECHEVERRÍA[1], D. RAMÍREZ-ZAPATA[1], C. RAMOS[2], J. MURILLO[1]. *[1]Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Universidad Pública de Navarra, 31006 Pamplona, Spain. [2]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Área de Genética, Facultad de Ciencias, Campus Teatinos s/n, 29010 Málaga, Spain. E-mail: jesus.murillo@unavarra.es*

The *Pseudomonas syringae* complex includes several species of Gram negative bacteria causing economically relevant diseases in many cultivated plants. Most isolates of *P. syringae* contain native plasmids collectively carrying many pathogenicity and virulence genes, which are readily exchanged intraspecifically. Gene flow is promoted by a diverse array of repeated sequences, among which insertion sequences and miniature inverted-repeat transposable elements (MITEs) are particularly abundant and active. The virulence plasmid pPsv48C from *P. syringae* pv. *savastanoi* NCPPB 3335 is extremely stable, and we showed that it contains two independent functional replicons (*repA* and *repJ*) and 29.5% of its sequence occupied by putative mobile elements. This plasmid spontaneously suffers the deletion of an 8.3 kb fragment, with a frequency greater than 10^{-3}, by recombination between two direct copies of MITE*Psy2*. Likewise, we showed that insertion sequence IS*801* promotes deletions of pPsv48C by one-ended transposition, with an average frequency greater than 10^{-4}, half of these resulting in the loss of a virulence gene. These deletion derivatives were maintained in the population by replication mediated by *repJ*, which is adjacent to IS*801*. We demonstrated that IS*801* also promotes deletions in plasmid pPsv48A and in the large plasmid from *P. syringae* pv. *phaseolicola* 1448A, either by recombination or failed transposition. The accumulation of these types of deletions *in vivo* is prevented by the occurrence in these plasmids of functional post-segregational killing systems, contributing to the maintenance of pathogenicity genes in *P. syringae* populations.

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Detection and phylogenetic analysis of *Grapevine virus A* from important vineyards in Iran. R. MORADI[1], D. KOOLIVAND[1], O. EINI[1], M. HAJIZADEH[2]. [1]*Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.* [2]*Department of Plant Protection, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran. E-mail: Koolivand@znu.ac.ir*

Grapevine virus A (GVA) belongs to the *Vitivirus* genus (family Betaflexiviridae). This virus is one of the most destructive agents in vineyards worldwide. From 2016 to 2017, leaf samples from grapevines with leafroll, reddening of leaf margins and petioles, as well as samples from symptomless grapevines, were collected from vineyards in different regions in west Iran (Abhar, Zanjan and Tarom). Total RNA was extracted from the different tissues of samples according, and was subjected to reverse transcription polymerase chain reaction (RT-PCR) with random hexamer primers. The synthesized cDNAs was used as template to amplify a DNA fragment (865 bp) in PCR by specific primers (GVA-HSS7/GVA-C7273) corresponding to coat protein (CP) gen of GVA. The full-length CP gene was amplified from suspected samples, and the amplified products were sequenced by Macrogen (Korea). The obtained sequences were aligned with other sequences from Genbank, and a phylogenetic tree was prepared using the MEGA6 program. We conclude that the most common leafroll were observed in grapevines as well as growth reductions in vineyards, and these symptoms were reported in grapevines infected with GVA. The sequence identities between the GVA isolates from Iran and the other isolates were 85 to 90% at the nucleotide level. CP-based phylogenetic trees also showed that the new Iranian isolates grouped in a subclade together with GVA isolates from diverse geographical regions, including China, Israel, and Greece.

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Distribution of ToxA, the necrosis virulence gene of the wheat tan spot agent, in North African and Middle East wheat-growing areas. N. OUAAR[1], A. YAHYAOUI[2], A. BENBELKACEM[3], H. BENSLIMANE[1]. [1]*Ecole Nationale Supérieure d'Agronomie, Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, 1 Avenue Pasteur, Hassen Badi, Algiers, Alggérie.* [2]*International Maize and Wheat Improvement Center, Apdo. Postal 6-641, 06600, Mexico DF, Mexico.* [3]*Institut National de la Recherche Agronomique d'Algérie, Unité de Recherche de Constantine, Station ITGC, Elkhroub, Algérie. E-mail: n.ouaar@st.ensa.dz*

Tan spot of wheat (caused by *Pyrenophora tritici-repentis*) is an economically important disease worldwide, and has long been important in North African and Middle East wheat-growing wheat regions. These areas are close to the wheat origin centre. *Pyrenophora tritici-repentis* uses at least three host-specific toxins, PtrToxA, PtrToxB and PtrToxC. Virulence of an isolate is correlated with the presence of these toxins, and host resistance is associated with absence of the sensitivity loci. Synthesis PtrToxA is under control of a ToxA gene, which is the most common virulence gene. To improve knowledge of pathogen populations in northern Africa, and because breeding for resistance to tan spot can be improved by knowledge of the distribution of toxin-encoding genes, 238 isolates sampled from Algerian, Tunisian and Syrian wheat-growing areas were analyzed. Using PCR, a molecular test was applied to the isolates populations to screen for the ToxA gene. ToxA occurred in isolates from all three countries, and the distribution of the ToxA gene was mapped in the study areas. These results allow breeders to better target genotypes in field, and use them according to deployment of the ToxA virulence gene.

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Allelic diversity analysis for Verticillium wilt resistance candidate genes in olive (*Olea europaea*). A. SERRANO, L. LEÓN, A. BELAJ, B. ROMÁN. *Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Centro Alameda del Obispo, Avda, Menéndez Pidal s/n. 14004, Córdoba, Spain. E-mail: alicia.serrano.gomez@ juntadeandalucia.es.*

Verticillium wilt (caused by *Verticillium dahliae*) a destructive soil-borne disease affecting olive crops in traditional production areas. The use of genomic tools could help to overcome some of the difficulties associated with pathogen infestation homogeneity, differences in colonization, ambiguous symptom ex-

pression or lack of high throughput screening techniques in selection for host resistance. Different *Verticillium* resistance genes from olive have been identified in recent transcriptomic studies. We explored the allelic diversity of four of these genes among 77 olive genotypes with different levels of resistance. The olive collection belongs to the World Olive Germplasm Bank, the Wild Olive tree collection and the Olive Breeding Program of IFAPA (Córdoba). The selected genes are among those identified in a suppression substractive library already reported: a disease resistance-responsive family protein (DRR), a transcription factor (GRASS), a caffeoyl-o-methyltransferase (CO-MT) and an acetone-cyanohydrin lyase (ACL). Primers for amplification of exonic regions of these four genes were designed, and amplified fragments were subjected to allele specific sequencing that allowed SNP detection. The overall nucleotide diversity of the identified alleles was determined, and the ratio of synonimous and non-synonimos substitution per respective site were calculated. Predicted proteins and phylogenetic analysis among alleles of each gene were also examined. The information from this study can be used for association analysis in wider germplasm collections. If validated, this knowledge may be useful for enhancing host resistance and/or assisting selection in olive breeding programmes.

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Elucidation of the rust resistance genetic control in Portuguese common bean through a genome-wide association study. S.T. LEITÃO[1], D. RUBIALES[2], M.C. VAZ PATTO[1]. [1]*Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Avenida da República, 2780-157 Oeiras, Portugal.* [2]*Instituto de Agricultura Sostenible, CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain. E-mail: sleitao@itqb.unl.pt*

Common bean (*Phaseolus vulgaris*) is the most important grain legumes for human consumption worldwide, with recognized nutritional and environmental benefits. However, its vulnerability to several diseases leads to significant yield losses and limited cultivation in Europe. It is therefore essential to identify disease resistant sources and to uncover the genetic control of resistance to improve production. Portugal holds unique common bean germplasm resulting from more than five centuries of natural adaptation and farmer's mass selection, not yet fully explored in breeding. We screened 158 accessions from this germplasm against *Uromyces appendiculatus*, the fungus responsible for bean rust. Each host accession was inoculated three times and, on average, 14 plants per accession were tested. Infection type (IT, scored using a 0-4 visual scale) and disease severity (% leaves covered by pustules) were analysed 12 d after inoculation. The most frequent IT was 4, indicating compatible plant-pathogen interactions. Disease severity of plants with IT = 3-4 ranged from <1 to 80%. Three accessions showed low (0-2) IT scores, indicative of incompatible interactions, and were considered resistant or partially-resistant. Eighty-six accessions showed chlorotic halos surrounding rust pustules (IT = 3). The same germplasm collection was also screened with 12k single nucleotide polymorphisms, uniformly distributed throughout the genome. Currently, we are searching for genomic regions controlling rust resistance through a genome-wide association study, using a mixed linear model accounting for genetic structure and familial relatedness. This approach will allow the development of molecular selection tools to assist future precision breeding of rust resistance in common bean.

The Research Unit of Biotechnology and Genetic Resources, INIAV, Oeiras, Portugal provided the common bean seeds. This research was supported by Fundação para a Ciência e Tecnologia (FCT, Portugal) through the grant SFRH/BD/92160/2013, IF/01337/2014 FCT Investigator contract, the project Exploiting bean genetics for food quality and attractiveness innovation (PTDC/AGR-TEC/3555/2012), the Research unit GREEN-it "Bioresources for Sustainability" (UID/Multi/04551/2013), and by COST Actions FA1208 and FA1306.

Phylogenetic and recombination analysis of the partial silencing suppressor NSs gene of *Tomato spotted wilt virus* from Iran. M. ABADKHAH, D. KOOLIVAND and O. EINI. *Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan-Iran. E-mail: Koolivand@znu.ac.ir*

Tomato spotted wilt virus (TSWV), is the type member of *Tospovirus (*family *Bunyaviridae*). This virus contains three single-stranded RNA segments (S,

M and L RNAs). Non-structural NSs protein has been identified as the suppressor silencing and is located on the S segment. Samples suspected to be infected by TSWV were collected from omato fields, and total RNAs were extracted using the RNX plus kit (SinaClone), based on the manufacture's instructions. Reverse transcription-polymerase chain reaction was carried out using a pair of specific primers (TSWV-NSsF/TSWV-NSsR) corresponding to a part of NSs gene of TSWV. A DNA fragment (724bp) was amplified in the PCR. The best result was obtained when the annealing temperature was set to 50°C. The amplified DNA fragment was sequenced and the new sequence was aligned with the reported sequences in the GenBank. The identities between the Iranian and other reported isolates were 96%-99%. Alignment was performed with other international sequences using Clustal W software, then a phylogenetic tree was generated using the MEGA7 program and the Neighbour-Joining method. Phylogenetic analysis showed that the new isolate was in a subclade with three isolates from France (LYE40, LYE47 and STMB3). Recombination events were identified using the Maxchi method in the RDP4 Beta 80 program, and showed that there was evidence for recombination of the Iranian isolate with other isolates (TSW(RT)/T15-2/wt).

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***Fig mosaic virus* as a pathogen in fig orchards in Iran; molecular aspects.** N. KHOSHKHATI, O. EINI, D. KOOLIVAND. *Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran. E-mail: Koolivand@znu.ac.ir*

Fig trees (*Ficus carica*) are one of the most important economic crops in some parts of Iran, and mosaic is one of the most common diseases in fig orchards. The causal agent of this disease is *Fig mosaic virus* (FMV). FMV is naturally transmitted by Eriophyid mites (*Aceria ficus*), and through grafts, but is not seed-transmissible. Based on mosaic symptoms in fig orchards, 30 leaf samples were collected that showed chlorosis, mosaic and leaf deformities. Total RNA was extracted and subjected to reverse transcription polymerase chain reaction (PCR). The amplified fragments were amplified optimized by a pair of specific primers, E5-s (Forward primer) and E5-a (reverse primer), which amplified a part of RNA1 with about 300 bp length. Positive samples came from leaves showing mosaic, deformation and chlorosis symptoms. PCR products were purified and sequenced (Bioneer) with specific primers. The obtained sequences from new isolates were compared with previously reported isolates around the world in GenBank sequence database, and showing high similarity between the new PNRSV isolates and some from Iran, Poland and India. In addition, a phylogenetic tree was generated with the Maximum Parsimony (MP) method using the MEGA6 program, and this demonstrated that the new isolates were grouped with isolates from diverse geographical regions, including Iran and Turkey. This showed that there are geographical relationships between isolates based on their phylogenies.

This research was supported by the University of Zanjan.

Physical structure of the chromosome ends and their implications in *Fusarium oxysporum* genome plasticity. L. GÓMEZ, G. BRAVO, A. DI PIETRO, M.I.G. RONCERO. *Departamento de Genetica, Campus de Rabanales, Edif. C5, Universidad de Cordoba, E14071-Cordoba, Spain. E-mail: b12gogil@uco.es*

The genome of *Fusarium oxysporum* is highly dynamic and contains lineage specific (LS) regions rich in transposable elements that are involved in pathogenic behavior. Unexpectedly, Southern blot hybridisation and sequence analysis in the tomato pathogenic isolate *F. oxysporum* f. sp. *lycopersici* 4287 revealed a high degree of structural and physical conservation in the telomeric and subtelomeric regions, extending approximately 30 kilobases from the chromosome ends. The evolutionary origin of this highly conserved region is currently unknown. Our mail goal has been to analyse the function of the conserved telomeric and subtelomeric regions in the maintenance and plasticity of the chromosome structure, as well as in the regulation of the adjacent genes.

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Integrated disease management

Development of the root-knot nematodes in zucchini and associated yield losses. S. VERDEJO-LUCAS[1], M. TALAVERA[2], A. PÉREZ-DE-LUQUE[3]. *IFAPA. [1]Camino de San Nicolás 1, 04745, La Mojonera, Almería, Spain. [2]Camino de Purchil s/n, 18004 Granada, Spain. [3]Alameda del Obispo, 14080 Cordoba, Spain. E-mail: soledad.verdejo@juntadeandalucia.es*

The interaction of *Cucurbita pepo* genotypes and *Meloidogyne* populations resulted in a poorer host condition from *M. incognita* than from *M. javanica*. The critical event in the *M. incognita*-zucchini interaction was the development from fourth stage juveniles to adult females. *Meloidogyne incognita*-induced feeding sites contained more and larger highly vacuolated giant cells than those of *M. javanica*, but 74% of the *M. incognita* feeding sites deteriorated before life cycle completion. In contrast, 96% of the invading *M. javanica* reached the egg-laying female stage. The extent of yield losses in zucchini depended on the interaction of (large) initial population densities, planting time and length of the growth period. Average yield losses in zucchini range from 20-36%, although densities below 125 nematodes/250 cm^3 of soil have negligible impact. Populations greater than 1100 nematodes/250 cm^3 of soil reduced yield by 52%. Population increases were greater in autumn cropping cycles than in spring, but yield reductions were less in autumn than spring cycles. Limited yield losses were observed in growth periods of ca. 2 months, in contrast to those of ca. 3-4 months. Root galling and yield were negatively related, indicating that the root-knot nematode damage was critical for plants, as it decreased yield. The leaf chlorophyll content decreased with increased population densities and post-infection time, with significant reductions at densities greater than 450 nematodes/250 cm^3 soil. Modification of planting date and cropping nematode resistant or non-host crops before planting zucchini effectively reduced nematode damage in zucchini.

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Meta-analysis of the effect of the application period in the management of Botrytis bunch rot in vineyards. G. FEDELE[1], E. GONZÁLEZ-DOMÍNGUEZ[1,] L. DELIÈRE[2], P. SAURIS[2], E. DÍAZ-LOSADA[3], J.L. RAMOS SÁEZ DE OJER[4], D. GRAMAJE[5], V. ROSSI[1]. *[1]Department of Sustainable Crop Production, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84 29122 Piacenza, Italy. [2]SAVE, Bordeaux Sciences Agro, INRA, 33140 Villenave d'Ornon, France. [3]Estación de Viticultura e Enoloxía de Galicia - INGCAL. Ponte San Clodio s/n, 32427, Leiro, Ourense, Spain. [4]Consejería de Agricultura, Ganadería y Medio Ambiente. Finca La Grajera, Ctra. LO-20 salida 13, 26071, Logroño, Spain. [5]Instituto de Ciencias de la Vid y del Vino (Consejo Superior de Investigaciones Científicas, Universidad de La Rioja, Gobierno de La Rioja). Finca La Grajera, Ctra. LO-20 salida 13, 26071, Logroño, Spain. E-mail: vittorio.rossi@unicatt.it*

Botrytis cinerea is one of the most important pathogens of grapevines. The management of *B. cinerea* is challenging, mainly because the pathogen produces large numbers of conidia on multiple inoculum sources, grapevines are susceptible at multiple growth stages, and different infection pathways exist. This complexity has caused growers to rely heavily on routine application of fungicides at four growth stages: flowering (A), pre-bunch closure (B), veraison (C) and before harvest (D). Recently, a weather-driven model has been developed to predict the development of Botrytis bunch rot. The model was validated in 21 epidemics using a discriminant function analysis (DFA) with 81% accuracy; the DFA also showed that the infections occurring during flowering and fruit set may play a key role in determining the severity of *B. cinerea* rot on mature bunches. These results are in apparent contrast with recommendations from the research carried out mainly in the 1990s, which suggested that sprays in growth stages B and in C to D as the most important for disease control. To better understand the contributions of the fungicides applied either in A, B, C or D on final Botrytis bunch rot, we performed a meta-analysis of 115 studies, conducted from 1970 to 2016 in eight countries, covering a wide range of epidemics. Raw data showed that average efficacy of treatments in A was 31% (range from 0.98 to -0.30), B, 22% (range from 0.84 to -0.67), C, 40% (range from 0.96 to -0.81) and D, 39% (range from 0.92 to -0.76). average

efficacy of a four-treatment schedule (at stages A, B, C and D) was 79% (range from 0.79 to 0.23). Studies were weighted in inverse proportion to their sampling variances for model fitting purposes. Results of the meta-analysis, combined with the epidemiological model, provide new information on how to schedule fungicides treatments for controlling *B. cinerea* in grapes.

Copper: a basic active ingredient in the control of olive diseases. L.F. ROCA, J.R. VIRUEGA, A. ÁVILA, J. MORAL, F. MARCHAL, J. ROMERO, P. MIRANDA, C. AGUSTÍ-BRISACH, A. TRAPERO. *Dpto. de Agronomía (Patología Agroforestal), Universidad de Córdoba. Campus de Rabanales, Edificio C-4, 14071, Córdoba. E-mail: trapero@uco.es*

Copper is extensively used in the control of olive diseases. More than 80% of the authorized fungicides in this crop in Spain contain this element as the active ingredient. Among the main diseases affecting the aerial part of olive trees are leaf spot (caused by *Venturia oleaginea*), cercosporiose (*Pseudocercospora cladosporioides*) and anthracnose (*Colletotrichum* spp.) These diseases cause defoliation and weakness of the trees and anthracnose also produces fruit rot, being especially severe in humid, temperate autumns. Other diseases, such as leprosy (*Phlyctema vagabunda*) or tuberculosis (*Pseudomonas savastanoi* pv. *Savastanoi*) are becoming increasingly relevant, mainly due to the intensification of olive culture. All of these diseases have been traditionally controlled using copper products. Copper treatments are preventive. Persistence and mode of action, which prevent development of pathogen resistance, are responsible for the success of these products. Future limitation of the amount of copper applied per hectare and year in olive plantations, imposed by the European Union, will force optimization of fungicide applications, with reduction of copper doses, use of alternative active ingredients and disease prediction models. Strategies of mixing copper and systemic fungicides (e.g. triazoles and strobilurins), have allowed reductions of up to 67% the amount of copper in the control of olive leaf spot. Dodine and bentiavalicarb are also examples of alternative and efficient active ingredients against olive leaf spot. Research must be intensified to achieve effective control of anthracnose, leprosy and tuberculosis.

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Tomato defence responses to nematodes and viruses induced by ozone treatments. M.I. PRIGIGALLO, P. VERONICO, F. CILLO, M.T. MELILLO, N. SASANELLI, G. BUBICI. *Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, 70126, Italy. E-mail: giovanninicola.bubici@cnr.it*

Ozone is widely used as a disinfectant, and ozonated water has been known to confer some protection of plants against several biotic stresses. By applying four foliar spray treatments of ozonated water (10 ppm ozone) on tomato seedlings, i.e. two pre- and two post-inoculation with *Tomato spotted wilt virus* (TSWV), we observed reduction of disease incidence and severity by 20%, as well as a virus titrr reduction by 80% at 19 days post-inoculation. The same treatments also reduced the number of galls induced by root knot nematode (RKN; *Meloidogyne incognita*) by 29%. Soil drenching with ozonated water for four consecutive days before inoculation reduced RKN gall formation by 60%, but not TSWV infection. Overall, in mock-inoculated plants, foliar sprays induced *PR1b1* expression in leaves, though other salicylate- (*PAL* and *PR-5x*) or jasmonate-dependent genes (*LoxD*, *AOS* and *PinI*) were substantially unaffected. Soil drenching promptly enhanced transcription of *PAL* and *PR1b1* in roots and leaves, down-regulated *PR-5x* and did not affect expression of *LoxD* and *AOS*. *PinI* was significantly down-regulated only in leaves. The impact of ozonated water applications on the expression of these genes did not correlate with that of benzothiadiazole, a known inducer of systemic acquired resistance. This demonstrates that ozonated water may protect tomato from two very different biotic stresses, especially when applied at the sites of their infection, and modulates salicylate and jasmonate pathways differently from benzothiadiazole.

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Early assessment of late wilt of maize (*Harpophora maydis*) and the control effect of *Lycium europaeum* extracts. C. RODRÍGUEZ-MALLOL[1], R. TEJ[1,2], L. MOLINERO-RUIZ[1]. [1]*Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain.* [2]*Physiology and Biochemistry of Plant Response to Abiotic Stresses Unit, Faculty of Sciences of Tunis, University of Tunis El Manar, 1060 Tunis El Manar, Tunisia. E-mail: lmolinero@ias.csic.es*

Maize late wilt (MLW), caused by the soilborne fungus *Harpophora maydis*, is characterized by the sudden appearance of symptoms from plant flowering onwards. Since genetic resistance is the most effective control method, protocols for the early evaluation of maize lines are required. As well, extracts of *Lycium europaeum* inhibiting the *in vitro* growth of *H. maydis* are promising as a potential control method. Three experiments were conducted under greenhouse conditions. In the first two experiments, inoculated plants were grown in different artificially colonized substrates for 6 weeks. The most disease conducive substrate was used in the third experiment, where effects of methanolic leaf extracts of *L. europaeum* were assessed. In the first experiment, plant development after inoculation was delayed an average of 3 d in phenological stages V_4 to V_5. In the second experiment, reductions of weights of aboveground parts and roots were recorded in inoculated plants compared with the controls. Necrotic lesions were apparent in the roots of the plants as early as 4 weeks after inoculation. In the third experiment, weight reductions only occurred in the plants inoculated with *H. maydis*. When the plants were inoculated and treated with different extracts of *L. europaeum*, one extract with a high chlorogenic acid content resulted in weights of roots that did not differ to those of the controls. This study has established a protocol for early evaluation of MLW, and has demonstrated bioactivity of *L. europaeum* against this disease.

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Maternal and paternal effects on the heritability of Verticillium wilt resistance in olive progenies. P. VALVERDE, C. TRAPERO, D. BARRANCO, C.M. DÍEZ, FCO.J. LÓPEZ ESCUDERO. *Departamento de Agronomía. Escuela Técnica Superior de Ingenieros Agrónomos y Montes Universidad de Córdoba. Edificio C-4, Planta baja. Campus Universitario de Rabanales. 14071, Córdoba. Spain. E-mail: pedrovalverde@uco.es*

Verticillium wilt, caused by the fungus *Verticillium dahliae*, is currently the most important disease of olive trees, causing important losses in olive growing countries worldwide. The use of genetic resistance is likely to be the most efficient, economically convenient and environmentally friendly control method to combat the disease, and will be a key component in integrated disease management. Twelve reciprocal crosses corresponding to all pairwise combinations were performed with the olive cultivars 'Picual', 'Frantoio', 'Sikitita' and 'Arbosana' acting as male or female parents. Additionally, the crosses 'Koroneiki' × 'Arbosana', 'Arbosana' × 'Koroneiki', 'Arbequina' × 'Arbosana' and 'Arbosana' × 'Arbequina' were investigated. The main goal was to evaluate the differential effect of the cultivars acting as mothers or fathers, in the level of resistance of the progenies to Verticillium wilt. Fruits were harvested during October and then seeds were sown and germinated in controlled conditions. Five-week-old olive seedlings were inoculated by dipping their bare root systems in conidial suspensions of the defoliating *V. dahliae* isolate V117. Weekly, during 10 weeks, plants were evaluated for symptom development, using a 0 to 4 scale, and every 2 weeks plant growth was also measured. The evaluated parameters presented variable values, depending on the reciprocal crosses. For instance, no differences in disease severity were recorded in the reciprocal crosses of 'Arbosana' and 'Arbequina', but differences up to 50% occurred in the reciprocal crosses of 'Frantoio' and 'Sikitita'. No differences plant death were recorded in the progeny of the reciprocal crosses of 'Arbosana' x 'Sikitita', but important differences up to 40% occurredin the reciprocal crosses of 'Sikitita' and 'Picual'.

Evaluation of selected soils for suppression of *Fusarium* diseases. M. SANTOS[1], F. DIÁNEZ[1], F. CARRETERO[1], F.J. GEA[2]. [1]*Agronomy Department, University of Almeria, Carretera Sacramento s/n. Almería 04120. Spain.* [2]*Departamento de Agronomía, Escuela Politécnica Superior, Universidad de Almería, Almería, Spain. E-mail: msantos@ual.es*

The study of naturally occurring disease-suppressive soils has produced significant progress towards acquiring understanding of the biotic and abiotic forces that inhibit plant disease development in such soils. This research evaluated possible suppressiveness of four different soils where Fusarium diseases have low severity (disease-suppressive, S). Soils used in all experiments were collected from a commercial organic carnation production glasshouse from Cádiz (Spain) (S1, S2 and S3) and Sevilla (Spain) (S4), and were inoculated with *Fusarium oxysporum* f. sp. *melonis* (Fom; race 0) and *Fusarium oxysporum* f. sp. *niveum* (Fon; race 0) (), at concentration 10^3 or 10^6 cfu g^{-1}. Experimental controls of Conductive soils (C), disinfested soils (D) and vermiculite (V) were included with and without inocula. The suppressiveness of the soils to Fusarium yellow of melon and Fusarium wilt of watermelon were evaluated for 50 d. Severity of Fom was reduced by 0%, and and Fon by -50%, in comparison to inoculated vermiculite ($P < 0.05$). The most suppressive soil was S4 for both pathogens. The growth of both pathogens in S-soils was suppressed compared with C soils and V, which suggested that S-soils displayed greater fungistasis than C-soils or V. The suppressiveness to the pathogen was reduced in D-soils. The suppressiveness to the pathogen was therefore likely to be due to microorganisms colonizing the roots grown in S-soils, and the suppression may be due to antagonistic microorganisms.

Reaction of some watermelon varieties to the races of *Fusarium oxysporum* f. sp. *niveum*. B. GEÇİOĞLU-ERİNCİK[1], M.T. DÖKEN[2]. [1]*Adnan Menderes University, Koçarlı Vocational School, Aydın.* [2]*Adnan Menderes University, Faculty of Agriculture, The Department of Plant Protection, Aydın. E-mail: bgerincik@adu.edu.tr*

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (Fon), is one of the major limiting factors for watermelon production. In recent surveys, this disease has been found to be widespread in the Province of Aydın. One of the best control measures against the disease is the use of resistant cultivars. The watermelon varieties (Crimson Sweet, Crimson Tide, Galaxy, Wonder and Anthem F1), commonly grown in the Aydın Province, were tested against the three races (Race 1, 2, and 3) of Fon. Root dip inoculation was used, and the experiment was conducted in a growth chamber. The reaction of each watermelon variety differed, depending on the race of the pathogen. Cultivar Wonder exhibited the least disease severity, ranging from 9% to 35% depending on the races. Crimson Sweet was the most susceptible cultivar, developing up to 78% disease severity.

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Identification of optimal cereal/legume combinations for Mediterranean rainfed farming systems. A. VILLEGAS-FERNÁNDEZ, A. AMARNA, D. RUBIALES. *Institute for Sustainable Agriculture, CSIC, Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. E-mail: diego.rubiales@ias.csic.es*

Modern agriculture based on monocultures suffers from a lack of biodiversity. Increasing the diversity of crop systems offers opportunities to improve yield stability, reduce pest and disease damage, and to enhance stress resilience in agricultural systems. This diversification should be adapted to each situation, considering a wide range of factors from the specific crop to local weather conditions. In an attempt to optimize cereal/legume combinations for Mediterranean rainfed farming systems, two field experiments were established at Córdoba in growing season 2016/2017 that will be extended to other areas in 2017/18. Crop combinations of wheat/Faba bean and barley/pea were tested, respectively, in the two experiments. Mixed intercropping was chosen, with a 50/50 proportion of cereal/legume. Two varieties for each crop were included. A split-plot experimental design was adopted, with main plots being management (two levels: conventional, i.e., applying the normal levels of fertilizers and pesticides; and low-level, with limited application of fertilizers and pesticides), and subplots being crop combination (including all possible combinations of the cereal and legumes varieties, as well as monocrops). Plot size was 3 × 4 m. Evaluations will include a wide number of parameters, including germination rates, vegetative biomass, weed biomass, plant heights, ground coverage, yields and disease incidence.

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Alternative seed treatments as a substitute for chemical seed treatments to control common bunt of wheat. M. NOCENTINI[1], T. CINELLI[1], C. COMPARINI[1], S. BENEDETTELLI, L. MUGNAI[1]. [1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: marconoce@gmail.com*

In recent years, the cultivation of ancient wheat varieties has been relaunched, due to their high nutrient and protein composition linked to low gluten content, and good organoleptic characteristics, mainly through the work of organic small-scale farmers. For organic production the seed cannot be treated with synthetic chemicals. This created a serious issue, due to the difficulty to obtain organic seed free from common blunt spores, and all the issues related to "homemade" treatments. For the above reasons, common bunt (caused by *Tilletia* sp.) became a major seed- and soil-borne disease for organic wheat producers. This study investigated alternative control measures to chemical seed treatments, that are environmentally friendly to support cultivar resistance, are easy to use and can be applied on small farms. Following good results obtained *in vitro*, against the germination of *Tilletia* teliospores, several organic products were used *in vivo* on seed of an ancient bread wheat cv. Sieve, artificially inoculated with *Tilletia* teliospores. The products being tested are: monoglycerides, *Sinapis alba* flour, *Pseudomonas chlororaphis*, copper complexed with a carrier, copper and zinc mixture complexed with citric acid, and peracetic acid. These products were compared with a traditional copper formulation registered for seed treatment, and two synthetic chemical products, one based on fludioxinil and the other a mixture of sedaxane, fludioxonil and difenoconazole. The results obtained in *in vitro* and *in vivo* trials will be presented.

Development of integrated disease management of fire blight using biocontrol agents and plant defense activators. S. AIT BAHADOU[1,2], A. OUIJJA[1], M.A. BOUKHARI[2], A. TAHIRI[2]. [1]*Laboratory of Plant Biotechnology and Molecular Biology, Moulay Ismail University, Faculty of Sciences; BP 11201, Ave Zitoune Meknes, Morocco.* [2] *Department of Plant Protection and Environment of the National School of Agriculture-Meknes, Km10, Rte Haj Kaddour, BP S/40, Meknès 50001, Morocco. E-mails: s.aitbahadou@edu.umi.ac.ma, ismail.bahadou@gmail.com*

The bacterial antagonists *Bacillus subtilis GB03, B. subtilis QST713, B. subtilis Y1336 and Pantoea agglomerans P10c*, and plant defense activators acibenzolar-S-methyl (ASM), fosetyl aluminium (F-Al), potassium phosphites (PH) and prohexadione-Ca (ProCa) were evaluated individually and in combinations for control of fire blight in Morocco. Under laboratory conditions, on detached blossoms of apple and pear, only biocontrol treatments based on *P. agglomerans P10c* and its mixture with *B. subtilis QST713* showed reduced the incidence of the disease when compared to other treatments. Under field conditions, the above mixture of biocontrol agents, as well as all other strains, were tested alone or combined with plant defense activators, using a split-split-plot trial design. The treatments were applied on trees at timings based on their respective modes of action. Results showed that *P. agglomerans P10c* reduced blossom infection by 66%, *B. subtilis QST713* by 64%, their 1:1 mixture by 62%, *B. subtilis GB03* by 64%, and *B. subtilis Y1336* by 53%. For the plant defense activators this reduction was 62% for ASM, 57% for ProCa, 50% for F-Al, and 49% for PH. On shoots, disease reductions ranged from 40% to 80% for the biocontrol agents, and 46% to 97% for the plant defense activators. Two applications of ProCa was the most effective treatment for reducing shoot blight incidence. The combination of plant defense activators and biocontrol agents allowed the greatest protection against blossom and shoot blight, ranging from 76% to 98%. The greatest protection was resulted from *B. subtilis QST713, P. agglomerans P10c* or their mixture combined with ASM or ProCa.

***Harpophora maydis* affecting maize in Southern Europe: different growth media, long-term storage and *in vitro* effects of extracts of *Lycium europaeum* L.** C.M. ORTIZ-BUSTOS[1], Y. MONGELÓS[1,2], R. TEJ[1,3], L. MOLINERO-RUIZ[1]. [1]*Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain.* [2]*Multidisciplinary Center of Technological Research (CEMIT), General Direction of Scientific and Technological Research (DGICT), National University of Asunción (UNA), Mcal. Estigarribia Km 10,5, 2169 San Lorenzo, Paraguay.* [3]*Physiology and Biochem-*

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Late wilt of maize is a vascular disease caused by the soilborne fungus *Harpophora maydis*. The disease is frequent in warm climates where high temperatures occur at maize crop flowering and tasseling stages. Genetic resistance is the most effective control method, but intermediate reactions of resistance highly dependent upon environmental conditions are frequent. Complementary and alternative control strategies are needed within integrated pest management programmes. The genus *Lycium* (Solanacecae) is well known as a herbal medicine with broad biological activities including antimicrobial effects. We selected the most appropriate culture media for *H. maydis* and evaluated different methods for its long-term storage. We also assessed *in vitro* effects of water and/or methanol extracts from *L. europaeum* on the pathogen. *Harpophora maydis* did not grow on acidified corn meal agar (CMAa). Greatest growth was recorded on lactic acid-potato dextrose agar (PDAa) and CMA, but fungal sporulation on CMA was less than on both PDA or PDAa. Glycerol and a sterilized soil mixture were appropriate to maintain viability of the fungus for at least one year. Maintenance at room temperature favoured mycelial growth. Four methanol extracts from *L. europaeum* (two from leaves and two from stems) reduced growth of *H. maydis*. The greatest antifungal effect was from an extract with the greatest total phenolic content, particularly with a large content of chlorogenic acid.

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Chemical composition and antifungal activity of essential oils from two Labiatae species. N. ISSIAKHEM-TAMDA[1], F. DAVIS[2], M. HAZZIT[3], M. AMIALI[3], N. ZERMANE[1].[1]*Agricultural National High School - El-Harrach-Algeria-Laboratory of Plant Physiology, Department of Botany, Avenue Hassan Badi, El Harrach-16004, Algiers, Algeria.* [2]*School of Chemistry, Food Biosciences & Pharmacy, University of Reading, UK.* [3]*Agricultural National High School, El-Harrach, Algeria, Laboratory of Chemistry, Department of Technology. E-mail: issiakhem.nadia@gmail.com*

The excessive use of synthetic chemicals to reduce the severity of pests and diseases in agriculture has generated pesticide resistance to some of the active ingredients, from selection pressure due to high doses and continuous applications, causing important economic losses. Consequently, it is important to examine alternative control strategies, such as using natural products from plant origin which are unlikely to adversely affect the environment and human health. Analysis and identification were undertaken of essential oils hydrodistilled from aerial parts of the Lamiaceae aromatic plants, origanum (*Origanum floribundum* Munby) and spearmint (*Mentha spicata* L.), using gas chromatography and mass spectroscopy. Their antagonistic activity was evaluated against pathogenic fungi isolated from legume crops, including *Botrytis* sp., *Fusarium* sp., *Alternaria* sp., and *Ascochyta* sp.. The major components of the oregano oil were carvacrol, thymol and p-cymene, and of the spearmint oil were carvone, limonene and eucalyptol. Mycelia discs taken from the margins of 7-d-old cultures were placed in the middle of PDA plates together with 1, 3 and 5 μL of essential oils (applied individually) added on 5 mm sterile Whatman paper discs placed in the middle of each Petri dish cover. The Petri dishes were sealed with parafilm and incubated in darkness at 25°C for 8 d. Control plates were treated with the same amounts of sterile distilled water. Oregano essential oils showed the greatest inhibition activity, and causing greater than 70% reduction of the mycelial growth for the three concentrations of essential oil. The spearmint oil was less active with the greatest inhibition activity against mycelia growth obtained with 5μL (about 32% of growth reduction).

Evaluation of microbial antagonists and siderophore production in the tomato phyllosphere as biocontrol agents. F. DIÁNEZ[1], J. YAU[2], M. SANTOS[1]. [1]*Departamento de Agronomia, Universidad de Almeria, Carretera Sacramento s/n, Almería 04120, Spain.* [2]*National Agriculture Research Institute of Panamá, Buildings 161, 162, Knowledge City, Clayton,. Carlos R. Lara Street, Panama Republic. E-mail: msantos@ual.es*

Microbial antagonists from phyllospheres of healthy greenhouse-grown tomatoes were isolated and

evaluated for their effectiveness in inhibiting mycelial growth of pathogenic fungi in dual culture and detached leaf assays, and for their siderophore production. A total of 63 bacterial and 68 fungal isolates were isolated from the tomato leaves and their antagonistic activity was evaluated in dual culture assay. The isolates inhibited mycelial growth against *Botrytis cinerea* (41% reduction), *Fusarium oxysporum* f. sp. *lycopersici* (66%), *Fusarium oxysporum* f. sp. *radicis-lycopersici* (33%), *Mycosphaerella pinodes* (53%), *Phytophthora parasitica* (47%), *Pythium aphanidermatum* (37%) and *Verticillium dahliae* (83%). To evaluate the antagonistic activity against *B. cinereal*, a total of 22 fungal and three bacterial isolates were tested in a detached leaf assay. Five of 22 fungus isolates prevented mycelial growth when compared to untreated controls when these were observed at ×40 magnification. Nineteen 21 fungus isolates produced siderophores, as indicated by CAS assays. These results indicate that organically-grown plants could be an good source of potential biocontrol agents. The future use of biological–chemical combinations, of endophytes in combination with commercial pesticides applied to the seeds or seedlings, could give synergistic effects on one or multiple disease-causing agents.

Evaluation of E.M., Zeolite and Agri-fos 600® to control Verticillium wilt. C. LAGOGIANNI[1], K. SOTIROPOULOS[1], A. KOULOUVARI[1], G. ZAKYNTHINOS[2], D.I. TSITSIGIANNIS[1]. [1]*Laboratory of Phytopathology, Department of Crop Science, School of Agricultural Production, Infrastructure and Environment, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece.* [2]*Department of Food Technology Technological Educational Institute (A.T.E.I.) of Kalamata, Kalamata, Greece. E-mail: dimtsi@aua.gr*

Induced resistance is part of plant immune systems developed after the stimulation of resistance mechanisms, resulting from non-pathogenic microorganisms or chemical inducers. This study evaluated the non-pathogenic beneficial microorganism formulation E.M. and the chemical inducers zeolite and Agri-fos 600®, to induce resistance mechanisms and control Verticillium wilt. E.M. is a formulation based on Effective Microorganisms, and is used to improve the quality-fertility of soil and the growth-quality of crops. Zeolite is a microporous, aluminosilicate mineral used as a commercial adsorbent and catalyst, and as a soil improvement substance. Agri-fos 600® is a formulation of potassium phosphonate anions that induce plant defense mechanisms. Pathogenicity experiments were performed in *Arabidopsis thaliana,* tomato and eggplants infected with *Verticillium dahliae*. Zeolite, E.M. and Agri-fos 600® were applied as root drenches. Virulence assays in greenhouse experiments showed that only zeolite and Agri-fos 600® reduced Verticillium wilt in tomato and *A. thaliana*, by 5-20%, but did not have any effect on eggplant. In contrast, E.M. reduced the disease only in eggplants by 20%. E.M. and zeolite were also evaluated in field experiments in naturally infested soil, where zeolite reduced Verticillium wilt in tomato plants by 25%, and E.M. by 45%. Quantification of *Verticillium* microsclerotia in soil showed that their numbers were reduced by 15% after treatment with E.M. compared to untreated control plants.

Plant pathology and food safety

Identification and characterization of *Acidovorax citrulli* strains from Serbia. N. ZLATKOVIĆ[1], A. PROKIĆ[1], K. GAŠIĆ[2], N. KUZMANOVIĆ[3], M. IVANOVIĆ[1], Ž. PAVLOVIĆ[1], A. OBRADOVIĆ[1]. [1]*University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Department of Plant Pathology, Nemanjina 6, 11080 Belgrade, Serbia.* [2]*Institute for Plant Protection and Environment, Department of Plant Pathology Teodora Drajzera 9, 11000 Belgrade, Serbia.* [3]*Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany. E-mail: nevena_blagojevic@yahoo.com*

In August 2014, typical bacterial fruit blotch symptoms were observed on mature watermelon fruit originating from fields in the Vojvodina province of Serbia. In the summer 2015 and 2016 we registered two more occurrences of the disease, in, respectively, the east and west areas of the country. White, glistening, convex and circular colonies with regular edges were predominantly isolated from diseased watermelon fruit collected from the affected fields. A total of 33 bacterial strains were subjected to further analyses. They were Gram-negative, aerobic, oxidase and catalase positive, nonfluorescent, and did not produce potato soft rot. All but two strains (KFB

359 and KFB 363) induced hypersensitive reactions in tobacco leaves. They grew at 41˚C and produced beige to tan-coloured, nonmucoid, convex colonies on yeast extract-dextrose-$CaCO_3$ agar. All strains studied used L-arabinose, did not reduce nitrate, nor utilized sucrose. Conventional PCR was performed using *A. citrulli*-specific primers BX-L1/BX-S-R2. The 16S rRNA gene sequence from two strains (KFB 343 and KFB 344) showed 100% identity to strains of *Acidovorax citrulli* from China, Thailand and the USA. These results identified the causal organism as *A. citrulli*. Genetic relatedness among strains was investigated by rep-PCR, using BOX and REP primers. All tested strains except one (KFB 358), showed the same BOX-PCR profiles. In addition, all strains were assigned to the same REP-PCR group. These results show that *A. citrulli* strains isolated in Serbia during three years belong to an homogeneous population. These occurrences of bacterial fruit blotch are considered to be of seed-borne origin, but is not known whether the pathogen will survive from season to season in Serbian climatic conditions.

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Screening of mycotoxin profile and mycotoxin gene clusters in toxigenic fungi of food crop plants reveals phenotypic and genetic variability at intraspecific levels. A. SUSCA, A. LOGRIECO, M. HAIDUKOWSKI, A. VILLANI,, A. MORETTI. *Institute of Sciences of Food Production, Italian National Research Council (ISPA-CNR), Via Amendola 122/O, 70126, Bari, Italy. E-mail: antonio.moretti@ispa.cnr.it*

There is concern regarding occurrence of toxigenic fungi on food and feed crops, since mycotoxin accumulation in the final products represents serious risks for human and animal health. Among the plant pathogens that produce mycotoxins *in planta*, *Aspergillus* and *Fusarium* spp. are the most common, and show great variability of their mycotoxin profiles, even in closely related species or at intraspecific levels. We report here results from studies conducted usingHPLC/FLD and LC-MS/MS measurements and whole genome sequencing: i) variability of Ochratoxin A (OTA) production related to the occurrence of the *ota* gene cluster in the *Aspergillus niger* clade, where both the intact and deleted clusters coexist; ii) variability of beauvericin (BEA) production and occurrence of the BEA gene cluster in *Fusarium subglutinans* and *F. temperatum*, two phylogenetic sister species where toxigenic potential is not related to real production capacity *in vitro*; iii) variability of fumonisin production and FUM gene cluster occurrences in *F. proliferatum* isolated from fig and maize, two populations with the same toxigenic potential but different fumonisin production capacity; iv) variability of trichothecene production in the *F. equiseti/incarnatum* species complex and related variability in the trichothecene gene cluster. Taken together, these data show that mycotoxin gene clusters can differ within a single species, or among very closely related species. The lack of a given mycotoxin production, at least in *in vitro* conditions, is frequently, but not always, related to the absence of gene clusters.

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Identification of pathotypes and analysis of the genetic structure of *Fusarium oxysporum* f. sp. *lentis* populations. H.R. POURALIBABA[1], Z. SATOVIC[2], M.J. COBOS[3], D. RUBIALES[3], S. FONDEVILLA[3]. [1] *Dryland Agricultural Research Institute, Education and Extension Organization (AREEO), Maraghe 119, Iran.* [2] *Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska 25, 10000 Zagreb, Croatia.* [3]*Institute for Sustainable Agriculture, CSIC, 14004 Córdoba, Spain. E-mail: hpouralibaba@ias.csic.es*

Lentil cultivation is threatened worldwide by Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lentis* (*Fol*). Knowledge on pathogenic diversity and genetic structure of *Fol* populations is fundamental for breeding for resistance and managing the disease. We therefore studied virulence diversity within a collection of *Fol* isolates. Twenty-eight resistant lentil accessions were inoculated with six *Fol* isolates from different geographical origins. The lentil accession × *Fol* isolate effect was highly significant, which allowed four accessions to be selected as a differential set. Inoculation of this set with 48 *Fol* isolates from Iran, Syria and Algeria, allowed the identification of seven different virulence patterns, designated pathotypes 1 to 7. In addition, the genetic structure of this *Fol* collection was analyzed using twelve SSR markers, eight of which were designed in this study.

AMOVA showed that there was large molecular variation within groups but also between groups, showing that the Iranian populations were different from non-Iranian populations. STRUCTURE and Fitch-Margoliash tree analyses concluded the presence of two ancestral *Fol* lineages, one distributed in all regions while the other was only present in Iran. Our results suggest that Iran could be the origin of the diversity demonstrated in this study.

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Comparison of microbial quality of lettuce grown under three crop systems in Cyprus. C. MENELAOS CHRISTODOULOU, E. SAVVA, D. TSALTAS. *Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology.E-mail: dimitris.tsaltas@cut.ac.cy*

Leafy greens, which are usually consumed raw, are increasingly recognized as important vehicles for transmission of foodborne pathogens. Contamination with pathogenic bacteria can occur at different stages of the production and distribution chain, making food safety of vegetables an important priority. Lettuce is the most commonly consumed vegetable worldwide, growing in close proximity to soil, while rain and irrigation water facilitate microbial movement and contamination. It is of scientific and public interest to explore how different production systems affect the presence of foodborne pathogens, since there is skepticism about safety of organic produce, hydroponics is increasing, aquaponics is a new appealing production trend. Lettuce and irrigation water samples of four years (2013-2016) were used for analysis. Three hundred and sixty lettuce samples (110 conventional (C), 105 organic (O), 90 hydroponic (H) and 55 aquaponics (A)), were collected and analyzed. For water analysis, 64 irrigation water samples (39 C/O, 15 H and 10 A) were analyzed. Total aerobic microbe counts, and Enterobacteriaceae for aquaponics, was 2 log less than in other samples. *Escherichia coli* were 0.61 $\log_{10}$cfu g^{-1} for C, O, 1.24 $\log_{10}$ cfug for H, while *E. coli* in aquaponics was below the detection limit. All *Salmonella* counts were also below detection limits. *Enterococcus* counts were 3.5-4.5 $\log_{10}$ less in aquaponics, while *E. coli* in irrigation water for aquaponics was under the detection limit. This study indicates that the microbiological quality of organic and aquaponics vegetable growing systems are equal to, or in better than, other systems. Some concerns, however, may rise for hydroponic systems, probably due to specific practices.

Genetic diversity of *Botrytis cinerea* between tomato greenhouses in Northern Algeria. A. ADJEBLI[1*], C. LEYRONAS[2], K. AISSAT[1], P.C. NICOT[2]. [1]*Laboratoire d'écologie Microbienne, Faculté des Sciences de la Nature et de la Vie, Université Abderrahmane Mira, Bejaia 06000, Algérie.* [2]*INRA, UR407 Pathologie Végétale, Domaine St Maurice CS 60094, F-84143 Montfavet Cedex, France. E-mail: ahmed.adjebli@univ-bejaia.dz*

To estimate the genetic diversity for a better understanding of the spread of *Botrytis cinerea*, we genotyped with nine microsatellite markers 174 isolates collected from four greenhouses during three growing seasons in the region of Bejaia. Four of these isolates were identified as *Botrytis pseudocinerea* according to the allele size at locus Bc6. For all other isolates further studied, all loci were polymorphic, with the mean number of alleles per locus ranging from 2.77 to 5.22. Considerable genetic variability was detected in all subpopulations ($D^* > 0.87$; $Hnb > 0.40$). Based on standardized index of association analysis, significant but low levels of clonality occurred, not excluding the possibility of recombination ($Rd = 0.07$, $P < 0.001$). A total of 109 haplotypes were characterized among the isolates, few of which were shared between subpopulations. This, together with moderate genetic differentiation among subpopulations according to the geographical origin ($0.080 < F_{ST} < 0.167$), suggested a low level of inoculum exchange among greenhouses, and little carryover of inoculum from one sampling season to the next. The importance of genetic structure of *B. cinerea* populations should be taken into consideration for management of grey mould in tomato greenhouses.

Postharvest fungal diseases of loquat cv. 'Algerie' in Spain. L. PALOU, P. SÁNCHEZ-TORRES, C. MONTESINOS-HERRERO, V. TABERNER. *Laboratori de Patologia, Centre de Tecnologia Postcollita (CTP), Institut Valencià d'Investigacions Agràries (IVIA), Apartat Oficial, 46113 Montcada, València, Spain. E-mail: palou_llu@gva.es*

Spain is the second largest producer and the greatest exporter of Japanese loquat (*Eriobotrya japonica* (Thunb.) Lindl.) for fresh consumption. More than 50% of the cultivated area is in Alacant province (SE of Spain), where approx 98% of total production is of loquat cv. 'Algerie', which is mainly exported to European Union (EU) markets. For two consecutive seasons, commercially grown 'Algerie' loquats from two orchards were assessed for disease caused by latent and wound pathogens. Selected healthy fruit were either surface-disinfected or artificially wounded in the rind and incubated in humid chambers at 20°C for up to 5 weeks. Additionally, disease was also assessed on commercially handled fruit (manually selected and packaged) stored at 5°C for up to 12 weeks; no loquat postharvest treatments are currently authorized in the EU. Isolated fungi were incubated on potato dextrose agar (PDA) plates at 25°C for purification and subsequent morphological and molecular identification. Pathogenicity of common isolates was demonstrated by fulfilling Koch's postulates. Disease development was assessed on artificially inoculated loquats stored at either 20 or 5°C. Regardless of the type of infection and postharvest fruit management, the most frequent postharvest diseases were black spot, caused by *Alternaria alternate*, and blue mold caused by *Penicillium expansum*. In addition, gray mold, caused by *Botrytis cinereal*, was frequently observed on both artificially wounded and commercially handled fruit, whereas anthracnose, caused by *Colletotrichum gloeosporioides*, was frequently observed on surface-disinfected loquats. Other minor pathogens that were found causing latent infections, especially in the fruit stem-end, were *Pestalotiopsis clavispora* and *Diplodia seriata.*

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The severe threat for sweet cherry production in Turkey: identification of causal agents of bacterial canker. H. ÖZAKTAN[1], M. AKBABA[1] [1]*University of Ege, Faculty of Agriculture, Department of Plant protection, Bornova, İzmir, TURKEY. E-mail: hatice.ozaktan@ege.edu.tr*

Turkey is the world's greatest producer of sweet cherries (*Prunus avium* L.), with production of 400.000 metric tons per year. An old but suitable for export variety, '0900 Ziraat-Salihli', played the main role in this industry. Cherry production in Turkey has been threatened by emerging and increasingly severe losses due to bacterial canker, caused by *Pseudomonas syringae* pathovars. This study identified fluorescent *Pseudomonas* isolates originating from different symptomatic tissues of sweet cherry trees from the Aegean Region in Turkey. Identification of bacterial canker causal agents was on isolation on microbiological media, phenotypic features of bacteria, including pathogenicity tests on immature fruit, and molecular diagnosis of toxins produced (yercinibactine, coronatine, syringomycin). Eleven of 16 fluorescent *Pseudomonas* isolates were identified as *Pseudomonas syringae* pv. *syringae* (*Pss*), four as *P. syringae* pv. *morsprunorum* race 2 (*Psm2*), and one as *Pseudomonas viridiflava*. The pathogenicity tests divided the tested strains into two groups: one including isolates causing black-brown necrosis, and the second of isolates inducing water-soaked superficial lesions. Phenotypic and genetic studies on toxin production showed that all *Pss* isolates produced syringomycin (*syrb* gene). However, *Psm*2 isolates did not show evidence for the coronatine production gene (*cfl* gene). All pathogenic isolates were also subjected pathogenicity test on micropropagated sweet cherry plantlets, using different inoculation methods. The best inoculation technique for production of typical disease symptoms was dipping non-wounded plantlets in bacterial suspensions.

Diversity of culturable bacteria in Spanish *Pleurotus eryngii* crops. A.J. GONZÁLEZ[1], E. TRAPIELLO[1], M.J. NAVARRO[2], F.J. GEA[2]. [1]*Laboratorio de Fitopatología, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Carretera de Oviedo s/n, 33300 Villaviciosa, Asturias, Spain.* [2]*Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintanar del Rey, Cuenca, Spain.*

Pleurotus eryngii, king oyster mushroom, is one of the most valuable cultivated mushroom species in Spain, where it is grown on sterilized substrates. Several bacterial species have been described as causing diseases on edible mushrooms, such as brown blotch or internal stipe necrosis. This study identified bacteria present on *P. eryngii* fruit bodies harvested from several mushroom growing farms located in Castilla-La

Mancha (Spain), and assessed their diversity. From the ten batches of samples analyzed, 39 isolates were obtained. Classical and molecular techniques were used for identification. The phenotypic tests carried out were: Gram, fluorescence under UV light, oxidation/fermentation of glucose and the presence of cytochrome c-oxidase. The LOPAT scheme (levan, oxidase, pectinolysis of the potato, hydrolysis of arginine, hypersensitivity in tobacco leaves) was applied to isolates of the genus *Pseudomonas*. In addition, the 16S rDNA was amplified and sequenced in at least one of the directions and the sequences obtained were compared to those deposited in databases by BLAST. Only 18% of the isolates were Gram positive. Among Gram negative bacteria, *Pseudomonas* was the best represented with 22 isolates, only one of which was non-fluorescent. Among the identified *Pseudomonas* spp., the most relevant were *P. tolaasii* and *P. azotoformans*. Other genera present were *Acinetobacter*, *Providence*, *Brochotrix*, *Myroides*, *Lactococcus*, and *Wautersiella*.

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Use of natural products to control key pathogens of typical Mediterranean crops. A. LA TORRE, G. PASCALI, L. RIGHI, S. BERTIN, V. BATTAGLIA. *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di ricerca Difesa e Certificazione (CREA-DC), Via C. G. Bertero 22, 00156-Rome, Italy.*

This study evaluated the use of essential oils for control of *Plasmopara viticola* and *Fusarium oxysporum* f. sp. *lycopersici*, two important pathogens of, respectively, grapevine and tomato. Clove oil (*Eugenia caryophyllata*) and BIOXEDA formulation containing 20% (w/w) of clove oil (Xeda International S.A.) were tested *in vitro* and *in vivo*. The *in vitro* tests consisted of evaluating the development of *P. viticola* on grapevine leaf discs, or *F. oxysporum* f. sp. *lycopersici* on agar medium supplemented with the tested products at various concentrations. In addition, spore germination was determined after using the products at different concentrations. The *in vivo* tests evaluated activity of the products against against grape downy mildew in open field, and *F. oxysporum* f. sp. *lycopersici* on tomato plants in a greenhouse. *In vitro* tests showed inhibitory activity of the products on mycelial growth and spore germination. *In vivo* tests revealed effectiveness of products although they were not as effective as a reference product. This study suggests that application of essential oils could to reduce the use of synthetic pesticides in agriculture, in accordance with European laws, and avoid the environmental pollution.

Seasonal distribution of *Apple mosaic virus* in infected apple and hazelnut tissues. A.BALTACI[1], F.ERTUNC[2]. [1]*Blacksea Agricultural Research Institute, Samsun, Turkey,* [2]*Ankara University, Faculty of Agriculture, Department of Plant Protection 06110 Ankara, Turkey. E-mail: ertunc@agri.ankara.edu.tr*

This research was carried out to detect the seasonal variation of *Apple mosaic virus* (ApMV) on apple and hazelnut tissues by DAS-ELISA and RT-PCR which the coat protein region was targeted. Tissues were sampled between March – January 2011 and five ApMV infected apple trees (all were *Granny Smith* variety) and three local hazelnut varieties (Foşa, Yassı badem and Mincane) were selected as five replicates in Hazelnut germplasm culture collection in Giresun. Shoots, leaves, male and female flowers, fruits and husk tissues were collected from infected hazelnut trees, shoots, leaves, flowers and fruits were collected from apple trees according to their set. *Apple mosaic virus* was detected in the shoots and leaves of apple trees collected in May, June and July by DAS-ELISA and similar results were also obtained by RT-PCR. The virus was present in shoots, leaves and female flowers of hazelnut especially collected in April, May, June and very reduced amount in July detected by DAS-ELISA. When the hazelnut tissues analyzed by RT-PCR, the tissues collected in March were also positive for the presence of ApMV. The remaining collected tissues were all negative in RT-PCR and DAS-ELISA, therefore, according to our results, May was the most suitable time for apple sampling while the April was found the most suitable period for hazelnut collection for the detection of ApMV

Distribution, molecular detection and characterization of corn viruses in Turkey. K. DEGIRMENCI[1], F. ERTUNC[2]. [1]*Ankara University Natural and Applied Sciences Institute. Ankara University, Faculty of Agri-*

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Leaf samples bearing virus-like symptoms of systemic mosaic, and asymptomatic leaves, were collected in 2010 and 2011 from Bartın, Düzce, Sakarya and Zonguldak provinces of the Blacksea region, and were assessed, using DAS-ELISA tests, for virus infections. The samples were tested for *Maize dwarf mosaic potyvirus* (MDMV), *Sugarcane mosaic potyvirus* (SCMV), *Barley yellow dwarf luteovirus* PAV and MAV strains (ByYDV), *Maize stripe tenuivirus* (MStV), *Maize mosaic rhabdovirus* (MMV), *Maize whiteline mosaic aureusvirus* (MWLMV), *Johnson grass mosaic potyvirus* (JGMV), *Wheat streak mosaic tritimovirus* (WSMV), *Barley stripe mosaic hordeivirus* (BSMV), *Maize chlorotic mottle machlomovirus* (MCMV) and *Cucumber mosaic virus* (CMV),A total of 424 plant samples were collected from the research area. Of the samples, 171 were infected by one of the above viruses and 60 had double or triple virus infections. No viruses were detected in 193 samples. MDMV was widespread, followed by BYDV-MAV, BYDV-PAV and MMV. Infection rates were 43% in Bartın, 52% in Duzce, 38% in Sakarya and 27% in Zonguldak. The other virus infections were rare in the research area. The main infection in maize cobs and seeds were MDMV and MMV. RNAs of infected leaves were isolated and subjected to RT-PCR amplification for MDMV, MMV, BYDV-PAV and MAV, and 336 bp, 457 bp, 320 bp, 320bp amplified products were obtained. Thirty-five MDMV isolates were sequenced and the sequences were deposited in the NCBI database, and phylogenetic analysis were performed. Our isolates were closely related to European MDMV isolates.

This research was supported by the Tagem Project funded by Ministry of Food, Agriculture and Animal Husbandry of Turkey.

Detection of resistance in corn varieties by molecular markers. K. DEGIRMENCI[1], F. ERTUNC[2]. [1] *Ankara University Natural and Applied Sciences Institute.* [2]*Ankara University, Faculty of Agriculture, Department of Plant Protection, 06110 Ankara, Turkey. E-mail: ertunc@agri.ankara.edu.tr*

Maize dwarf mosaic virus (MDMV) is the most important and widespead virus infection of maize in Turkey. In order to determine the genetic suceptibility of our corn varieties, one isolate of MDMV was selected according to symptom expression and virulence, and tested on 88 local maize varieties, ten commercial varieties, two resistant(D21 and D32) and two susceptible (Dcmv 1145 and D408) for resistance against MDMV. Field trials were organized as randomised block designs with three replicates and six plants in each plot. DNA was isolated from the infected plant leaves. Two CAPS markers (Pic 13L2 and Pic 19LX) and one indel marker (M12) were used for the detection of Scmv1 and Scmv2 resistance genes. Amplified products of Pic 13L2 CAP marker were digested with Rsa1 and the others were digested with NlaIII. From symptom expression of the plants and results of the molecular amplifications, four self-pollinated maize lines, M112, M100, M108 and M 96 were shown to be resistant to MDMV. The Scmv1 gene was present in nine samples and the Scmv2 gene was detected in five samples.

This research was supported by the Tagem Project, funded by Ministry of Food, Agriculture and Animal Husbandry of Turkey.

Development of a biocontrol agent against *Fusarium spp.* using culture media fermented by lactic acid bacteria. C. LUZ[1], F.B. LUCIANO[2], J. MAÑES[1], G. MECA[1]. [1]*Laboratorio de Química de los Alimentos y Toxicología de la Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s / n, 46100 Burjassot, España.* [2] *Departamento de ciência animal, Escola de Ciências da Vida, Pontificia Universidade Católica do Paraná, Rua Imaculada Conceição 1155, 80901-215 Curitiba, Paraná, Brasil.*

Bioconservation is a biotechnological application that promotes shelf-life extension and food safety using microorganisms or their metabolic products. Some LAB strains produce low molecular weight compounds related to phenolic acids, with important antifungal activities. Provided LAB are food grade organisms and comply with the "Qualified Presumption of Safety" (QPS) introduced by the "European Food Safety Agency" (EFSA), they have considerable potential as biopreservatives in food applications. Reduction of food spoilage caused by mycotoxigenic fungi is one of the main problems in food security. Seven strains of lactic acid bacte-

ria (LAB) were tested for antifungal activity against nine mycotoxigenic *Fusarium spp*. LABs were grown on MRS broth for 48 h at 37°C in anaerobic conditions. The cell free supernatants (CFS) were concentrated by lyophilization, filtered and tested for the antifungal properties using a diffusion agar method. Minimum inhibitory and minimum fungicidal concentrations of each CFS were determined in in 96-well microplates. All LABs tested produced growth inhibition of the nine fungi on solid medium. The minimal inhibitory concentrations ranged from 4 to 16 g L^{-1}, and minimum fungicidal concentrations from 8 to 31 g L^{-1}. Further investigations will focus on development of a natural biocontrol agent against *Fusarium spp.* contamination in cereals and derivate products.

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Detection of *Southern tomato virus* in seed and seedlings of commercial tomato varieties

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Southern tomato virus (STV) is a member of the new genus *Almalgavirus* (family *Amalgaviridae*), with a 3.5 kb double stranded RNA (dsRNA) genome containing two partially overlapping open reading frames (ORFs), coding for the putative coat protein gene and with typical motifs of the RNA-dependent RNA-polymerase (RdRp). This virus is related to families *Totiviridae* and *Partitiviridae*. STV is efficiently seed transmitted, with a transmission rate >70%. Since the first report of STV in Spain in 2013, it was detected in several commercial and local varieties of tomato from different Spanish tomato production areas. STV-infected fruits are symptomless or show uneven ripening, and the virus is often found in mixed infections with other typical tomato-infecting viruses. This project assessed whether tomato germplasm is generally infected with STV. Twenty seed lots and more than 30 seedling samples of different commercial and local tomato varieties from commercial nurseries, were analyzed. These assessments showed that STV is widespread through the tomato germplasm. Although STV is a cryptic virus characterized by no developing important plant diseases, its presence could interfere in the evolution of other symptomatic viruses and also in the host. The role of STV in infected tomato plants requires clarification.

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Effects of the co-infection of *Pepino mosaic virus* and *Southern tomato virus* on tomato plants.

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Southern tomato virus (STV) was detected in tomato plants showing stunting, fruit discolouration and size reduction, and also in symptomless plants. This virus is efficiently seed transmitted and its role in the tomato infected plants is currently unknown. We evaluated effects of STV in single and mixed infections with *Pepino mosaic virus* (PepMV) in affected tomato plants. The assay consisted of four different combinations: single STV and PepMV infected plants, plants co-infected with PepMV and STV and non-infected plants (four plants of each treatment). Plants were grown a growth chamber, and different parameters were evaluated, including; time for symptom development, symptom severity index, virus concentration and plant biomass. All of the plants infected with PepMV developed symptoms. However, in plants co-infected with STV symptoms appeared 15 d later. Plants infected only with STV did not develop any symptoms during the assay. The co-infected plants presented greater biomass at the end of the assay than those with single infections of either PepMV or STV, and were similar to those of the uninfected plants. The concentration of STV remained almost constant during the assay, and PepMV concentration was greater at 15 d after infection and decreased in following evaluations, regardless of the single or mixed infections in the plants. These results indicate that co-infection of PepMV and STV could improve the development of tomato plants compared to those only infected with PepMV or STV, with similar biomass to the non-infected plants. Further studies are being undertaken to confirm these results.

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Identification and mycotoxigenic ability of *Aspergillus* spp. associated with black rot of pomegranate fruit. A. SUSCA[1], L. KANETIS[2], M. HAIDUKOWSKI[1], A. VILLANI[1], S. TESTEMPASIS[3], S. SAMUEL[4], A. LOGRIECO[1,] G. KARAOGLANIDIS[3]. [1]*Istituto di Scienze delle Produzioni Alimentari. Consiglio Nazionale delle Ricerche, via Amendola 122/O, 70126 Bari, Italy.* [2]*Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603, Limassol, Cyprus.* [3]*Aristotle University of Thessaloniki, Department of Agriculture, Plant Pathology Laboratory, 55132, Thessaloniki, Greece.* [4]*Department of Agriculture, Ministry of Agriculture, Rural Development and Environment, 1412, Nicosia, Cyprus. E-mail: antonella.susca@ispa.cnr.it*

Due to their nutritional value pomegranate is a rapidly expanding crop with promising prospects, consumed mainly as fresh fruit, juices and jams. Pomegranate fruit rots contribute significantly to crop losses, with black rot (caused by *Aspergillus* spp.) being a common disease. Black rot damages external fruit surfaces, resulting in fungal invasion of arils that are covered by spore masses of black aspergilli (*Aspergillus* section Nigri). This fungus group is considered the main source of ochratoxin A (OTA) contamination in numerous food commodities, and species of the section have been reported as fumonisin (FB) producers. Therefore, black rot may not only reduce yield, but also deteriorate products due to mycotoxin production, and compromise consumer health safety. Our purpose was to identify black aspergilli associated with pomegranate fruit rots and investigate their mycotoxin capacities. Thirty seven *Aspergillus* spp. isolates from pomegranate fruit showing black rot symptoms were collected from Greece, Cyprus and Italy. Species identification was performed at three genetic loci, beta-tubulin, calmodulin and translation elongation-1a. Thirty-five isolates belonged to *A. niger* "aggregate", mostly *A. tubingensis*, one to *A. japonicus* and one to *A. violaceofuscus*. OTA and FB capacity of the isolates was also investigated, with negative results, respectively, on YES and CY20S media. To our knowledge this is the first report of multi-locus characterization of black aspergilli associated with pomegranate black rot. Further studies on an enlarged set of strains, and evaluation of natural occurrence of the toxins, are required to better elucidate the potential mycotoxin risks on pomegranate fruit.

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Population structure of *Phytophthora infestance* causing to potato late blight in the Çukurova Region of Turkey. H. GÜNAÇTI[1], T. AY[1], C. CAN[2]. [1]*Biological Control Research Institute Koprukoy/Adana, Turkey.* [2]*Gaziantep University, Department of Biology. Gaziantep/Turkey.*

Potato late blight (caused by *Phytophthora infestans*) is one of the most destructive diseases of potato in Turkey. The pathogen can infect stems, leaves, and tubers. Late blight is becoming increasingly difficult to control, leading to intensified use of fungicides in the potato production. This study explored the molecular and biochemical characters of *P. infestans* populations in Turkey. Metalaxyl sensitivity, mating type analyses, and phenotypic characterization were carried out in 2013-2014, with surveys in the Çukurova potato cultivation areas, and 186 *P. infestans* isolates were obtained. Through these analyses metalaxyl resistance profile, mt DNA haplotypes, occurrence of A2 type in the region and genetic differences were determined. These results are the first characterization data on for the P. infestans populations in Turkey.

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Survey of distribution of the fire blight pathogen (*Erwinia amylovora*) on pome fruits in Montenegro. J. LATINOVIĆ, B. KANDIĆ and N. LATINOVIĆ. *University of Montenegro, Biotechnical Faculty, Mihaila Lalića 1, 81000 Podgorica, Montenegro. E-mail: jelenalat@ac.me*

From 2013 to 2016, a national survey of *Erwinia amylovora*, the causal agent of fire blight, was carried out in Montenegro, focusing on pome fruits (apple, pear and quince) as the most important host plants. The study included field observations and laboratory identification of the pathogen. The most common symptoms related to fire blight were "shepherd's crook" on the tips of tree shoots, with brown discolouration under the bark and blighted leaves. Samples from symptomatic plants were examined in the laboratory, and bacteria were isolated nutrient agar. Pathogenicity of obtained isolates was confirmed by artificial inoculation of immature pear fruits, and hypersensitive reaction in tobacco leaves. Identification of isolates was by Gram staining, studying cultural characteristics on nutrient sucrose agar and King's B medium), and by rapid immunochromatography. Occurrence and distribution of *E. amylovora* in Montenegro, mostly in northern areas, but also in central areas. The disease was most prevalent in Bijelo Polje and Berane, and quince was the most common host plant. Infection of apple and pear trees was usually noticed if they were in a proximity to old, diseased quince trees. Adequate phytosanitary measures need to be implemented to control the disease.

This research was supported by the Phytosanitary Directorate of Montenegro.

Biological and chemical control of *Aspergillus flavus* and aflatoxins in maize. C. LAGOGIANNI, D.I. TSITSIGIANNIS. *Laboratory of Phytopathology, Department of Crop Science, School of Agricultural Production, Infrastructure and Environment, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece. E-mail: dimtsi@aua.gr*

Aspergillus spp. cause significant rots in maize and produce the carcinogenic aflatoxins. Several chemical and biocontrol formulations and other non-pathogenic biotic factors were evaluated for the control of *A. flavus* in maize. *In vitro* experiments were performed on maize kernels with the following factors: a) Zeolite, a mineral with physicochemical properties; b) Agri-fos 600®, a product based on potassium phosphonate anions that induce plant immune responses; c) Trianum®, a product based on the fungus *Trichoderma harzianum* that inhibits the infection and colonization by pathogenic fungi; d) Botector®, a product containing the yeast *Aureobasidium pullulans* whose action is based on inhibition of pathogen colonization; e) *Paenibacillus alvei* K-165, an antagonistic bacterium that induces systemic resistance of plants; f) Serenade Max®, a product that stimulates natural plant defense mechanisms; g) Vacciplant®, a product that contains laminarine, an inducer of the plant immune system; and h) a non-toxigenic strain of *Aspergillus flavus*. The fungicides Switch®, Geoxe®, Granuflo®, Cantus®, Chorus® and Quadris® were also tested. The experiments demonstrated that the chemical formulations reduced infection *A. flavus* in *in vitro* and field experiments. In particular, the fungicide Switch® reduced disease severity and aflatoxin production by 70%. Additionally, the biopesticides Botector® and Mycostop® reduced aflatoxin contamination of maize by 50%. Applying good agricultural practices and combinations of biological agents and fungicides at the maize growth stage of anthesis and silking can significantly reduce aflatoxin contamination.

Effects of the viroids and rootstocks on fruit yield and juice quality of Tunisian citrus variety "Maltese half-blood". A. NAJAR[1], L.HAMROUNI[2], R. BOUHLEL[1], A. JEMMALI[1], B. JAMMOUSSI[3], N. DURAN-VILA[4]. [1]*National Institute of Agricultural Research of Tunisia, Street Hedi Karray, 1004 El Menzah, Tunis, Tunisia.* [2]*National Research Institute of Rural Engineering, Water and Forests, Street Hedi Karray, 1004, El Menzah, Tunis, Tunisia.* [3]*Higher Institute of Education and Continuing Education, Tunisia.* [4]*Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain. E-mail: asmanajara@yahoo.fr*

In Tunisia, citrus varieties are commonly grafted on sour orange rootstocks. Considering the present strategy to prevent damage which could be associated with tristeza disease, the substitution of sour orange rootstocks with symptomless rootstock/scion combinations is a desirable approach. However, some promising rootstocks are known to be sensitive to viroid infection. The performance of Tunisian 'Maltese half-blood' sweet orange infected with Citrus exocortis viroid (CEVd), Hop stunt viroid (HSVd), Citrus bark cracking viroid (CBCVd), Citrus bent leaf viroid (CBLVd) and Citrus dwarfing viroid in single or mixed infections was evaluated on eight rootstocks [sour orange (SO), 'Carrizo' citrange (CC), volkamer lemon (CV), 'Cleopatra' mandarin (MCL), 'Swingle' citrumelo (Citru), 'Rangpur' lime alemow (LR) and trifoliate orange (PT)], at the INRAT station in Cap Bon region. The trees were planted in 2005 and size, fruit production and fruit quality were evaluated every year from 2008. Mixed viroid infections decreased the canopies of Maltese grafted on CC by 41%, Citru by 40%, MCL by 39%, LR by 50%, CV by 46% and PT by 60%. The cumulative yield of Maltese grafted on CM and inoculated with HSVd was 76% less than the control. Mixed infections decreased production from the rootstocks Citru by 30% and PT by 60%. The only viroid effect on fruit quality was increased vitamin C content. This was more pronounced from mixed infections where the greatest amounts of vitamin C were recorded for fruit juice of Maltese grafted on CM, CV, LR or PT.

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Effect of temperature on *Lactuca sativa* cultivars infected naturally with *Sclerotinia sclerotiorum* - a field study. P. KRÓLIKIEWICZ, V.K. MACIOSZEK, T. JĘCZ,, A.K. KONONOWICZ. *Department of Genetics, Plant Biology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12, 16, 90-237 Lodz, Poland. E-mail: andrzej.kononowicz@biol.uni.lodz.pl*

Sclerotinia sclerotiorum is one of the most destructive fungi of cultivated *Lactuca sativa* (lettuce), causing lettuce drop. Four lettuce cultivars (iceberg lettuce cvs *Diamentinas* and *Templin*, green lettuce cv. *Lollo Bionda* and red lettuce cv. *Lollo Rossa*) were grown in a horticultural holding in the central Poland (Lodz voivodeship), in the field naturally infested by *S. sclerotiorum*. In parallel, control lettuces were grown in a non-infested field. The experiment was conducted in 2016, in three yields/repetitions. Lettuce seeds used were purchased by Rijk Zwaan and Nunhems Companies. Seedlings were prepared by Schwanteland GmbH, Jungpflanzen, Germany. Each of lettuce cultivar was grown in 8 × 1 m plots, and each plot was divided into five sectors, each of 20 lettuce heads (100 heads per plot. Fertilizers (5 kg each of ammonium nitrate, potassium sulphate and triple superphosphate) were applied to each plot. Temperature was measured daily, and numbers of infected lettuce heads were counted at 4 and 8 weeks. Severity of lettuce drop was assessed using 5 point scale, and survival of individual plants was estimated. total phenolic and flavenoid content was also assessed. The most resistant cultivar to low temperature as well as to *S. sclerotorium* infection was *Lollo Rosa*. This cultivar also had the greatest phenolic and flavonoid contents.

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The effects of sulfur dioxide pads on postharvest grey mold and quality of sultana table grapes. P. KINAY TEKSUR, F. SEN[2], H.B. ÜNAL[3], A.K. SELVİ[1], A.KALIN[4], A.M. AGHDAM[1], B. CENBERCI COŞKUN[5]. [1]*Ege University Faculty of Agriculture Department of Plant Protection, 35100 Bornova, Izmir, Turkey.* [2]*Ege University Faculty of Agriculture Department of Horticulturae, 35100 Bornova, Izmir, Turkey.*

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Sultana seedless table grapes are widely grown in Aegean Region. One of the most important problems in the storage of table grapes is latent infections by *Botrytis cinerea* originating from vineyards. SO_2 generators are used on grapes during the storage to control grey mold. However, these pads, produced by different companies, vary in their activity, which results both from the SO_2 content and different *B. cinerea* infection levels on grapes. The activities were investigated of SO_2 pads from different companies against different *B.cinerea* loads, and on fruit quality. Studies were carried out in cold storage rooms (-0.5 ± 0.5°C, 90% relative humidity) at two separate periods (2012-2013 and 2013-2014) for 3 months of storage. Effects were measured on development of decay, microbial populations, and quality parameters on grapes inoculated with *B. cinerea* (at 10^5 and 10^6 spores mL^{-1}) and untreated grapes that had been harvested from two different vineyards. Decay rate was 56% in the second month of storage for the grapes taken from the first vineyard, where different spraying programs were applied, whereas this rate was 92% for grapes taken from the other vineyard, where another control programme was applied. In both year trials, Fresca and Uvas SO_2 generators showed similar success in preventing decay development, especially during the first 2 months of storage. There were no differences in decay development for different SO_2 generators on grapes where *B. cinerea* was artificially inoculated.

Network of Mediterranean Culture Collections for preserving biodiversity of phytopathogenic and toxigenic microorganisms. A.F. LOGRIECO[1], G. PERRONE[1], L. MUGNAI[2], R.R.M. PATERSON[3], A. VENÂNCIO[3], L. LÓPEZ[4], M.C. MACIAN[4], R. AZNAR[4], N. LIMA[3]. [1]Institute of Sciences of Food Production, National Research Council, Via Amendola 122/O, 70126, Bari, Italy. [2]Dipartimento di Scienze Produzioni Agrialimentari e dell'Ambiente, University of Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. [3]CEB-Centre of Biological Engineering, Campus de Gualtar, University of Minho, 4710-057 Braga, Portugal. [4]CECT-Spanish Type Culture Collection, University of Valencia, 46980 Paterna, Spain.

In recent years, there has been increasing concern for the importance of biodiversity. Various actions and initiatives have been applied at the European level to preserve the biodiversity of life (B4Life project), and in particular of microorganisms, such as European Consortium of Microbial Resources Centres (EMbaRC) and Microbial Resource Research Infrastructure (MIRRI) projects. These aimed to establish a self-sustainable community of European Microbial Resource Centres, representing a large biodiversity and offering a wide range of bioresources, experts and services. The particular aspect relevant to preservation and identification of plant pathogenic microorganisms (fungi, bacteria and viruses) is crucial to face the new challenges related to climate change and emerging plant diseases in the Mediterranean region (e.g. *Xylella fastidiosa* on olive trees, ToLC-NDV virus on *Solanacee* and *Cucurbitacee*). In addition, some fungal strains can produce mycotoxins with severe effects on human and animal health *(e.g. Aspergillus flavus* on maize; *Fusarium* spp. on wheat), and can be human pathogens. Biodiversity is changing in relation to global temperature increases, and the relevant plant pathogen issues will be closely dependent to the pathogen species (i.e. thermophiles or others) dominating in the new climate scenarios. Currently, many studies on species characterisation at genetic and biochemical levels are generating a large and numerous datasets, and the scientific community can gain from organising and sharing biological resources and related information. A network on Mediterranean Culture Collections represents an important and strategic initiative to strengthen research activities, to face emerging phytopathogenic and toxigenic microorganisms and to assist food security/safety.

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Comparative genomic analysis of secondary metabolite gene clusters in *Fusarium* species. V.C. LIUZZI[1], F. FANELLI[1], M. CHIARA[2], J. F. LESLIE[3], A. F. LOGRIECO[1], G. MULÈ[1]. [1]Institute of Sciences of Food Production, National Research Council, Bari, Italy. [2]Dipartimento di Bioscienze, Università degli Studi di

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The lack of standard analysis workflows next generation sequencing (NGS) data for toxigenic fungi prevents systematic comparative genomic studies. We have developed guidelines for the assembly and annotation of NGS data derived from genomic projects on phytopathogenic/toxigenic fungi. To demonstrate the potential of our workflow, we have carried out a comparative genomic analysis of different *Fusarium* species, focusing on secondary metabolite (SM) biosynthetic gene clusters.The workflow was structured as follow: 1) retrieval of *Fusarium* genome sequences available from public databases; 2) sequencing of new *Fusarium* genomes; 3) assembling genomes using *Spades v5.0*; 4) annotation of genomes using *Augustus v3.1*; 5) retrieval of information concerning known/unknown-SM clusters; 6) annotation of *Pfam* domains and SM biosynthetic gene clusters prediction; 7) calculation of clusters of orthologous genes (COGs) and gene families; 8) studying the presence/absence, order, orientation of each gene within clusters and the distribution of clusters among the isolates; 9) construction of "pancluster" phylogenetic trees; and 10) collecting all the data obtained in a *Fusarium* SM cluster database. Preliminary data show that phylogenies and SM cluster distribution among the isolates included in the current study are coherent with published data, and recapitulate the discontinuous distribution of SM clusters and thus in the genetic potential of species to produce secondary metabolites. Detailed analysis of individual clusters enabled the identification and the study of different mechanism of inheritance of SM clusters, showing the advantages of systematic strategies for the analysis and annotation of these genomes.

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Occurrence of spot and net forms of net blotch of barley in Algeria. I.H. LAMMARI[1], Z.E.A FELLAHI[2], A. BENBELKACEM[3], H.BENSLIMANE[1]. *[1]Ecole Nationale Supérieure d'Agronomie (ENSA), Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, 1, Avenue Pasteur, Hassen Badi, Algiers, Algeria. [2]Université El Bachir El Ibrahimi, Bordj Bou Arreridj. [3]Institut National de la Recherche Agronomique d'Algérie, Unité de Recherche de Constantine, Station ITGC, Elkhroub, Algérie. E-mail: h.lammari@st.ensa.dz*

In Algeria, cereal products are very important for local food and in the national economy. Barley (*Hordeum vulgare*) is the second most cultivated cereal after wheat. This crop is affected by several foliar diseases. Net blotch is one of the most common diseases in Algeria, caused by *Pyrenophora teres*. The pathogen occurs in two forms, *P. teres* f. *teres* (*Ptt*) causing the net form (NFNB) disease and producing longitudinal or transversal necrotic bands, and *P. teres* f. *maculata* (*Ptm*) causing the spot form (SFNB) and producing dark brown circular or elliptical lesions. Since the two pathogen forms are morphologically similar but genetically distinct, PCR primer sets have been developed to allow their differentiation, without symptom or morphological analyses. Since correct identification of pathogens is important for effective disease management and epidemiological study, we to explored the occurrence of *P. teres* and populations in Algeria, using two specific primer pairs. *Ptt* is the more prevalent among all the prospected provinces. This evidence will help characterization of the causal agent populations, and assist epidemiological research, to improve control of the net blotch disease in this country.

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Identification and characterization of *Acidovorax citrulli* strains from Serbia. N. ZLATKOVIĆ[1], A. PROKIĆ[1], K. GAŠIĆ[2], N. KUZMANOVIĆ[3], M. IVANOVIĆ[1], Ž. PAVLOVIĆ[1], A. OBRADOVIĆ[1]. *[1]University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Department of Plant Pathology, Nemanjina 6, 11080 Belgrade, Serbia. [2]Institute for Plant Protection and Environment, Department of Plant Pathology Teodora Drajzera 9, 11000 Belgrade, Serbia. [3]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany. E-mail: nevena_blagojevic@yahoo.com*

In August 2014, typical bacterial fruit blotch symptoms were observed on mature watermelon fruits

originating from fields in the Vojvodina province. In the summers of 2015 and 2016 we registered two more occurrences in, respectively, the east and west of the country. White, glistening, convex and circular colonies with regular edges were predominantly isolated from diseased watermelon fruits collected in the affected fields. A total of 33 bacterial strains were subjected to further analysis. They were Gram-negative, aerobic, oxidase and catalase positive, non-fluorescent and did not produce potato soft rot. All but two strains (KFB 359 and KFB 363) induced hypersensitive reactions in tobacco leaves. They grew at 41˚C and produced beige to tan-coloured, non-mucoid, convex colonies on yeast extract-dextrose-$CaCO_3$ agar. All the strains utilized L-arabinose, did not reduce nitrate, nor utilized sucrose. Conventional PCR was performed using *A. citrulli*-specific primers BX-L1/BX-S-R2. The 16S rRNA gene sequence from two strains (KFB 343 and KFB 344) showed 100% identity to strains of *A. citrulli* from China, Thailand and the USA. According to physiological and biochemical tests, PCR assay and 16s rRNA gene sequencing analysis, the causal organism was confirmed as *A. citrulli*. Genetic relatedness among strains was investigated by rep-PCR, using BOX and REP primers. All tested strains except one (KFB 358), showed the same BOX-PCR profiles. In addition, all strains were assigned to the same REP-PCR group. These results show that *A. citrulli* strains isolated in Serbia during 3 years belong to an homogeneous population. These isolated occurrences are considered to originate from seed-borne inoculum, but it remains unknown whether the pathogen will survive from season to season in Serbian climatic conditions.

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Genotypic and phenotypic characterization of *Bacillus amyloliquefaciens* strains active against fungal pathogens of wheat. R. CARACCIOLO[1,2], J. CABREFIGA[2], I. MORA[2], A. FABI[1], R. D'OVIDIO[1], E. MONTESINOS[2], L. VARVARO[1]. [1]*Department Of Agriculture and Forestry Science (DAFNE), University of Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo, Italy.* [2]*Center for Innovation and Development in Plant Health (CIDSAV)-Institute of Food and Agricultural Technology, University of Girona, Maria Aurèlia Capmany, 61, 17003 Girona, Spain. E-mail: caracciolo@unitus.it*

Wheat is one of the most important world food crops and its widespread cultivation constantly exposes this host to many abiotic and biotic stresses. pathogenic fungi represent a serious threat to the cultivation of this cereal. The use of biocontrol agents (BCAs) is one of the most successful strategies for prevention and control of plant diseases. This study dealt with (i) the *in-vitro* evaluation of the antifungal activity of three strains of *B. amyloliquefaciens* (Ba) against *Fusarium graminearum*, *Fusarium culmorum*, *Bipolaris sorokiniana* and *Septoria tritici*, and (ii) the molecular and chemical characterization of the compounds involved in the activity.Determiation of he inhibitory actions of filtrates and extracts from Ba growth broths against all the tested pathogens allowed us to understand the nature of the exoproduced, liposoluble and thermostable molecules involved. Analysis of the presence of *ituA, fenD, surfA, bmyB* and *mycA* genes confirmed the potential production of cyclic lipopeptides (CLPs), such as Iturin, Fengycin, Surfactin, Bacillomycin and Mycosubtilin, that are reported as antifungal compounds. The production of CLPs, was confirmed with the characterization of the culture filtrates by FPLC, and the antagonistic assays performed with the fractions obtained. IFengycins were mainly responsible of the antifungal activity.These results confirm that *B. amyloliquefacens* could be a valid BCA against the most important pathogens of wheat, and its use in the formulation of commercial bio-pesticides can be recommended..

Phenotypic and genetic characterization of *Pseudomonas syringae* strains isolated from hazelnut in Serbia. A. PROKIĆ[1], N. KUZMANOVIĆ[2], N. ZLATKOVIĆ[1], M. IVANOVIĆ[1], K. GAŠIĆ[3], Ž. PAVLOVIĆ[1], A. OBRADOVIĆ[1]. [1]*University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Department of Plant Pathology, Nemanjina 6, 11080 Belgrade, Serbia.* [2]*Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany.* [3]*Institute for Plant Protection and Environment, Department of Plant Pathology, Teodora Drajzera 9, 11000 Belgrade, Serbia. E-mail: andjelka@agrif.bg.ac.rs*

From 2010 to 2014, *Pseudomonas*-like bacterial strains were recovered from symptomatic hazelnut plants in different locations in Serbia. To confirm their identity, ten strains were characterized by morphological, biochemical, physiological and molecular tests (PCR detection of syringomycin production, repetitive-sequence PCR and *rpoD* housekeeping gene analysis). The strains were fluorescent, HR positive, oxidase negative, negative for arginine dihydrolase and pectinase activity, and variable in levan production. All strains showed uniform biochemical characteristics typical for *P. syringae* pv. *syringae*: they hydrolysed gelatin and aesculin, produced acid from glucose, sucrose and inositol, and grew at 36°C and in 5% NaCl. All but one strain had positive ice nucleation activity. Pathogenicity of the strains was tested by inoculation of immature sweet cherry fruits. Dark necrotic lesions varying in size developed 2 weeks after inoculation, indicating different levels of virulence of investigated strains. Genetic profiles generated with BOX primers were polymorphic for each strain revealing high genetic diversity. The *syr*B gene encoding syringomycin production, characteristic for pv. *syringae*, was detected by PCR analysis in all the strains. *RpoD* gene sequence analysis also indicated high similarity of the strains and *P. s.* pv. *syringae*. Results obtained in this study provided new information about the first detection of *P. syringae* pv. *syringae* on hazelnut in Serbia.

This research was supported by the project III46008 financed by Ministry of Education, Science and Technological Development, Republic of Serbia.

Cross inoculation assays confirms grass pea/pea phylogeny proximity at the *Fusarium oxysporum* host range level. A.M. SAMPAIO[1], N.F. ALMEIDA[1], N. RISPAIL[2], D. RUBIALES[2,] M.C. VAZ PATTO[1]. [1]*Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Avenida da República, 2781-157 Oeiras, Portugal.* [2]*Instituto de Agricultura Sostenible, CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain. E-mail: amsampaio@itqb.unl.pt*

Grass pea (*Lathyrus sativus*) has considerable potential as legume crop in dryland farming systems. It is superior in yield, protein value, nitrogen fixation, drought, flood and salinity tolerance when compared to other legume crops. However, yield inconsistency due to sensitivity to specific diseases strongly limits its cultivation. Fusarium wilt, caused by the soil borne fungus *Fusarium oxysporum*, is one of the most important diseases affecting grain legumes worldwide, and is becoming a threat for grass pea production in Portugal. Understanding its host specificity is important for epidemiology and disease management. No information is available in grass pea, so the host range of two new *F. oxysporum* isolates collected from grass pea was analysed in several other important legume crops, including pea (*Pisum sativum*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*) and barrel medic (*Medicago truncatula*). The responses were also studied of grass pea to *F. oxysporum* f. spp. infecting these crops, including *F. oxysporum* f. sp. *pisi* races 1 and 2, *F. oxysporum* f. sp. *lentis*, *F. oxysporum* f. sp. *ciceris* and *F. oxysporum* f. sp. *medicaginis*. There were very similar responses of pea and grass pea accessions to both of these new grass pea isolates, and to *F. oxysporum* f. sp. p*isi*, suggesting little specialization of *F. oxysporum* to *L. sativus*. This is not surprizing since grass pea is phylogenetically close to pea. However, we do not exclude that specialization may occur but remains undetected. More isolates should be sampled from grass pea fields to ratify this.

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Identification of pathotypes and analysis of the genetic structure of *Fusarium oxysporum* f. sp. *lentis* populations. H.R. POURALIBABA[1], Z. SATOVIC[2], M.J. COBOS[3], D. RUBIALES[3], S. FONDEVILLA[3]. [1] *Dryland Agricultural Research Institute, Education and Extension Organization (AREEO), Maraghe 119, Iran.* [2]*Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska 25, 10000 Zagreb, Croatia.* [3]*Institute for Sustainable Agriculture, CSIC, 14004 Córdoba, Spain. E-mail: hpouralibaba@ias.csic.es*

Lentil cultivation is threatened worldwide by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis*

(*Fol*). Knowledge on pathogenic diversity and genetic structure of *Fol* populations is fundamental for breeding for resistance and managing the disease. We studied virulence diversity within a collection of *Fol* isolates. We first inoculated 28 lentil resistant accessions with six *Fol* isolates from different geographical origins. The lentil accession × *Fol* isolate effect was highly significant, which allowed four accessions to be selected as a differential set. Inoculation of this set with 48 *Fol* isolates from Iran, Syria and Algeria, allowed the identification of seven different virulent patterns, designated pathotypes 1 to 7. In addition, the genetic structure of this *Fol* collection was analyzed using twelve SSR markers, eight of which were designed in this study. AMOVA showed that there is high molecular variation within groups but also between groups, differing Iranian populations from non-Iranian populations. STRUCTURE and Fitch-Margoliash tree analysis showed the presence of two ancestral FOL lineages, one distributed among all regions while the other was only present in Iran. Our results suggest that Iran could be the origin of the diversity covered in this study.

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Sources of resistance to *Fusarium oxysporum* f. sp. *lentis* in Spanish lentil germplasm. H.R. POURALIBABA[1], D. RUBIALES[2], A. PEREZ-DE-LUQUE[3], S. FONDEVILLA[2]. [1]*Dryland Agricultural Research Institute, Education and Extension Organization (AREEO), Maraghe 119, Iran.* [2]*Institute for Sustainable Agriculture, CSIC, 14004 Córdoba, Spain.* [3]*IFAPA, Alameda del Obispo, Córdoba, Spain. E-mail: hpouralibaba@ias.csic.es*

Fusarium oxysporum f. sp. *lentis* (*Fol*) causes serious disease of lentil crops globally, resulting in severe yield losses, especially under warm and drought conditions. Use of resistant cultivars is the most effective approach to control the disease, and lentil landraces could be a valuable resource of disease resistance. We evaluated 196 Spanish lentil landraces from CRF-INIA against an aggressive *Fol* isolate under controlled conditions. Using a four point severity scale, developed in this study, AUDPC and a Disease Index were calculated. Seventeen accessions were identified as resistant at the seedling stage. A field trial was also conducted in a naturally infested farm in the North West of Iran. Results based on percent plant mortality confirmed the resistance of twelve accessions. To further characterize the resistance identified, components of resistance were histologically studied on six lentil accessions with different levels of resistance against two contrasting *Fol* pathotypes. Uniform xylem occlusion with gum-like substance was observed as a quantitative mechanism of resistance in all accessions, whereas fast secretion of phenolic compounds was observed as a qualitative mechanism only in the combination of BG01969/pathotype 1.

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Molecular pathogen-host interactions

Genomic screens to identify next-generation MAMPs and their cognate pattern recognition receptors. A. G. MOTT[1], S. THAKUR[1], E. SMAKOWSKA[2], P. W. WANG[3], Y. BELKHADIR[2], D. S. GUTTMAN[1,3], D. DESVEAUX[1,3]. [1]*Department of Cell & Systems Biology, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada.* [2]*Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC), Dr Bohr Gasse 3, Vienna 1030, Austria.* [3]*Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, Ontario, Canada. E-mail: darrell.desveaux@utoronto.ca*

The front line of plant defence against pathogens depends on the action of extracellular leucine-rich repeat, receptor-like kinases (LRR-RLKs). These serve as Pattern Recognition Receptors (PRRs) to recognize essential, evolutionarily conserved, features of pathogens called Microbe-Associated Molecular Patterns (MAMPs). MAMP recognition by PRRs activates PRR-triggered immunity (PTI), which suppresses the growth of nearly all "non-host" microbes, as well as many potential pathogens. Next generation sequencing of *Pseudomonas syringae* pathovars has allowed the successful *in silico* prediction of MAMPs, through identification of positive selection signatures on proteins of the core genome. Although these "next-generation MAMPs" induce the hallmark responses of PTI, including virulence suppression, the

PRRs that recognize them remain to be identified. Latest genomic approaches are presented, to identify novel MAMPs and their cognate PRRs, as well as efforts to translate resistance conferred by these genes into agricultural crops.

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Morphological, pathological and molecular characterization of a new virulence type of *Pyrenophora tritici-repentis*, the causal agent of tan spot. H. BENSLIMANE. *Ecole Nationale Supérieure d'Agronomie, Département de Botanique, El-Harrach, Alger, Algérie. E-mail: h.benslimane@ensa.dz*

Pyrenophora tritici-repentis causes tan spot, a disease responsible for large economic losses in several wheat-growing areas worldwide. According to their ability to produce necrosis and/or chlorosis on a set of four differential bread wheat lines, the isolates of this fungus are currently grouped into eight races. When durum wheat genotypes were added to the differential set, a new virulence type was identified. The isolates (Ptr24, Ptr65, Ptr68 and Ptr76) showing this virulence pattern are unable to attack bread wheat; neither symptoms of chlorosis nor necrosis have developed, while the isolates caused necrosis in durum genotypes. These isolates were further characterised using morphological, pathological and molecular methods. The sexual stage was induced *in-vitro* to confirm the species identity through a new and simple method, using wheat straw and water agar. The isolates were inoculated onto five bread wheat genotypes (Glenlea, 6B-662, 6B-365, Salamouni, Katepwa) and three durum wheat genotypes (4B-160, Coulter, 4B-1149). Katepwa was included because this genotype possesses sensitivity genes to Ptr ToxA and Ptr ToxB. The pathogen genomes were submitted to a virulence gene investigation using PCR and sequencing analysis. All isolates caused resistance reactions on all bread wheat lines, while they produced necrosis in durum wheat. The resistance of Katepwa is further evidence of the absence of ToxA and ToxB gene products. ToxA and ToxB genes were amplified from all isolates, whereas *toxb* gene was absent. Sequence analysis for both genes (ToxA and ToxB) on the new virulence type isolates revealed no apparent differences with those found in the two known functional genes, and coding for PtrToxA and PtrToxB toxins. For the analyzed genes, the sequences did not harbour any mutation. However, these areas (almost the coding regions) were limited to a restricted part of the genes; mutations could be in another regions of both genes and prevented expression of symptoms. The presence of ToxA and ToxB, despite the absence of symptoms usually caused by their products, suggests the existence of an unknown homologue for these two genes. The presence of ToxA in the isolate unable to produce necrosis in Glenlea is reported for the first time. Several hypotheses are presented to explain the lack of ToxA and ToxB gene expression.

Primary metabolism modulation caused by *Onion yellow dwarf virus* infection in 'Rossa di Tropea' onion. A. TIBERINI[1], F. ARANITI[1], A. CIAMPA[3], S.B. GRANDE[1], A. TAGLIENTI[2], M.R. ABENAVOLI[1], M.T. DELL'ABATE[3], L. TOMASSOLI[2], G. ALBANESE[1]. [1]*Università degli Studi Mediterranea di Reggio Calabria, Dipartimento di AGRARIA, Località Feo di Vito - 89122 Reggio Calabria, Italy.* [2]*Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22 - 00156 Roma, Italy.* [3]*Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per la Studio delle Relazioni Pianta Suolo, Via della Navicella 2/4 - 00184 Roma, Italy. E-mail: antonio.tiberini@unirc.it*

Onion yellow dwarf virus (OYDV, genus *Potyvirus*), an aphid transmitted virus, is one of the most limiting pathogens for onion (*Allium cepa* L.) production worldwide, with infection rates in field up to 100% during the biennial host life cycle. The virus causes severe leaf symptoms and plant stunting, reduced bulb size and seed yield. Recently, OYDV has been found responsible for an severe agronomic decline of 'Rossa di Tropea' onion, a cultivar of southern Italy (Calabria), granted by European Union with the Protected Designation Origin (IGP) trademark. This cultivar is known for its mild to sweet flavour but also richness in flavonols and anthocyanins. A research project SI.ORTO (SIR-MIUR grant – SIORTO-RBSI149LD5) has been activated to study the effects of

OYDV on accumulation of nutraceutical compounds in 'Rossa di Tropea'. Gas Chromatography-Mass Spectrometry (GC-MS) and primary metabolism profiling has compared onion bulb samples, healthy *versus* OYDV-infected, collected at three infection times (bulb harvesting, leaves drying and after bulb storage) during a trial conducted in Calabria. Several metabolites connected to sugar and amino acid metabolism, and the TCA cycle decreased in OYDV-infected bulbs compared to control plants at the first testing time. A pronounced increase of theses metabolites then occurred. Magnetic resonance microimaging (MRI) on whole bulbs highlighted structural alteration of OYDV-infected bulbs as compared to healthy bulbs. This research is the first study on metabolic modulation in the onion/virus pathosystem 'Rossa di Tropea'/OYDV.

Downregulation of violaxanthin cycle metabolites is associated with the lesions observed in *mlo* resistant barleys. G. MONTILLA-BASCÓN[1], M. ROCA[2], L.A.J. MUR[3], E. PRATS[1]. [1]*Department of Plant Breeding, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14004 Córdoba, Spain*. [2]*Food Phytochemistry Department, Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), University Campus Pablo de Olavide, Building 46, Sevilla 41013, Spain* [3]*Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, UK.*

In many temperate regions cereal powdery mildew, caused by *Blumeria graminis* ff. spp., seriously constrains crop production. A durable host resistance mechanism that prevents cell penetration by the pathogen is the formation of papillae, which are localized cell wall appositions at attack sites. Papillae provide race non-specific defense, conferring broad-spectrum resistance. Barley genotypes carrying the *mlo* gene display highly effective papilla-based penetration resistance to powdery mildew, that has been durable for over 30 years. However, the *mlo* gene shows adverse pleiotropic effects such as large necrotic/chlorotic flecks on leaves, accelerated leaf senescence and reduced grain yield, and these adverse effects are particularly dramatic under stress conditions. For this reason, *mlo* cannot be used in winter barley varieties. Despite its importance for crop production, the mechanism(s) leading to these pleiotropic effects are still not understood, nor are its molecular and cellular bases. We have previously observed that the damage was associated with particular genetic backgrounds and was linked to stomatal and photosynthetic dysfunctions. We have investigated changes in xanthophyll cycle metabolite profiles and the chlorophyll degradation pathway in two sets of *mlo*-isogenic lines with different genetic backgrounds. A decrease in chlorophyll a and b occurred in the resistant isoline, characterized by necrotic flecking but accumulation of chlorophyllide or pheophorbide were not detected. Overall, xanthophyll metabolites increased following pathogen inoculation in the resistant *mlo* line lacking lesions. Furthermore, anteroxanthin responded to inoculation with increases in all genotypes but greater differences compared with healthy plants in the resistant *mlo* genotype lacking lesions.

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Early and late transcriptome changes in a tomato cultivar carrying the *Sw-5* resistance gene upon infection by a resistance-breaking strain of *Tomato spotted wilt virus*. G. BUBICI, F. CILLO, M.I. PRIGIGALLO, R. MONFREDA. *Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, 70126, Italy. E-mail: giovanninicola.bubici@cnr.it*

We analyzed the transcriptome (RNA-Seq) of leaf samples collected from a field crop of tomato cv. Docet (*Sw5* resistance gene) in Apulia, southern Italy, with different symptom severity and accumulation levels of a resistance-breaking strain of *Tomato spotted wilt virus* (TSWV). Four groups of samples were assumed to be different stages of plant tissue colonization by the virus: plants without symptoms and a null virus titre (group A) or 1 $\times 10^2$ TSWV reads per million (*rpm*; B), and plants with symptoms and 1 $\times 10^4$ *rpm* (C) or 2 $\times 10^5$ *rpm* (D). Transcriptome sequencing revealed that plant response to TSWV infection is profoundly related to its accumulation level in the tissues. At an early stage of infection (B *vs*. A comparison), genes related to photosystem I were down-regulated, and oxidoreductase activity increased. Considerable virus colonization (C

vs. B) activated defense-related mechanisms such as cell surface receptor signalling, phenylpropanoid biosynthesis and transcription factor activity. In contrast, photosynthesis, transmembrane transporter activity, and biosynthesis of monosaccharides and peptides were down-regulated. This scenario increased at an advanced stage of colonization (D *vs.* C), with attenuation of response to stimuli (e.g., surface receptor signaling and protein kinase activity) and an increase of catalytic activities such as ubiquitin-protein transferase and ribonuclease. TSWV infection constantly injured tomato cell metabolism (e.g., photosynthesis, monosaccharide and peptide biosynthesis, ion transporter activity) while plant defense (e.g., cell surface receptor signaling, phenylpropanoid pathway), clearly ineffective in such compatible plant-virus interaction, occurred late and disappeared soon after.

Photosynthetic efficiency differs between *Brassica napus* cultivars that are susceptible or resistant to *Alternaria brassicicola*. V.K. MACIOSZEK[1], T. JĘCZ[1], P. KRÓLIKIEWICZ[1], H. SCHOONBEEK[2], C. RIDOUT[2]& A.K. KONONOWICZ[1]. [1]*Department of Genetics, Plant Biology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12, 16, 90-237 Lodz, Poland.* [2]*John Innes Centre, Norwich Research Park, UK. E-mail: violetta.macioszek@biol.uni.lodz.pl*

Brassica napus (oilseed rape, OSR) cultivars, winter and spring types, have been phenotyped for their changes in photosynthetic efficiency and resistance/susceptibility to the necrotrophic fungus *Alternaria brassicicola*, which causes black spot on all *Brassica* species worldwide. 160 OSR cultivars were grown under laboratory conditions in triplicate. Leaf inoculation (3.5×10^5 conidia mL^{-1}) was performed. Win_DIAS3 system necrosis formation, and FluorCam7 system Kaukasky effect, and photosynthetic dyes and spectroscopy were used to analyse the third leaf of each plant at 5 d post inoculation, when symptoms developed. Analysis of necroses showed that 111 of the cultivars were susceptible or highly susceptible to *A. brassicicola*, and 49 were resistant or highly resistant. Photosynthetic efficiency expressed as FV/Fm (QY_{max}) indicated whether *A. brassicicola* infection affected photosystem II in a dark-adapted state. Uninfected (control) leaves showed QY_{max} values of 0.63 to 0.87, whereas infected leaves were 0.50 to 0.86. The most spectacular decrease in QY_{max} was observed in two susceptible cultivars Zairai Chousenshu and Ningyou7, with differences between control and infected leaves at 0.29. Resistant OSR cultivars showed no changes in QY_{max} values (0.01 to 0.05). Two the most susceptible spring cultivars (MONTY-028DH and Zairai Chousenshu) and the two most resistant winter ones cultivars (Savannah and Askari) were selected for further analyses such as changes in levels of photosynthesis-related proteins (RUBISCO, LHCa and LHCb) by Western blot analyses.

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The role of the necrosis and ethylene inducing gene *VdNEP* in virulence of *Verticillium dahliae*. A. TRIANTAFYLLOPOULOU[1], A.K. TZIMA[1], S. KANG[2], E.I. PAPLOMATAS[1]. [1]*Laboratory of Phytopathology, Agricultural University of Athens, Iera Odos 75, Athens, Greece.* [2]*Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, Pennsylvania, USA. E-mail: alextriantafyl@gmail.com*

The VdNEP protein from the fungus *Verticillium dahliae* is a member of the Nep1-Like proteins (NLPs), that have been isolated from plant pathogenic fungi and induce necrosis and ethylene production in dicotyledonous plants, functioning as toxins or effectors. Over-expression of the *VdNEP* gene under the control of a strong promoter in three races of *V. dahliae* (the tomato race 1, the cotton non-defoliating and defoliating strains) resulted in elevated transcript levels of the *VdNEP* transgene in transformed strains compared to wild type strains. *VdNEP* overexpressing strains caused increased disease symptoms on different hosts, compared to the respective wild type strains. Differentiation between the defoliating and non-defoliating races of the pathogen was possible when the *VdNEP* gene was used as a molecular marker. Furthermore, a *VdNEP-EGFP* fusion construct was generated and inserted in the three races of *V. dahliae*, to observe localization and/or secretion of the protein. Observation of the fusion protein within fungal cells is currently in progress.

Developmental processes regulated by small RNAs during *Arabidopsis*-Root knot nematode interaction. F.E. DÍAZ-MANZANO[1], J. CABRERA[1], M. BARCALA[1], R. OLMO[1], A.C. SILVA[1], M.F. ANDRÉS[2], I. MARTÍNEZ[1], V. RUIZ-FERRER[1], C. FENOLL[1], C. ESCOBAR[1]. [1]*Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla-La Mancha, Avenida de Carlos III, s/n, 45071, Toledo, Spain.* [2]*Departamento Protección Vegetal, Instituto Ciencias Agrarias-CSIC, Calle Serrano, 115, 28006, Madrid, Spain. E-mail: Fernando.Diaz@uclm.es*

Root knot nematodes (*Meloidogyne* spp.) currently cause major agricultural losses. They infect plants in the root elongation zone and penetrate intracellularly into the vascular cylinders, inducing galls containing nematode feeding cells, the giant cells (GCs). We studied the differential transcriptome of *Arabidopsis* GCs and galls after *Meloidogyne* spp. Infection, as compared to vascular cells, revealing high-repressed genes probably due to gene expression reprogramming during their differentiation. Sequencing of small RNAs (sRNAs) showed profiles consistent with a role of sRNAs in gene silencing. The 24 nt-sRNAs, known to be involved in epigenetic regulation, were highly induced in early formed galls (3 d post infection), and together with differentially regulated miRNAs could be mediating the large gene repression that occurs during early development of GCs/galls. We have studied the roles of miR390 and miR172, accumulated in galls at early infection stages. Loss of function *Arabidopsis* lines for both miRNAs showed reduced numbers of galls after nematode infection. The two miRNAs participate in plant developmental processes in different ways. *TAS3* precursor is cleaved by miR390 triggering tasiRNAs biogenesis that inhibits *ARF2-4*, releasing repression of lateral root growth. In contrast, miR172 downregulates the *AP2*-like genes during flowering via a translational mechanism rather than by mRNA cleavage. We discuss the putative molecular networks induced by plant-nematodes in this biotic interaction through miR172 and miR390.

Role of the PTC1 protein phosphatase in stress response in *Fusarium oxysporum* f. sp. *lycopersici*. P.P. FERREIRA LEMOS and C. HERA. *Departamento de Genética. Campus Universitario de Rabanales. Universidad de Córdoba. 14071 Córdoba, Spain.*

Type 2c Ser/Thr phosphatases (PTCs) are a class of protein phosphatases, conserved in eukaryotes. The PP2C proteins are involved in the regulation of many cellular functional processes, addressed by their role on MAPK cascades. Seven putative PTC proteins have been identified in *Fusarium oxysporum* f. sp *lycopersici* 4287, using the BLAST algorithm, with PTCs from *Saccharomyces cerevisiae* and *Fusarium graminearum*. The expression of these genes in different stress conditions and plant infection was evaluated by RT-qPCR. Upregulation of *ptc1* was observed after cell wall stress, osmotic stress and plant infection, while downregulation was detected after invasive growth. A mutant strain, Δ*ptc1*, was obtained by the split marker strategy. The Δ*ptc1* strain was more sensitive to SDS (0.125%) and menadione (20 μg mL^{-1}) than the wild type, indicating possible roles of PTC1 in, respectively, cell wall/membrane stress (MPK1 pathway) and oxidative stress (HOG1 pathway). In addition, the Δ*ptc1* strain showed greater tolerance to LiCl (0.15M and 0.30M) on different media pH (5, 7 and 8.5) than wild type, suggesting a role of PTC1 in lithium efflux mediated by the Nha1 (Na+/K+/Li+/Rb+ antiporter). The phosphorylation level of HOG1, MPK1 and FMK1 proteins was evaluated by western-blot; the Δ*ptc1* strain showed increased phosphorylation level of HOG1 compared to the wild type. These results suggest an important role of PTC1 on the HOG1 pathway of *Fusarium oxysporum* f. sp *lycopersici*.

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Transient silencing of the *FaWRKY1* strawberry gene (*Fragaria x ananassa*) in fruit induces resistance to *Colletotrichum acutatum* infection. J.J. HIGUERA-SOBRINO[1], F.J. MOLINA-HIDALGO[1], I. ARJONA-GIRONA[2], F. AMIL-RUIZ[1], J. GARRIDO-GALA[1], A. LEKHBOU[1], J.A. MERCADO[3], F. PLIEGO-ALFARO[3], J. MUÑOZ-BLANCO[1], C.J. LÓPEZ-HERRERA[2], J.L. CABALLERO[1]. [1]*Departamento de Bioquímica y Biología Molecular, Edif. Severo Ochoa-C6, Planta Baja-Ala Norte. Campus de Rabanales s/n. Universidad de Córdoba-14071, Córdoba, Spain.* [2]*Departamento de Protección de Cultivos, Instituto de Agricultura Sostenible, C.S.I.C. C/Alameda del Obispo s/n, Apartado 4084, Córdoba, Spain.* [3]*Departa-*

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Anthracnose, caused by *Colletotrichum acutatum*, is responsible for significant yield losses in commercial strawberry production worldwide. For this reason, it is of interest to uncover the molecular basis underlying this strawberry/pathogen interaction. Previously, *FaWRKY1* was identified as an important element mediating defence responses. This gene encodes an AtWRKY75-like transcription factor (type IIc), which is upregulated in strawberry following *C. acutatum* infection. In this study, *Agrobacterium*-mediated transient transformation was used to both silence and overexpress the *FaWRKY1* gene in fruit, with the aim to clarify its function in the strawberry defense mechanism. Analyses of FaWRKY1-RNAi strawberry fruits showed resistance to *C. acutatum* infection, 5 d after inoculation with this pathogen. Overexpression of this gene in strawberry fruit showed increased susceptibility to *C. acutatum*. Molecular analysis is being carried out with these fruit samples to elucidate candidate genes transcriptionally regulated by FaWRKY1. Furthermore, *in vitro* DNA-binding assays have revealed a tentative consensus sequence [G/T][T/C]TGAC[T/C], containing the core sequence TGAC (W box), as the likely target sequence for FaWRKY1 binding. These analyses will strengthen genome-wide promoter target site prediction for FaWRKY1.

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Evidence that the putative movement protein (MP2) of *Broad bean wilt virus 1* is a pathogenicity determinant. C. CARPINO[1,2], I. FERRIOL[1], L. ELVIRA-GONZÁLEZ[1], L. RUBIO[1,3], E. PERI[2], S. DAVINO[2,4], L. GALIPIENSO[1,3,4]. [1]*Instituto Valenciano de Investigaciones Agrarias (IVIA), Ctra. CV-315, 46113 Moncada, Valencia, Spain.* [2]*Department of Agricultural and Forestry Science, University of Palermo, Piazza Marina 61, 90133 Palermo, Italy.* [3]*Euro-Mediterranean Institute of Science and Technology (IEMEST), Vía Michele Miraglia 20, 90139 Palermo, Italy.* [4]*Departamento de Biotecnología, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.*

Broad bean wilt virus 1 (BBWV-1, genus *Fabavirus*, family *Secoviridae*) infects crops of economic importance, such as broad bean, pepper, tomato, spinach, and ornamental plants. The virus genome is constituted by two molecules of positive single stranded RNA, each encoding a polyprotein which is further processed by proteolytic cleavage. RNA1 encodes the proteins involved in viral replication and expression, while RNA2 encodes the movement protein (MP) and two coat proteins (LCP and SCP). RNA2 contains an alternative second start codon rendering a smaller putative movement protein, called MP2. To date, the BBWV-1 proteins related to pathogenicity are unknown. The roles of MP2 in symptom determination, post-transcriptional gene silencing (PTGS) and elicitation of hypersensitive response (HR) were examined. Expression of MP2 in *Nicotiana benthamiana* through *Potato virus X* (PVX) caused necrotic lesions, indicating that MP2 is a symptom determinant. Analysis of O_2^- accumulation and necrosis staining revealed that this protein elicited the cellular HR. Transient expression of MP2 in *N. benthamiana 16C*, that constitutively expresses Green Fluorescent Protein (GFP), and a complementation assay with a vector based on *Turnip crinkle virus* sequence (TCV-sGFP) showed that this protein acts as a suppressor of PTGS.

Chlorophyll degradation pathway is linked to stomatal and photosynthetic dysfunctions observed in oats resistant to powdery mildew. G. MONTILLA-BASCÓN[1], M. ROCA[2], L.A.J. MUR[3], PRATS E[1]. [1]*Department of Plant breeding, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14004 Córdoba, Spain.* [2]*Food Phytochemistry Department, Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), University Campus Pablo de Olavide, Building 46, Sevilla 41013, Spain.* [3]*Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, UK.*

Cost of resistance is usually associated with the energy and nutritional penalties linked to induction of defenses. Currently, a mechanistic understanding of the sources of these costs is lacking, other than vague

suggestions of the energy "lost" in inducing the defense. We have shown that penetration resistance or hypersensitive response (HR) provoke stomatal and photosynthetic dysfunctions, which could be important components of the disease resistance cost. More importantly, the stomatal dysfunctions (lock-up) are genotype, but not response-type, dependent, since genotypes with similar resistance responses show very different locking patterns when assessed histologically. We have assessed the content of several photosynthetic pigments including chlorophyll *a*, and *b*, several metabolites of the xanthophyll cycle, and metabolites of the chlorophyll degradation pathway in healthy and powdery mildew (*Blumeria graminis* f. sp. *avenae*) inoculated oat seedlings. Resistant genotypes associated with stomatal and photosynthetic dysfunctions activate the chlorophyll degradation pathway early after pathogen inoculation, increasing the level of pheophytin *a* content. These genotypes also showed a reduction in chlorophyll *a* and *b* contents, whereas the resistant genotypes lacking physiological dysfunctions showed no variation in the level of these compounds.

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Early signalling during *mlo*-based papilla resistance involve a subtle crosstalk between jasmonate, salicylic acid and abcisic acid pathways. F. CANALES-CASTILLA[1], G. MONTILLA-BASCÓN[1], N. RISPAIL[1], A. GÓMEZ-CADENAS[2], V. ARBONA[2], PRATS E[1]. [1]*Department of Plant breeding, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14004 Córdoba, Spain.* [2]*Ecofisiologia i Biotecnologia. Dpt. Ciències Agràries i del Medi Natural. Universitat Jaume I - Campus Riu Sec. E-12071 Castelló de la Plana, Spain.*

Powdery mildew is one of the most widespread and damaging crop diseases. One of the most efficient and durable powdery mildew resistance mechanisms was originally found in barley lines carrying homozygous recessive alleles at the Mlo locus. These lines show efficient resistance to pathogen penetration based on formation of papillae, which are localised cell wall appositions at attack sites. The *Mlo* gene encodes a protein considered a negative regulator of the defence response so that its loss leads to more rapid and/or enhanced papilla formation. Although it is known that host plants sense powdery mildew fungi and start to activate defenses as early as 30 min following pathogen challenge, very little is known of the signaling that leads to the efficient papillae formation of *mlo* genotypes. We have explored the profile of several signaling molecules in two sets of *mlo*-isogenic lines with different genetic background, over a time course ranging from 30 min to 24 h. Abcisic acid decreased following inoculation in all susceptible and resistant genotypes whereas salicylic acid increased only in the resistant *mlo* genotypes from 2 h post inoculation, with a maximum at 24 h. Jasmonic acid and its derivative, Ile-jasmonic increased in resistant genotypes at 10-12 h after inoculation, whereas its biosynthetic intermediate 12-OPDA accumulated in resistant genotypes as early as 4 h and following. These data, showing a subtle and very early regulation of these signaling pathways, will shed light on the mechanisms of papilla formation.

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Biocontrol, natural compounds and plant defense stimulants

Characteristics of the biocontrol rhizobacterium *Pseudomonas chlororaphis* PCL1606. S. TIENDA, C. VIDA, A. DE VICENTE, F.M. CAZORLA**.** *Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: sandratienda@uma.es*

The major disease affecting avocado crops in the Mediterranean area is avocado white root rot, caused by *Rosellinia necatrix*. The biocontrol rhizobacterium *Pseudomonas chlororaphis* PCL1606 has been isolated from rhizosphere of healthy avocado trees, growing in an area affected by white root rot. As a main characteristic, PCL1606 showed strong *in vitro* antagonism against *R. necatrix* and other important soil-borne pathogens. This is mainly due to produc-

tion of the antimicrobial compound 2-hexyl, 5-propyl resorcinol (HPR). Production of other antifungal compounds by PCL1606 has also been detected. PCL1606 has the ability to persist on and colonize avocado roots, where the bacterium interacts closely and colonizes *R. necatrix* hyphae, leading to negative effects on the fungus. Those phenotypes, acting together, allowed PCL1606 to display biocontrol activity towards *R. necatrix* in avocado plants. We have observed that PCL1606 shows no plant growth promoting activities. The availability of the complete genome sequence of PCL1606 will allow identification of additional features of the strain involved in biocontrol.

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Biological characterisation of *Pochonia chlamydosporia* isolates associated with root-knot nematodes. J. HORTA, I. ABRANTES, I.L. CONCEIÇÃO *Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Universidade de Coimbra, P-3000 456 Coimbra, Portugal. E-mail: isabelluci@gmail.com*

The nematophagous fungus *Pochonia chlamydosporia* (Pc) is a ubiquitous facultative parasite of eggs of potato cyst nematode (PCN; *Globodera* spp.), and root-knot nematode (RKN; *Meloidogyne* spp.). This study assessed the potential of Portuguese Pc isolates as biocontrol agents (BCA) of nematodes. Four isolates associated with *Meloidogyne* spp. eggs (PcI, PcII, PcIV and PcV), three associated with tomato infected roots (PcVI, PcVIII and PcIX), one(Pc2) from PCN eggs, as well as an exotic isolate (Vc10), were evaluated, using *in vitro* assays, for their abilities to produce chlamydospores, colonise tomato rhizospheres, and parasitise *G. rostochiensis* and *M. incognita* eggs. The isolates had marked differences in performance. Isolate PcI colonised the host rhizosphere extensively (89%), whereas PcIX was a poor coloniser (<50%). Isolates PcII and Pc2 produced greatest numbers of chlamydospores on solid medium (>20 × 10^5 chlamydospores g^{-1}). The proportion of RKN eggs parasitised was low (<60%) for all isolates. PcI and PcVIII were the best parasites against RKN eggs (>50%), and PcV and Pc2 parasitised more than 50% of the PCN eggs. These results suggest that PcI is the native isolate with the greatest potential as a BCA, since this isolate revealed desirable traits such as good rhizosphere competence and prolific chlamydospore production, thus having a greater potential than the other isolates for exploitation as a BCA.

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The efficacy of plant-derived protein hydrolysates against zucchini powdery mildew is affected by their biochemical characteristics. M. CAPPELLETTI[1,2], M. PERAZZOLLI[1], A. NESLER[1,3], O. GIOVANNINI[1], I. PERTOT[1,4]. *[1]Department of Sustainable Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige, Italy. [2]DI4A, Department of Agrifood, Environmental and Animal Sciences, University of Udine, 33100 Udine, Italy. [3]Bi-PA - Biological Products for Agriculture, B-1840 Londerzeel, Belgium. [4]Center Agriculture, Food and Enviroment, University of Trento, 38010 San Michele all'Adige, Italy. [*]E-mail: martina.cappelletti@fmach.it*

The substitution of pesticides has become a priority in agriculture, and induction of plant resistance by protein hydrolysates may offer a sustainable solution. Peptide fragments can act as elicitors of plant immunity, and a protein extract was shown to reduce powdery mildew symptoms by stimulating grapevine defense responses under field conditions. The present research investigated potential correlations between the efficacy of plant-derived hydrolysates against the zucchini powdery mildew (caused by *Podosphaera xanthii*) and their biochemical features, such as peptide and amino acid composition, in order to clarify their modes of action. Under greenhouse conditions, soybean, rapeseed and guar hydrolysates were tested. These were obtained by enzymatic and acid hydrolysis of low-cost protein meals. Preventative foliar treatments with guar hydrolysates produced with Alcalase and 6N sulfuric acid demonstrated disease reduction compared with

the non-hydrolysed protein source. A positive correlation was found between efficacy of guar acid hydrolysates and degree of hydrolysis, suggesting that this hydrolysis method may enhance the functional properties of the original protein source. Positive correlations were also found between efficacy and concentrations of specific peptides and amino acids, which may contribute to the induction of resistance. Different from acid hydrolysates, no toxic effect on *P. xanthii* conidium germination was observed for the enzymatic hydrolysates, suggesting the activation of plant defense responses. Our results confirmed that the biocontrol activity of protein hydrolysates is affected by the original source, and the method and degree of hydrolysis. Further research is required to fully clarify the mode of action of these compounds.

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***Geosmithia* spp. and *Ophiostoma novo-ulmi*, a new fungus-fungus association mediated by elm bark beetles.** A.L. PEPORI, A. SANTINI. *Institute for Sustainable Plant Protection, CNR, Via Madonna del Piano, 10 50019 Sesto Fiorentino, Italy. E-mail: alberto.santini@cnr.it*

Geosmithia is a mainly insect-associated monophyletic morphogenus of Ascomycota. Some recent highlights show that some Geosmithias may play roles in the elm-Dutch elm disease (DED) pathosystem. Geosmithia and *Ophiostoma novo-ulmi* (ONU) have been found to be spread by elm bark beetles; they share the same habitat for consistent parts of their life cycles. Highly frequent horizontal gene transfer has been observed between the two fungi. We investigated the relationship between ONU and several elm Geosmithias, using: dual culture and colony interaction trials; ONU fertility tests in the presence of *Geosmithia*; microscopy observations of the interaction between ONU and *Geosmithia*; and pathogenicity trials on elm trees. The relationship between the two fungi was close and stable, and characterised by parasitism of ONU by *Geosmithia*. Our results add new complexity to the DED pathosystem, by demonstrating that *Geosmithia* conducts mycoparasitic activity against ONU. The rise of *Geosmithia* modifies the relationships and the dynamics among the DED components, potentially reducing the overall impact of the disease. For this reason, *Geosmithia* can be exploited as a possible biocontrol agent against ONU. Such a holistic approach to plant pathology strengthens the idea that different management of diseases in natural environments is possible.

Endo- and epi-phytic fungal communities of olive twigs is influenced by cultivar and olive knot infection. T. GOMES[1,2], J.A. PEREIRA[1], T. LINO-NETO[2], A. BENNET[3], P. BAPTISTA[1]. [1]CIMO/ *Polytechnic Institute of Bragança, School of Agriculture, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. pbaptista@ipb.pt.* [2]*Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center (CBFP), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.* [3]*Ecological Sciences, The James Hutton Institute, Errol Road, Invergowrie, Dundee, DD2 5DA UK. E-mail: teresa.mdg@gmail.com*

Olive tree phyllospheres are colonized by a diverse microbial assemblage that may interact with pathogenic fungi, making them potential candidates for disease supression. Olive knot (OK) is caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Psv.), causing significant economic losses in olive tree (*Olea europaea*). We evaluated the effects of cultivar and OK infection on endo- and epi-phytic fungal communities inhabiting olive twig tissues. For this, fungal composition and diversity was assessed in asymptomatic and OK-symptomatic twigs co-occurring olive cultivars with different susceptibilities to OK disease. Isolated species were identified using ITS rDNA sequencing. The cultivar and OK infection were important in shaping the endophytic and epiphytic fungal communities. Fungal community composition differed ($P = 0.005$) between olive tree cultivars, being Nectriaceae - the dominant family in cvs Cobrançosa and Verdeal Transmontana, whereas Pleosporaceae was dominant in the cv. Madural. Epiphytic and endophytic fungal communities also differed in size and composition in asymptomatic and OK-symptomatic twigs, for the three cultivars. In general, asymptomatic twigs had more diverse and rich populations (up to 1.4-fold) when com-

pared to OK-symptomatic twigs. Among the species identified in the asymptomatic tissues, *Cladosporium cladosporioides* was most frequently isolated within the epiphytic community, and *Chromelosporium carneum* within the endophytic community. In the OK-symptomatic tissues, *Cladosporium* sp. was the most frequently isolated within epiphytic community and *Fusarium lateritium* within the endophytic community. According to indicator species analysis, *C. carneum*, *Pyronema domesticum* and *Phoma aloes* (IndVal up to 0.56) may be promising species for the OK suppression. Better acknowledgement should be developed to uncover their roles on olicve tree health.

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Constitutive secretion of pisatin in root exudates participates in pea defence against *Fusarium oxysporum* f. sp. *pisi*. N. RISPAIL[1], M. BANI[1,3], A. CIMMINO[2], A. EVIDENTE[2], D. RUBIALES[1]. [1]*Instituto de Agricultura Sostenible, CSIC, Avenida Menendez Pidal s/n, Córdoba, Spain.* [2]*Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte. S. Angelo, Via Cintia 4, 80126 Napoli, Italy.* [3]*Ecole Nationale Supérieure de Biotechnologie, Ville universitaire Ali Mendjeli, BP E66 25100 Constantine, Algeria. E-mail: nrispail@ias.csic.es*

Root exudates are important regulators of plant rhizospheres. They can modulate the composition and dynamics of soil micro-organisms and participate in the dialogue between plants and microorganisms. They are known to modulate germination and growth of soil microorganisms. As such, they may contribute to crop resistance to soil-borne pathogens. To determine whether root exudates can contribute to defense against the pea root pathogen *Fusarium oxysporum* f. sp. *pisi* (*Fop*), and to identify the active metabolites, we studied the effects of the root exudates of 12 pea accessions with differential responses to the disease. Most root exudates stimulated the germination of *Fop* conidia in liquid bioassays. The root exudates of three accessions, by contrast, inhibited germination, indicating the presence of inhibitory substances in these root exudates. Ethyl acetate extraction of root exudates indicated that the inhibiting substances were contained in the apolar fraction, that contains most secondary metabolites. Further fractioning and analysis identified the pea phytoalexin pisatin as the most active metabolite, and pisatin was identified in the active fraction of pea root exudate extracts. This compound to inhibit *Fop* germination in liquid bioassay and its amount in root exudates was negatively correlated with the extent of *Fop* germination. These results indicate the existence of a pre-penetration mechanism in pea that can delay the build-up of pathogenic populations in soil. Our results also suggest an important role of pisatin in the constitutive defense of pea against *Fop*.

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Control of bacterial plant diseases using licorice extract with a new controlled release formulation. S. JACOBS[1], H. ÖZAKTAN[2], M. AKBABA[2], A. AKKÖPRÜ[2], I. KLEEBERG[1], A. SCHIKORA[3]. [1]*Trifolio-M GmbH, Dr.-Hans-Wilhelmi-Weg 1, 35633 Lahnau, Germany.* [2]*Department of Plant Protection, Faculty of Agriculture, University of Ege, 35100 Bornova Izmir, Turkey.* [3]*Federal Research Centre for Cultivated Plants (JKI), Institute of Epidemiology and Pathogen Diagnostic, Messeweg 11/12, 38104 Braunschweig, Germany. E-mails: Sophie.Jacobs@trifolio-m.de; Adam.Schikora@julius-kuehn.de*

The lack of active substances for the control of bacterial diseases in plants causes serious problems. Only a few agrobactericides are available, but all have distinct shortcomings. The European Union banned the agricultural use of antibiotics in 2004 fearing antibiotic multi-resistant strains, which occurred in fire blight control/prevention in orchards in USA, New Zealand and Israel. In Europe, permission for antibiotic use is limited to cases of "clear and present danger". Alternatively, different copper-based products are authorized as bactericides, but natural resistances in pathogenic *Pseudomonas* and *Xanthomonas* strains have become prevalent, and intensive copper sprays used to control the diseases resulted in

reduced efficacies of these products. In addition, there is an increasing body of evidence that the use of copper products is environmentally damaging due to harmful effects on plants and soil. Nevertheless, in European organic agriculture copper-based products are the main agents used against bacterial diseases in vegetable and fruit production systems. Recently, the use of copper compounds has been restricted by regulations of the European Community (Directive 2009/128/EC). The limited number of existing products requires effective alternatives, which adhere to organic farming principles. We examined the bactericidal effects of licorice extract and a new control release formulation against the main bacterial targets in organic agriculture practice. We tested its efficacy on the model plant *Arabidopsis* as well as numerous crop plants using different patho-systems.

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Assessment of biological control agents against *Gnomoniopsis smithogilvyi* (syn. *castanea*), the fugus causing chestnut brown rot and canker. M. CONTI[1], J. CROVADORE[1], B. COCHARD[1], R. CHABLAIS[1], M. JERMINI[2], F. LEFORT[1]. [1]*Plants and pathogens Group, Institute Land Nature Environment, hepia, University of Applied Sciences and Arts Western Switzerland (HES-SO), 150 route de Presinge, 1254 Jussy, Switzerland.* [2]*Agroscope, Cadenazzo Research Centre, A Ramél 18, 6593 Cadenazzo, Switzerland. E-mail: francois.lefort@hesge.ch*

In vitro challenge tests were carried out between *Gnomoniopsis smithogilvyi* and seven antagonistic bacterial strains and nine fungal strains. Two genotypes of *G. smithogilvyi* from Geneva (GE1) and Ticino (TI1) were used during these tests. These *in vitro* challenge tests allowed selection of five fungal and three bacterial strains, which demonstrated strong inhibitory activity on growth of *G. smithogilvyi*. These were: *Trichoderma harzianum B05, T. harzianum F1, T. hamatum, T. aureoviride* and *T. asperellum*; and *Pseudomonas putida, Bacillus amyloliquefaciens Ba4* and *B. amyloliquefaciens Ba2*. These organisms were retained for biological control experiments on chestnut scions. Batches of eight chestnut scions were inoculated with each fungal or bacterial antagonists by soaking them for 48 h at room temperature in bacterial or fungal suspensions in water. The scions were transferred individually into glass culture tubes and placed in a climatic chamber for 3 weeks. to allow a uniform endophytic growth of the antagonists. A suspension of *G. smithogilvyi* conidia was then applied to the scions of all modalities. and half of the control scions. The development of fructifications on bark of the scions, and the condition of the scions, were observed before and after inoculation, for a total duration of 6 weeks. Most of the organisms did not reduce disease *in vivo*, but the bacterial strain *P. putida* UASWS0946 and the fungal strain *T. hamatum* UASWS1405 totally inhibited the growth of *G. smithogilvyi* and *C. parasitica.*

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Preliminary characterization of the bioactive metabolites produced by *Ascochyta lentis* var. *lathyri*, responsible for a grasspea disease. A. BOARI[1], A. CIMMINO, A. EVIDENTE[2], A. INFANTINO[3], M. MASI, M.C. ZONNO[1], and M. VURRO[1]. [1]*Intitute of Sciences of Food Production, National Research Council, via Amendola 122/O, 70126 Bari, Italy.* [2]*Department of Chemical Sciences, University of Naples "Federico II", Complesso Universitario Montesant'Angelo, via Cinthia 4, 80126, Naples, Italy.* [3]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale, Via C.G. Bertero 22, 00156 Rome, Italy. E-mail: evidente@unina.it*

Ascochyta lentis var. *lathyri* causes necrotic lesions on leaves and stems of grasspea (*Lathyrus sativus* L.) plants, recently described for the first time in Italy. This fungus was not pathogenic to seedlings of nine other leguminous species, including lentil (*Lens culinaris* Medik.). For this reason, and in consideration of its morphological characteristics, the fungus was considered a pathogenic, and morphological variant, of *Ascochyta lentis* (pathogenic to lentil), despite genetic similarities. Considering, (a) the increasing interest for the cultivation of grasspea as a source of protein and genetic resistance to diseases; (b) the known capability of the genus *Ascochyta* to produce biologically active secondary metabolites; and (c) the potential of comparative metabolic analysis to

provide valuable information on fungal ecology and evolution, studies were instigated to investigate the production of bioactive metabolites by *A. lentis* and *A. lentis* var. *lathyri*. Two strains of each pathogen were grown on a defined liquid medium. Culture filtrates were extracted with organic solvents, and tested for bioactivity using different assays. The successive steps of the purification, bioassay guided, were performed by CC and TLC. This provided different bioactive fractions and metabolites, which are being characterized using spectroscopic and physical methods. Preliminary results have confirmed differences in the metabolic profiles of the strains, in agreement with other differences previously described.

Inhibition of early development stages of rusts and powdery mildew by fungal and plant metabolites. E. BARILLI[1], A. CIMMINO[2], M.J. GONZALEZ BERNAL[1], M. MASI, D. RUBIALES[1], A. EVIDENTE[2]. [1]*Institute for Sustainable Agriculture, CSIC, Córdoba, 14004, Spain.* [2]*Department of Chemical Sciences, University of Naples "Federico II", Complesso Universitario Montesant'Angelo, via Cintia 4, 80126, Naples, Italy. E-mail: evidente@unina.it*

Foliar diseases caused by biotrophic pathogens, such as rusts and powdery mildews, are major limiting factors in legume and cereal production worldwide. Crop protection is largely based on chemical control, although there is renewed interest in the discovery of natural products as alternatives to synthetic fungicides. A plant and a fungal metabolite (respectively, *inuloxin A* and *sphaeropsidin A*), belonging to different classes of naturally occurring compounds, have been evaluated, together with a synthetic fungicide, at different concentrations on pea and oat plants for their potential to inhibit spore germination and subsequent fungal growth. Pathogens responsible of rust (*Uromyces pisi*) and powdery mildew (*Erysiphe pisi*) on pea, andthese diseases on oat (*P. coronata* f. sp. *avenae* and *Blumeria graminis* f. sp. *avenae*), were artificially inoculated on their susceptible hosts under controlled conditions. Spore germination, plant penetration and fungal development were microscopically scored on detached leaves at 24 and 48 h after inoculation. *Inuloxin A* and *sphaeropsidin A* both reduced spore germination and fungal development of all the pathogens, at values comparable to those obtained by the synthetic fungicide, offering potential as natural fungicides for management of these diseases.

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Secondary bioactive metabolites produced by emerging forest pathogens in Sardinia. M. MASI[1], B. T. LINALDEDDU[2], A. CIMMINO[1], P. NOCERA[1], L. MADDAU[3], A. EVIDENTE[1]. [1]*Department of Chemical Sciences, University of Naples "Federico II", Complesso Universitario Montesant'Angelo, via Cinthia 4, 80126, Naples, Italy.* [2]*Università degli Studi di Padova - Dipartimento di Territorio e sistemi agro-forestali (TESAF), viale dell'Università 16, 35020 Legnaro (PD).* [3]*Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi di Sassari, Viale Italia 39, 07100, Sassari, Italy. E-mail: evidente@unina.it*

Several studies have demonstrated the important role of fungal species in the aetiology of serious disease affecting forest trees in Sardinia (Italy). Among them, *Diplodia corticola* has serious impacts on oak ecosystems, adversely affecting vitality and productivity of trees. Recently, a severe trunk and branch disease caused by a newly described species, *Diaporthella cryptica*, was observed in several hazelnut groves in Sardinia. The considerable ecological relevance of forests, and severe damage caused by these fungi, requires increased knowledge of the bio-ecology of these invasive species, and particularly identifying the virulence factors involved in the pathogenesis processes. Extensive studies on liquid cultures of *D. corticola* have indicated the potential of this fungus to produce an array of bioactive secondary metabolites *in vitro*, some of which have shown potential in application studies. Sphaeropsidin A has been studied as novel therapeutic strategy to combat drug-resistant cancer. Other metabolites, such as diorcinol and diplopyrone B, have shown activity against important plant pathogens belonging to different phyla, especially *Phytophthora* spp. which are effectively controlled with few synthetic fungicides in forestry. Investigations carried out with organic extracts of *D. cryptica* have shown the ability of this pathogen to produce bioactive compounds. Organic extracts and column chromatographic fractions were

phytotoxic on tomato leaves at 2 mg mL^{-1} in a leaf puncture assay.

Biofumigant action of *Brassica* seed meals against *Phytophthora cinnamomi* in dehesa ecosystems. M. GONZÁLEZ[1], P. RÍOS[1], P. FERNÁNDEZ[1], A. DE HARO[2], M.S. SERRANO[1], M.E. SÁNCHEZ[1]. [1]*ETSIAM, Universidad de Córdoba, Ctra. Madrid-Cádiz km 396, 14014-Córdoba, Spain.* [2]*Instituto de Agricultura Sostenible (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. E-mail: ag1sahem@uco.es*

Phytophthora cinnamomi causes a highly destructive root rot that seriously affects oaks in seminatural woodlands (dehesa systems). Disease management using soil biofumigation is promising, but requires further validation. The effectiveness of ground seeds from *Brassica carinata* and *B. juncea* to inhibit mycelial growth and reduce chlamydospore viability of *P. cinnamomi* in soil was established, in contrast with the inability shown by *B. napus* seed meals. Reduced root necrosis in inoculated *Lupinus luteus* plants was also achieved when infested soils were treated with *B. carinata* or *B. juncea* seed meals. Glucosinolate content analyses of these seed meals indicated that effectiveness was related to large content of sinigrin (2-propenyl glucosinolate). Biofumigation with seed meals rich in sinigrin should be considered as an effective measure to be incorporated in the integrated control of the oak disease caused by *P. cinnamomi* in dehesa ecosystems. Aas host seed production levels in dehesa are low, this approach should be best applied on agricultural lands.

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Control of *Phytophthora* root rot on Mediteranean *Quercus* spp. Using fosetyl-Al trunk injections. M. GONZÁLEZ[1], M.A. ROMERO[1], C. RAMO[2], M.S. SERRANO[1], M.E. SÁNCHEZ[1]. [1]*ETSIAM, Universidad de Córdoba, Ctra. Madrid-Cádiz km 396, 14014-Córdoba, Spain.* [2]*Estación Biológica de Doñana (CSIC), Américo Vespucio 26, 41092-Sevilla, Spain. E-mail: ag1sahem@uco.es*

Potassium phosphite (PP) is the most used plant defense stimulant against Phytophthora diseases, but PP formulations are prohibited in Spain when registered as fertilizers. In previous pot experiments, fosetyl-aluminium (fos-Al) demonstrated better efficacy than PP for prevention of root disease caused by *Phytophthora cinnamomi* on *Quercus suber* and *Q. ilex*. In November 2014, 4% fos-Al was applied by trunk injection (one 20 mL-injector at each 20 cm-perimeter) to trees in two different *Quercus* woodlands affected by *P. cinnamomi*. For each woodland, 20 asymptomatic trees (defoliation class, DC = 0), 20 trees with low crown symptoms (10-25% defoliation, DC = 1), and 20 with moderate crown symptoms (26-50% defoliation, DC = 2), were randomly chosen. For each DC, ten trees were injected with fos-Al and ten with water only. All trees were sampled (rhizosphere soil and rootlets) before treatments. At I year intervals, all trees were evaluated for defoliation class, re-sampled (roots and soil). Samples were checked for *P. cinnamomi* presence (roots) and *P. cinnamomi* inoculum density (chlamydospores g^{-1} dry soil).Disease severity (variation in DC with time) decreased in treated trees when compared with untreated trees, mainly because the treated trees had decreased DCs, but also because untreated trees had increased DCs. Detection of *P. cinnamomi* in roots increased in the water-treated trees, although soil inoculum densities decreased with time, not differing between treated or untreated trees. This trial has demonstrated the effectiveness of fos-Al for prevention of *P. cinnamomi* oak disease in the field, decreasing crown symptoms and pathogen detection in roots.

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Control of *Acanthoscelides obtectus* (Coleoptera: Chrisomelidae:Bruchinae) adults through trichodiene produced by *Trichoderma harzianum*. A. RODRÍGUEZ-GONZÁLEZ[1], V. SUÁREZ-VILLANUEVA[1], S. MAYO[1], G. CARRO-HUERGA[1], S. ÁLVAREZ-GARCÍA[1], P.A. CASQUERO[1], S. GUTIÉRREZ[2]. [1]*Grupo de Investigación en Ingeniería y Agricultura Sostenible, Instituto de Recursos Naturales, Medio Ambiente y Biodiversidad, Escuela Superior y Técnica de Ingeniería Agraria, Universidad de León, Avenida de Portugal 41, 24071-León, Spain.* [2]*Área de Microbiología, Universidad de León, Avenida de Astorga s/n, 24401-Ponferrada, Spain. E-mail: alrog@unileon.es*

Acanthoscelides obtectus (Coleoptera: Chrisomelidae: Bruchidae) of an emerging grape pest, *Xylotrechus arvicola* (Coleoptera: Cerambycidae) is a pest that attacks common bean (*Phaseolus vulgaris*). The use of biological agents, e.g. *Trichoderma*, to control pests can minimize detrimental effects on beneficial organisms and the environment. A *tri5* gene from *T. arundinaceum* (a trichothecene producer) has been expressed in the wild isolate of *T. harzianum* CECT 2413 (a trichothecene non-producing strain), a potential biocontrol agent (BCA) of phytopathogens, to determine if trichodiene (a volatile sesquiterpene precursor) had ability to control different stages of insect pests. *Trichoderma harzianum* (T34) and its trichodiene producer transformant (T34-*tri5*.27) were used to determine, under laboratory conditions, their insecticidal activity against *A. obtectus* adults. The susceptibility of *A. obtectus* adults against *T. harzianum* (T34 and T34-*tri5*.27) was evaluated with a spray tower. One mL of spore suspension (1×10^7 spores mL^{-1}) of *Trichoderma* strains was applied directly to the insects. Mortality monitoring was carried out every 2 d after treatment for 14 d. T34 accumulated a mortality of 59%, whereas T34-*tri*5.27 reached 66%. The effects of both fungi were different ($P < 0.05$) to the control treatment. The ability of *T. harzianum* to control *A. obtectus* adults was increased by the production of trichodiene. Production of non-toxic volatile terpenes by BCAs could be an effective tool to ameliorate their potential to control insect pests, and hence make them more appealing than conventional insecticides.

Evaluation of five essential oils from Mediterranean aromatic plants for use as bio-fungicides in the control of *Cladobotryum mycophilum* in cultivated button mushroom. F.J. GEA[1], M.J. NAVARRO[1], M. SANTOS[2], F. DIÁNEZ[2], G. ORTÍZ-DE-ELGUEA[3,4], R. SANCHEZ[4,5], D. HERRAIZ[4]. *[1]Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintanar del Rey, Cuenca, Spain. [2]Departamento de Agronomía, Escuela Politécnica Superior, Universidad de Almería, Almería, Spain. [3]Departamento de Ciencia y Tecnología Agroforestal y Genética, ETSIAM-IDR (UCLM), Albacete, Spain. [4]Centro de Investigación Agroforestal de Albaladejito (CIAF), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Cuenca, Spain. [5]Parque Científico-Tecnológico de Castilla-La Mancha, Abacete, Spain. E-mail: fjgea.cies@dipucuenca.es*

Cobweb, caused by the mycoparasite *Cladobotryum mycophilum*, is one of the most serious diseases that affect cultures of white button mushroom (*Agaricus bisporus*) worlwide. Effective control of this disease includes the application of fungicides (metrafenone or prochloraz) and strict higiene measures. However, biological control plays an important role. The essential oils (EOs) from five typically Mediterranean aromatic plant species belonging to the Labiatae family (*Lavandula x intermedia*, *Thymus mastichina*, *Thymus vulgaris*, *Salvia lavandulifolia* and *Satureja montana*) were tested against this pathogen. The plant material was cultivated in the experimental fields of CIAF Albaladejito – IRIAF. Essential oils were obtained by hydrodistillation in a Clevenger-type apparatus, and chemically characterized by Gas Chromatography (GC-FID). Essential oils were assayed *in vitro* using a macrodilution test for antifungal activity against six isolates of *C. mycophilum*. The sensitivity of *C. mycophilum* was estimated from EC_{50} values (mg L^{-1} of EO inhibiting radial mycelial growth by 50%). The most effective EOs for inhibiting *in vitro* growth of *C. mycophilum* were *T. vulgaris* (mean EC_{50} = 3.9 - 41.7 mg L^{-1}) and *S. montana* (EC_{50} = 9.4 - 27.8 mg L^{-1}). The main compounds in *T. vulgaris* EO were p-cymene (29.7%) and thymol (25.8%), while in *S. montana* EO carvacrol (17.2%), p-cymene (11.5%) and camphor (10.1%) predominated. *Thymus vulgaris* and *S. montana* EOs and their compounds, especially thymol and carvacrol, have potential alternatives to the synthetic fungicides that are applied in mushroom cultivation to prevent cobweb disease.

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Assessment of specific traits of *Pseudomonas fluorescens* PICF7 for involvement in endophytic lifestyle, rhizosphere survival and biocontrol of Verticillium wilt of olive. N. MONTES-OSUNA, J. MERCADO-BLANCO. *Department of Crop Protection, Institute for Sustainable Agriculture (CSIC), Avenida Menéndez Pidal s/n, Campus "Alameda del Obispo", 14004 Córdoba, Spain. E-mail: nuriamontes@ias.csic.es*

Pseudomonas fluorescens PICF7 is a natural colonizer of olive rhizospheres, able to endophytically colonize the root tissues and act as an effective biocon-

trol agent against Verticillium wilt of olive. This disease is difficult to manage, and single control measures are mostly ineffective. An integrated management strategy is therefore recommended. Within this framework, biocontrol approaches represent an excellent option, particularly if they are combined with other disease management tools. We aim to identify and characterize genes of strain PICF7 implicated in phenotypes such rhizosphere/soil persistence (copper resistance, 1-aminocyclopropane-1-carboxylate deaminase activity, ACC), root colonization (biofilm formation) and plant growth promotion (phytase activity). Previously, presence of a putative ACC gene (involved in degradation of the ethylene precursor) was suggested in the genome of PICF7. However, ACC deaminase activity was not demonstrated in this strain, whereas the presence of a putative D-cysteine desulfhydrase coding gene was found. Approx. 4,000 tetracycline-resistant colonies from an available Tn5 random insertion mutant bank were screened for phenotypes defective in some of the traits mentioned above. A collection of 80 mutants was selected, including 34 showing reduced (or no growth) or colour change in medium supplemented with copper, ten with impaired biofilm formation, 18 unable to grow or with altered morphology in YEM medium, and 18 displaying reduced or no production of phytase. Molecular characterization of these mutants is currently being performed, to identify the affected genes and determine their involvement in (endophytic) colonization, biocontrol performance, and rhizosphere survival of strain PICF7.

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Using endophytic bacteria to improve tomato growth and control bacterial spot caused by *Xanthomonas euvesicatoria*. A. AKKÖPRÜ. *Yüzüncü Yıl University, Faculty of Agriculture Deptartment of Plant Protection, Van, Turkey. E-mail: ahmetakkopru@yyu.edu.tr*

This study evaluates effects of endophytic bacteria (EB) isolates (*Ochrobactrum* sp. CB36/*1*, *Pantoea agglomerans* CC37/*2*, *Bacillus thuringiensis* CA41/*1*, *Pseudomonas fluorescens* CC44) on bacterial spot disease of tomato caused by *Xanthomonas euvesicatoria* (*Xe*), and on tomato (cv. Marmande) growth parameters. The EB strains were applied twice to seedlings as drenches at the second leaf stage, and 4 d before inoculation of *Xe*. Suspensions of *Xe* (10^8 cfu mL^{-1}) were applied by spraying on 4-week-old seedlings. Disease severity was evaluated using a 0-4 scale, 3 weeks after *Xe* inoculation. The seedlings were grown in peat medium at 24(±2)°C, 60% humidity, 14/10h day/night photoperiods in a climate chamber. Although the EB strains did not significantly affect plant length, all the strains increased fresh and dry weights of shoots by approx. of 6.5%. Root fresh weights were increased by 9.5% to 52% by the isolates, and *Bacillus thuringiensis* CA41/1 was the most effective isolate. Root dry weights were increased by 31% by the *Pantoea agglomerans* CC37/*2*, even under the disease pressure. Although all EB isolates except *B. thuringiensis* CA41/*1* decreased the disease severity compared with positive controls, the most effective isolate was *Ochrobactrum* sp. CB36/1 which decreased severity by 32%.

Assessment of water extracts and essential oils from Mediterranean plants against *Verticillium dahliae* in olive. A. MULERO-APARICIO, A. VARO, M. ADEM, L.F. ROCA, M.C. RAYA-ORTEGA, F.J. LÓPEZ-ESCUDERO, A. TRAPERO. *Departamento de Agronomía (Patología Agroforestal), Universidad de Córdoba, Campus Universitario de Rabanales, Edificio Celestino Mutis (C4), 14071 Córdoba, Spain. E-mail: z32muapa@uco.es*

Verticillium wilt of olive is considered the most concerning disease in all olive growing areas. In recent years, biological control within integrated disease management has gained importance, due to the lack of an effective treatment against this disease. There are several plant substances, including water extracts, essential oils or biological products, that possess antifungal activity, are easily degraded, and are safe for human use and the environment. This study describes the potential effect of 44 plant extracts and 20 essential oils against *Verticillium dahliae*. The results demonstrate the *in vitro* and *in planta* effectiveness of essential oil from Thymus, in particular *Thymus* sp. 04 (prepared in the laboratory) and the commercial product *Thymus* sp. 01, against *V. dahliae*. Inhibition of mycelial growth and microsclerotia reached 100% for both treatments.Disease re-

duction in olive plants reached 65% for Thymus sp. 04 and 42% for Thymus sp. 01. These treatments showed the potential for essential oils use in the control of this pathogenas part of an integrated disease management strategy. This is the first report of the use of essential oils for control of Verticillium wilt in olive plants. Further studies are warranted to identify the bioactive compounds in the essential oils that are active against *V. dahliae*, and evaluate their potential use as natural fungicides.

Effectiveness of a non-pathogenic strain of *Fusarium oxysporum* (FO12) against *Verticillium dahliae*.
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The strain FO12 of *Fusarium oxysporum*, isolated from cork, was one of the most effective treatments against *Verticillium dahliae*, from more than 200 natural products evaluated in previous studies. The present study evaluated potential of FO12 as biocontrol agent (BCA) against Verticillium wilt of olive (VWO). *In vitro* and *in vivo* studies were carried out to determine the mode of action of FO12. These included: dual cultures and mycelial growth of *V. dahliae* in potato dextrose agar amended with serial amounts of sterilized crude FO12 extract; efficacy of crude extract, conidia, supernatant and chlamydospores against VWO in controlled conditions and on inoculum reduction of *V. dahliae* in naturally infested soils; and possible induction of resistance in olive plants through foliar or irrigation treatments in controlled conditions. In addition, FO12 was evaluated under partially controlled and field conditions with irrigation treatments. Antibiosis and parasitism mechanisms were not observed in dual culture essays, but mycelial growth of *V. dahliae* was reduced in the presence of FO12. The treatments with the crude extract and chlamydospores were effective both for controlling VWO and reducing *V. dahliae* inoculum in naturally infested soil under *in vitro*, partially controlled and field conditions. FO12 can easily produce chlamydospores, favouring a durable and stable product formulation, for assessment of potential of FO12 as BCA against VWO in field conditions.

Biological control of quarantine bacterial diseases with selected strains of *Bacillus amyloliquefaciens*.
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Emerging quarantine bacterial plant diseases are important problems in crop production in the European Union. Some of the more relevant are caused by *Erwinia amylovora*, *Pseudomonas syringae* pv. actinidiae, *Xanthomonas arboricola* pv. pruni and *Xanthomonas fragariae*. There are considerable difficulties in control of fruit production losses and preventing the spread of these pathogens. Chemical control in EU countries is limited to copper compounds, because antibiotics are not authorized. Therefore, there is urgent need for alternative or complementary control methods. An extensive collection of strains of *Bacillus subtilis* and *B. amyloliquefaciens*, obtained from Mediterranean crops and forest, that have been screened for the control of these bacterial diseases. The first step was to build a collection of strains having the biosynthetic genes related with to production of antimicrobial compounds, the actual production of these compounds, and the range of antagonistic *in vitro* activity against these plant pathogenic bacteria. The best strains were tested for control of infections in the corresponding plant hosts (pear, peach, strawberry, kiwifruit), under controlled environment conditions in a greeenhouse, and under semi-field assays. Promising results were obtained in the efficacy of control in comparison with commercial biological products.

Study of diversity of endophytic communities from *Posidonia oceanica* and their *in vitro* antagonistic activities against *Pythium aphanidermatum*.
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Most of the Mediterranean sublittoral area occupied by seagrasses is dominated by the endemic plant *Posi-*

donia oceanica. Despite its ubiquity and dominance in the Mediterranean Sea, the mycoflora of *P. oceanica* has been rarely studied. We isolated and identified fungal endophytes in the roots of this plant. In addition, we selected fungal strains able to produce antagonistic substances *in vitro* against *Pythium aphanidermatum*. In summer 2016 at three sampling sites were investigated along Parque Natural de Cabo de Gata-Níjar, Almería (Spain). Roots of *P. oceanica* were collected using scuba diving. Roots of 45 plants were carefully excavated from the substrate, separated from the shoots, and placed in 100 mL capacity beakers filled with seawater. These were stored in the dark at 4°C. The rhizomes were carefully washed in tapwater. Roots were surface-sterilised by sequential washing in 5% NaOCl for 5 min, 95% EtOH for 1 min and 5% H_2O_2 for 3 min, and then rinsed three times in distilled sterile water. Root fragments were aseptically excised and placed on malt-extract agar (MEA, Oxoid) in Petri dishes (10-cm diam.) for preliminary morphological identification on the basis of macroscopic and microscopic characters. Fifteen fungal taxa (mainly Ascomycota, and in the *Pleosporales*), were identified. Antagonistic capacity of *Gliomastix* sp., *Papulaspora* sp. *Cladosporium sphaerospermum* had greater higher antagonistic activity against *P. aphanidermatum* than the other isolated fungi.

Antifungal activity of plant essential oils against *Trichoderma aggressivum* f. sp. *europaeum*. F. DIÁNEZ[1], C. PARRA[1], M.J. NAVARRO[2], F.J. GEA[2], M. SANTOS[1]. *Departamento de Agronomia. Universidad de Almeria. Carretera Sacramento s/n. Almería 04120. Spain.* [2]*Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintanar del Rey, Cuenca, Spain. E-mail: msantos@ual.es*

This study investigated the fungicidal activity against *Trichoderma aggressivum* f. sp. *europaeum* of essential oils (EOs) from: *Syzygium aromaticum*, *Pelargonium graveolens*, *Lavandula angustifolia*, *Cupresus sempervirens*, *Mentha piperita*, *Santolina chamaecyparissus*, *Citrus sinensis*, *Pogostemon patchouli*, *Thymus mastichina*, *Thymus vulgaris*, *Eucalyptus globulus* and *Rosmarinus officinalis*. The disc diffusion method was used to evaluate the inhibition of hyphal growth. Test discs were prepared with 8 µL of each oil at concentrations of 5, 10, 15, 20 and 30%, and control discs with 1% Tween-80. These discs (5 mm diam.) were arranged around the *T. aggtressivum* colony on each agar plate, at a distance of 4 cm, and incubated at 28°C for 1 week. The inhibition of hyphal growth was visually evaluated and photographed. EOs of rosemary (5%, growth inhibition >50%), mentha (15%, >70%), Patchouli and *Lavandula* (15%, >50%) inhibited hyphal growth of *T. aggressivum*. The other EOs did not reduce growth of hyphae at the concentrations tested. This study has indicated that rosemary, which is available and cost effective, is an attractive option for further investigations as an alternative to synthetic fungicides for the control of green mold caused by *T. aggtressivum*.

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Screening of potential biocontrol bacteria against *Pseudomonas savastanoi* pv. *Savastanoi*, and elucidation of their modes of action. D. MINA[1], J. PEREIRA[1], T. LINO-NETO[2], P. BAPTISTA[1]. [1]*CIMO / School of Agriculture, Polytechnic Institute of Bragança,* Campus de Santa Apolónia, 5300-253 Bragança, Portugal. [2]*BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre*, University of *Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: pbaptista@ipb.pt*

Over the last decades, the olive knot disease, caused by the bacterium *Pseudomonas savastanoi pv. savastanoi* (Psv), has been responsible for severe damage in olive orchards. Reduced vigour and stem dryness caused by the pathogen lead to decreased olive fruit production, and severe losses for farmers. Bacterial endophytes and epiphytes from olive tree phyllospheres were screened for the suppression of Psv. Several mechanisms for this activity were also studied by evaluating indoleacetic acid (IAA), siderophore and lytic enzyme production. Interspecific interactions were assessed on solid media with agar overlays. IAA was estimated spectrophotometrically, and siderophores and lytic enzymes were evaluated qualitatively. Several tested bacterial species reduced Psv growth by up to 70%, as well as its viability. The greatest inhibition was observed for *Frondihabitans* sp. and *Paenibacillus* sp. Reduced production of IAA and siderophores by Psv, which are associated with knot development, was detected in the presence of the most efficient bacteria. Produc-

tion of lytic enzymes by antagonists, such as lipase, chitinase, protease and amylase, was also identified. These results indicate that some of the bacteria tested have potential as biocontrol agents, due to their capacity to produce metabolites/lytic enzymes that can interfere with Psv growth and/or development of knots. These potential biological agents should be further evaluated under natural conditions.

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Molecular characterisation of *Pochonia chlamydosporia* isolates associated with root-knot nematodes. J. HORTA, I. ABRANTES, M.C. VIEIRA dos SANTOS. *Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Universidade de Coimbra, P-3000 456 Coimbra, Portugal. E-mail: mcvs@sapo.pt*

Root-knot nematodes (RKN; *Meloidogyne* spp.) are among the most economically damaging soil-dwelling parasites of agricultural crops. Exploitation of natural enemies of nematodes could lead to successful pest management strategies. *Pochonia chlamydosporia* is a widespread facultative parasite of nematode eggs that has been developed as a biocontrol agent. However, knowledge of the genetic diversity of naturally-occurring of *P. chlamydosporia* populations is still limited. This study identified and characterised Portuguese *P. chlamydosporia* isolates associated with RKN. Three tomato root samples infected with *Meloidogyne* spp. from three plots of a greenhouse in Setúbal, Portugal, were screened for the presence of *P. chlamydosporia*. Before screening, RKN females were identified by esterase phenotyping. Three phenotypes were detected: Hi4 (*M. hispanica*), I2 (*M. incognita*) and J3 (*M. javanica*). *Pochonia chlamydosporia* isolation was carried out by plating nematode eggs and roots on a semi-selective medium. Ten isolates were obtained and their identities confirmed by PCR using specific diagnostic primers derived from the β-tubulin gene. Intra-specific variation was evaluated by enterobacterial repetitive intergenic consensus (ERIC) PCR and restriction fragment-length polymorphism (RFLP) of the ITS region. A Portuguese isolate from *Globodera rostochiensis* eggs and two non-native isolates, Vc10 (IMI 331547) from Brazil and Pc3922 (IMI SD 187) from Cuba, both originally obtained from *M. incognita* eggs, were also analysed. Clustering analysis of ERIC-PCR profiles revealed similarities related to the geographic origin of the isolates, and there seems to be no relation between the clusters and the host nematode species.

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Assessment of specific traits of *Pseudomonas fluorescens* PICF7 for their involvement in endophytic lifestyle, rhizosphere survival and biocontrol of Verticillium wilt of olive. N. MONTES-OSUNA, J. MERCADO-BLANCO. *Department of Crop Protection, Institute for Sustainable Agriculture (CSIC), Avenida Menéndez Pidal s/n, Campus "Alameda del Obispo", 14004 Córdoba, Spain. E-mail: nuriamontes@ias.csic.es*

Pseudomonas fluorescens PICF7 is a natural colonizer of olive rhizospheres, able to endophytically colonize root tissues and act as an effective biocontrol agent against Verticillium wilt of olive. This disease is difficult to manage, and single control measures are mostly ineffective. An integrated management strategy is therefore recommended. Biocontrol approaches represent an excellent option, particularly if they are combined with other disease control methods. We identified and characterized genes of strain PICF7 implicated in phenotypes such as rhizosphere/soil persistence (copper resistance, 1-aminocyclopropane-1-carboxylate deaminase activity, ACC), root colonization (biofilm formation), and plant growth promotion (phytase activity). Presence, in the genome of PICF7, of a putative ACC gene (involved in degradation of the ethylene precursor), was previously suggested. However, ACC deaminase activity was not demonstrated in PICF7,

whereas a putative D-cysteine desulfhydrase coding gene was found. Approx. 4,000 tetracycline-resistant colonies from an available Tn5 random insertion mutant bank were screened to find phenotypes defective in some of the traits mentioned above. A collection of 80 mutants were selected, including 34 showing reduced (or no growth) or colour change in medium supplemented with copper, ten impaired in biofilm formation, 18 unable to grow or with altered morphology in YEM medium, and 18 displaying reduced or no production of phytase. The molecular characterization of these mutants is currently being performed to identify the affected genes and to determine their involvement in (endophytic) colonization, biocontrol performance, and rhizosphere survival of strain PICF7.

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Characteristics of the biocontrol rhizobacterium *Pseudomonas chlororaphis* PCL1606. S. TIENDA, C. VIDA, A. DE VICENTE , F.M. CAZORLA. *Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: sandratienda@uma.es*

The major disease affecting avocado crops in the Mediterranean area is white root rot, caused by *Rosellinia necatrix*. The biocontrol rhizobacterium *Pseudomonas chlororaphis* PCL1606 has been isolated from rhizospheres of healthy avocado trees, growing in an area affected by white root rot. As a main characteristic, PCL1606 showed strong *in vitro* antagonism against *R. necatrix* and other important soil-borne pathogens, mainly due to the production of the antimicrobial compound 2-hexyl, 5-propyl resorcinol (HPR). Production of other antifungal compounds has also been detected. PCL1606 persists and colonizes avocado roots, closely interacting and colonizing hyphae of *R. necatrix*, leading to negative effect on the fungus. These phenotypes, acting together, allowed PCL1606 to display biocontrol activity towards *R. necatrix* in avocado plants. We have observed that PCL1606 shows no plant growth promoting activities. The availability of the complete genome sequence of PCL1606 will allow identification of additional features involved in biocontrol by this bacterium.

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Characterization of new mycoviruses in *Fusarium oxysporum* f. sp. *dianthi*. A.T. TRENAS [1,2], M.C. CAÑIZARES[2], A. VALVERDE-CORREDOR[1], C.G. LEMUS-MINOR[1], M.D. GARCÍA-PEDRAJAS[2], E. PÉREZ-ARTÉS[1]. *[1]Depto. Protección de cultivos, Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas (IAS-CSIC), Alameda del Obispo s/n, 14004 Córdoba, España. [2]Depto. Protección de plantas, Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), 297550 Algarrobo-Costa, Málaga, España. E-mail: a.torres.trenas@csic.es.*

Mycoviruses that cause hypovirulence are potential biocontrol agents of their fungal hosts. In previous research, we characterized FodV1, a chryso-like mycovirus found in isolate Fod116 of *Fusarium oxysporum* f. sp. *dianthi* (Fod). The transference of FodV1 to a new Fod recipient isolate evidenced the induction of hypovirulence in the fungal host. We have analysed the prevalence of FodV1 as well as the incidence and diversity of mycoviral dsRNAs in a collection of 300 Fod isolates. RT-PCR using total RNA extracts and specific primers for the RdRp segment of FodV1, and subsequent sequence analysis, showed that mycovirus FodV1 was present in only three additional Fod isolates. Cellulose column chromatography analysis showed the presence of other dsRNA molecules in 40 isolates. These dsRNAs corresponded to at least five banding patterns, characteristic of different viral families, and three of them were selected for further characterization. Partial sequence data indicated that a monopartite 2.5 kb mycovirus corresponds to a mitovirus, and that a cuatripartite mycovirus shows high homology with Aspergillus foetidus dsRNA mycovirus, and probably corresponds to a new member of the family Alternaviridae. A third monopartite 9.5 kb mycovirus (FodV2) has been almost fully sequenced. This shows high homology with a number of previously described hypoviruses. To determine the putative hypovirulent nature of

FodV2, we transferred it by hyphal anastomosis to a new hygR-tagged recipient isolate, and analysed its effect on some hypovirulence-associated phenotypic traits. Results obtained indicated that FodV2 does not induce hypovirulence in its fungal host.

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Role of the gluconic acid production by the rhizobacterium *Rahnella aquatilis* in pH regulation and biocontrol of the vascular wilt fungus *Fusarium oxysporum*. D. PALMIERI[1], F. DE CURTIS[1], D. VITULLO[1], A. DI PIETRO[2], G. LIMA[1], D. TURRÀ[2]. [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis snc - 86100 Campobasso, Italy.* [2]*Department of Genetics, University of Cordoba, Campus Rabanales, Ed. Gregor Mendel - 14071 Cordoba, Spain. E-mail: davide.palmieri@studenti.unimol.it*

pH affects all aspects of life. Microbes have evolved efficient mechanisms of ambient pH adaptation and modification. In plant rhizospheres, secretions from roots promote the proliferation of microbes, which can alter the pH of this ecological niche. Previous research revealed that rhizosphere pH acts a key factor during infection of the vascular wilt fungus *F. oxysporum* f. sp. *lycopersici* (*Fol*) on its host plant tomato (*Solanum lycopersicum*). While non-infected roots acidify the extracellular environment, infection by *Fol* results in marked root alkalinization, which promotes fungal pathogenicity. We studied the role of pH modification by the soil-inhabiting Gram-negative bacterium *Rahnella aquatilis* (*Ra*) in its interaction with *Fol* in the tomato rhizosphere. Co-inoculation of tomato roots with *Ra* provided efficient protection from vascular wilt caused by *Fol*. *Ra* produced strong extracellular acidification, both in artificial media and in the tomato rhizosphere, most likely through production of gluconic acid from glucose through the enzyme glucose dehydrogenase (Gcd). Preventing rhizosphere acidification by *Ra*, either through application of a buffer solution or by targeted deletion of the bacterial Gcd gene, led to loss of the biocontrol activity against *Fol*. These results suggest that extracellular pH regulation plays a key role in the interaction between bacteria and fungi in the rhizosphere, with important consequences for plant health.

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Effects of farnesol production by *Trichoderma* on the development of bean (*Phaseolus vulgaris*). S. MAYO[1], A. RODRÍGUEZ-GONZÁLEZ[1], O. GONZÁLEZ-LÓPEZ[1], A. LORENZANA[1], G. CARRO-HUERGA[1], M.P. CAMPELO[1], S. GUTIÉRREZ[2], P.A. CASQUERO[1]. [1]*Research Group of Engineering and Sustainable Agriculture, Natural Resources Institute, University of León, Av. Portugal 41, 24071 León, Spain.* [2]*Area of Microbiology, Research Group of Engineering and Sustainable Agriculture, University School of Agricultural Engineers, University of León, Ponferrada Campus, Av. Astorga s/n, 24401 Ponferrada, Spain. E-mail: pacasl@unileon.es*

Common bean (*Phaseolus vulgaris*) is the third most important food legume worldwide, surpassed only by soybean and peanut. *Trichoderma* (Teleomorph: *Hypocrea*) is a fungal genus found in the soil. These fungi are secondary, fast growing, opportunistic invasive organisms, which produce enzymes that degrade fungal cell walls, and induce production of compounds with antimicrobial activity. We evaluated the effect of farnesol production of *T. harzianum* (T34) on the development of bean. *In vivo* assays were performed with this isolate and two transformants (T34dpp1.2 and T34dpp1.3) which were overexpressing the *dpp1* gene. Bean seeds were coated with a spore suspension of each *Trichoderma* isolate. They were sown and maintained with a photoperiod of 16 h light, 25°C/16 °C (day/night), and 60% RH. Plants were removed 45 d after sowing, evaluated for: hypocotyl diameter, , root system length,and dry weights of shoots and roots. T34dpp1.3 and control plants (without fungi) were larger than plants inoculated plant with T34, in hypocotlyl diameter, root system length, and shoot dry weight. However, T34 did not present differences in comparison with T34dpp1.3 for root system dry weight root system, but T34dpp1.2 did.

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Biological control of *Pseudomonas savastanoi* pv. *savastanoi* by two bacteria isolated from olive tree phyllospheres. D. MINA[1], A. SANTOS[1], J. PEREIRA[1], T. LINO-NETO[2], P. BAPTISTA[1]. [1]*CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.* [2]*BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: pbaptista@ipb.pt*

Olive knot disease, caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Psv), has been responsible for severe crop losses in olive orchards, especially in Mediterranean countries. Olive knot cannot be eradicated once it is established in an orchard, so control is based on preventative measures. Previous laboratory experiments showed the capacity of some bacterial species, isolated from olive tree phyllospheres, to inhibited Psv growth. The two most promising bacterial isolates (*Frondihabitans* sp. and *Paenibacillus* sp.) were evaluated for the control of Psv in olive plantlets (*Olea europaea*) under greenhouse conditions, to predict their effects in natural conditions. In pot experiments, 2-year-old olive plants (cv. Cobrançosa) were inoculated with the antagonistic bacteria and Psv, individually or in combination. Inoculations were performed in wounds previously made in three different sites of the main stem of each plant. Thirty replicate plants were used per strain. The plants were observed for symptom development and the number of bacteria on the inoculation sites was periodically evaluated, for up to 120 d after inoculation. To quantify the reduction of symptom expression, knots were excised from stems and their weights were compared between treatments. Inoculation with Psv resulted in the formation of knots with greater weights compared to plants inoculated simultaneously with Psv and antagonistic bacteria. Both tested bacteria also reduced the amount of Psv in the inoculation sites, suggesting their effectiveness for reducing multiplication of the pathogen. Data presented demonstrate, for the first time, this bacterial potential in supressing olive knot, and these two species should be considered in the future as potential biocontrol agents against *Psv.*

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Antimicrobial activity of natural plant compounds against phytopathogenic bacteria and interference with *quorum sensing*. A. CARUSO[1], A. ANZALONE[1], L. GURRIERI[1], S. PROVENZANO[1], P. BELLA [2], R. PALMERI[1], V. CATARA [1], G. LICCIARDELLO[1]. [1]*Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy.* [2]*Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze Ed. 4, 90128 Palermo, Italy. E-mail: gralicci@unict.it*

Natural plant products have received a great deal of attention as sustainable alternatives for management of plant diseases caused by bacteria. We evaluated the antimicrobial activity of citrus peel components and phenols with relevant antioxidant activity (cathecol, citronellol, esperidin, limonene, quercitin and rutin) against nine phytopathogenic bacteria in the genera *Clavibacter*, *Erwinia, Pectobacterium*, *Pseudomonas* and *Xanthomonas*. The greatest inhibitory activity was induced by cathecol against *Xanthomonas* species and *P. syringae* pv. *Tomato*, and by citronellol against *C. michiganensis* subsp. *michiganensis* and *E. amylovora*. Cathecol minimum inhibitory concentrations ranged from 0.5 to 0.0625 mg mL^{-1} , and those for citronellol were 1 to 0.125 mg mL^{-1}. In addition, the ability to inhibit the quorum sensing (QS) cell-to-cell signaling system, which controls the virulence behaviour of a broad spectrum of bacterial pathogens, was evaluated. Using *Chromobacterium violaceum* as a biosensor system, citronellol was active against medium chain N-acyl-homoserine lactones preventing the production of violacein, as indicated by the lack of pigmentation of the indicator organism in vicinity of the treated disks. To determine if this suppression was linked to anti-virulence activity, the effect of citronellol was tested in the QS active phytopathogen *Pseudomonas corrugata* strain CFBP 5454, causal agent of tomato pith necrosis, in which the PcoIR AHL- based signaling system regulates production of phytotoxic cyclic lipopeptides (CLPs). Consistently with QSI activity, the relative expression of genes contributing to the production of the CLPs cormycin and corpeptins was reduced in a concentration-dependent manner

in response to non-lethal concentrations of citronellol.

Innovative approaches in plant disease diagnosis and management

Establishment of specific molecular diagnostic tests for *Gnomoniopsis smithogilvyi* (syn. *castanea*) and *Cryphonectria parasitica*. M. CONTI[1], J. CROVADORE[1], B. COCHARD[1], R. CHABLAIS[1], J.B. MEYER[2], M. JERMINI[3], F. LEFORT[1]. [1]*Plants and Pathogens Group, Institute Land Nature Environment, hepia, University of Applied Sciences and Arts Western Switzerland (HES-SO), 150 route de Presinge, 1254 Jussy, Switzerland.* [2]*Unit Biodiversity and Conservation Biology, Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birsmendorf, Switzerland.* [3]*Agroscope, Cadenazzo Research Centre, A Ramél 18, 6593 Cadenazzo, Switzerland. E-mail: francois.lefort@hesge.ch*

Two fungi cause chestnut tree diseases in Switzerland: *Cryphonectria parasitica*, the endemic chestnut canker agent, and *Gnomoniopsis smithogilvyi*, an endophytic fungus, recently identified in Europe and Switzerland as the main agent of chestnut fruit brown rot, also causing chestnut canker. *Gnomoniopsis smithogilvyi* causes high plant mortality in young chestnut nurseries and orchards. Presence of these fungi was evaluated in plant material used for the multiplication of six of chestnut varieties in Ticino, using specific molecular diagnostic tests developed for both species. All sequences available in GenBank for the internal transcript spacer (ITS) of the ribosomal DNA, the elongation factor 1-alpha (EF1a) gene and the beta-tubulin gene (TUBB), were collected for these two fungi. Significant differences between *G. smithogilvyi*, *Gnomoniopsis spp.* and *C. parasitica* were sought. After analysing 164 ITS, 90 EF1a and 45 TUBB sequences, only the TUBB gene sequences showed any significant differences between the species. Specific PCR primers for each species were then designed from the TUBB sequences alignment. *In silico* analyses with BLAST (GenBank) confirmed the strict specificity of these primers. The two primer pairs were then tested with DNA extracted from previously characterised isolates of *G. smithogilvyi* and *C. parasitica* from Ticino, Wallis and Geneva, from roots and stems of germinated chestnuts or leaves of chestnut trees. These tests showed great robustness, and provide a tool to indicate the phytosanitary status of propagation material, especially for the endophyte *G. smithogilvyi*.

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Does resistance to *Plasmopara viticola* in grapevine influence infectivity of sporangia? F. BOVE, T. CAFFI, V. ROSSI. *Department of Sustainable Crop Production, Diproves, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy. E-mail: federica.bove@unicatt.it*

Partial plant resistance impacts on different epidemiological components of pathogens, which modify dynamics of disease epidemics. In *Plasmopara viticola*, the causal agent of grapevine downy mildew, different morphological characteristics have been observed between sporangia originated from lesions on susceptible and resistant hosts. This study evaluated whether, in addition to morphological modifications, partial host resistance can affect the infectivity of *P. viticola* sporangia, i.e., their ability to cause infection. Artificial inoculation experiments were performed between 2014 and 2016. A population of *P. viticola* sampled from susceptible vineyards was used for artificial inoculations on leaf discs of cv. Merlot and of fifteen grape breeding lines showing partial resistance, conferred by one or more *Rpv loci*. The sporangia produced on lesions originating on the susceptible and resistant varieties were then re-inoculated on leaf discs of cv. Merlot at three different vine growth stages (shoot elongation, full flowering, ripening of berries), and the infection efficiency was evaluated as the proportion of inoculation sites showing disease symptoms. There were no significant differences for the infection efficiency of sporangia produced on the different host varieties.

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Development of DDct Real Time RT-qPCR for the detection of *Onion yellow dwarf virus*. A. TIBER-

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Onion yellow dwarf virus (OYDV, genus *Potyvirus*), an aphid stylet-borne virus, was identified in Italy in 1993, and in the Italian onion variety 'Rossa di Tropea' in 2005. First investigations for OYDV were performed using serology, whereas, more recently, a specific RT-PCR test was used to examine the incidence of the virus in 'Rossa di Tropea', in bulb and seed production cycles. The correlation was assessed between OYDV infection and nutraceutical compounds in 'Rossa di Tropea', and a specific Real Time RT-qPCR assay was developed for OYDV. Specificity has been evaluated by including no target viruses related to OYDV and/or viruses generally found in onion. Analytical sensitivity was determined using ten-fold dilution series in crude extracts, either from leaf or bulb samples derived from field trials and from surveys carried out in Calabria (Southern Italy). The analytical sensitivity was directly compared with ELISA and end point RT-PCR, and allowed detection of the virus up to the dilution limit of 1×10^{-6} for leaves and 1×10^{-5} for bulbs. A DDCt Real Time RT-qPCR assay was performed using the 5.8S rDNA gene as reference to normalize the relative quantification data. This assay allowed investigation of the modulation of virus titre in the OYDV - 'Rossa di Tropea' pathosystem.

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Cytogenomic analyses reveal nuclear content variation along the life cycles of the Pucciniales (rust fungi). T. RIBEIRO[1], C. FEITEIRA[1], S. TAVARES[1,2,3], A.P. RAMOS[1], M. MONTEIRO[4], M COELHO[5], M.C. SILVA[1,2], J. LOUREIRO[6], L. MORAIS-CECÍLIO and P. TALHINHAS[1,2]. [1]*LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [2]*Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa. Quinta do Marquês, 2780-505 Oeiras, Portugal.* [3]*Section for Plant and Soil Science, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Frederiksberg Copenhagen, Denmark.* [4]*Instituto Gulbenkian de Ciência. R. Quinta Grande, 6. 2780-156 Oeiras, Portugal.* [5]*CREM-Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa. Quinta da Torre, Campus Universitário, 2829-516 Caparica, Portugal.* [6]*CFE, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra. 3001-401 Coimbra, Portugal. E-mail: ptalhinhas@isa.ulisboa.pt*

Rust fungi (Basidiomycota, Pucciniales) are biotrophic plant pathogens with complex life cycles (up to five spore types). The urediniosporic infection cycle is frequently the most important for pathogen dissemination, as the only stage capable of multiple uninterrupted repetition. The cell nuclear content of rust fungi is thought to follow that of other Basidiomycota, with haploid nuclei throughout the life cycle, only becoming diploid upon karyogamy in telia and immediately returning to the haploid state as meiosis takes place leading to the formation of basidiospores. The presence of 1C, 2C and a low proportion of 4C nuclei was recently detected in different stages of the urediniosporic cycle of several rust fungi, using genome size quantification techniques,. These results suggest the presence of diploid nuclei that supposedly only occur in teliospores, compatible with the occurrence of karyogamy and meiosis prior to urediniospore formation, although endopolyplody or other parasexuality phenomena cannot be ruled out. This unexpected phenomenon may be transversal to the Pucciniales, since it has been detected in over 60 rust species, with no apparent phylogenetic structural forms.

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A diagnostic microarray for the multiplex characterization of strains of the *Ralstonia solanacearum* species complex. G. CELLIER[1], S. ARRIBAT[2], F. CHIROLEU[2], P. PRIOR[3],, I. ROBENE[2]. [1]*Tropical Pests and Diseases unit, Plant Health Laboratory, ANSES,*

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Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (Rssc), is one of the most destructive plant diseases worldwide. Rssc affects a wide host range, and includes several ecotypes that represent major constraints and are under strict regulation (e.g. brown rot or Moko strains). The reliable characterization of epidemiological strains at the ecotype level is a challenge because of this complexity, and is generally achieved by combining several diagnostic protocols. We used microarray technology (ArrayTube) to develop a standard protocol that performs a multiplex characterization of RSSC strains in a single reaction, from the phylotype to the ecotype level (17 targeted groups of interest). Based on 27 sequenced genomes of RSSC, probes were designed with a 50-mer length constraint and thoroughly evaluated for any flaws or secondary structures. Validation data performed on 75 target and 12 non-target strains showed strong intra- and inter-repeatability, reproducibility, and good specificity, which allowed for the accurate detection of the 17 groups of interest. This custom microarray represents a significant improvement in the epidemiological monitoring of Rssc strains worldwide, and it has the potential to provide insights for phylogenetic incongruence of Rssc strains, based on the host of isolation. The microarray may be used to indicate potentially emergent strains.

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Selection of genetic variants of *Citrus tristeza virus* as a strategy to protect against severe seedling yellows strains. G. SCUDERI[1,2], R. FERRARO[2], M. RUSSO[1,2], M. C. BAZZANO[1], A. CATARA[2], G. LICCIARDELLO[1,2,3]. *[1]Agrobiotech Z.I. Blocco Palma I, Str. le V. Lancia 57- 95121 Catania Italy. [2]Parco Scientifico e Tecnologico della Sicilia ZI. Blocco Palma I, Str.le V. Lancia 57- 95121 Catania Italy. [3]Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy. E-mail: gralicci@unict.it*

Citrus tristeza virus (CTV) is a phenotypically complex virus causing severe economic losses to citrus industries worldwide. In Sicily (Italy) tristeza disease affects more than 5 million trees with devastating effects in some areas and mild symptoms in others, despite the same scion/stock combinations being grown. To investigate either the presence of different CTV strains or a natural cross protection phenomenon, a genetic assessment of the virus population structure has been carried out through an In-Check platform based on Lab-on-chip technology. This has revealed a prevalent diffusion of VT-like genotypes. Two genotypes showed symptomless phenotypes, despite the VT genotype. Appropriate biological tests showed they reduced severe symptoms in pre-inoculated sour orange seedlings challenged with the aggressive CTV-VT isolate SG29 (KC748392) prevalent in Sicily. A study of genetic variants has been undertaken to find genetic differences of the virus (if any) which interfere with the VT aggressive genotype. Deep sequencing of the two potentially cross-protective VT strains revealed they are genetic variants of isolate SG29, which differ for few nucleotides. Comparative analyses have shown eight conserved non-silent mutations in comparison to the VT aggressive strain, including three in the p33 gene, described as involved in cross-protection by the superinfection exclusion mechanism. This technology opens new prospects in the strategy against seedling yellows CTV, and may be suitable for other pathogens.

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CRISPR-Cas for genome-editing of fungi of interest in agriculture. S. SARROCCO[1], J. VANG[2], I. VICENTE MUÑOZ[1], L. MALFATTI[1], M. LÜBECK[2], G. VANNACCI[1]. *[1]Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124, Pisa, Italy. [2]Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University of Copenhagen, A.C. Meyers Vænge 15, 2450, Copenhagen, Denmark. E-mail: giovanni.vannacci@unipi.it*

Genome editing of filamentous fungi using CRISPR-Cas9 technology has increased in recent years. There are few reports about CRISPR-engineered filamentous fungi related to biocontrol and crop disease. Our goal was to use this technique, as proof of concept of its feasibility, to edit the genome of a *Trichoderma afro-harzianum* and a *T. gamsii* isolate, well known as biocontrol and biostimulating fungi, as well as in a mycotoxigenic *Fusarium graminearum* isolate, the causal agent of Fusarium Head Blight (FHB). A gene encoding a polyketide-synthase, disruption of which can be easily detected phenotypically, was chosen as the target gene in all the three isolates, and used to design the RNA-guide to be included in the RGR-cassette. The cassette was then assembled in a Cas9 expressing plasmid. The resulting vector will be used for fungal transformation by protoplasts. Resulting mutants from all the three fungi will be phenotypically and molecularly analyzed, to verify the knockout of the selected gene. The presence of a shortened AMA1 sequence will allow rapid removal of the plasmid from the edited strains, simply by reducing the selection pressure. Edited strains will be checked for the presence of foreign DNA, to contribute to the debate about the inclusion of this type of genetically manipulated microorganisms within GMOs. The ability to manipulate, beneficial and plant pathogenic isolates at a genetic level with these techniques represents a tool to increase knowledge of how these fungi interact with their hosts.

Spore trapping and quantitative PCR for monitoring airborne inoculum of *Mycosphaerella nawae* in persimmon. M. BERBEGAL[1], J.L. MIRA[2], J. ARMENGOL[1], A. VICENT[2]. [1]*Instituto Agroforestal Mediterráneo, Universitat Politècnica de València. 46022, Valencia, Spain.* [2]*Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA). Moncada 46113, Valencia, Spain. E-mail: mobermar@etsia.upv.es*

Circular leaf spot of persimmon, caused by *Mycosphaerella nawae*, includes symptoms of necrotic leaf lesions, defoliation and fruit drop. The disease is widespread in humid regions in Japan and South Korea, and, more recently, also in Mediterranean areas in Spain. The pathogen reproduces in leaf litter through ascospores formed in pseudothecia. Fungicide sprays are scheduled based on ascospore monitoring to define the periods of inoculum availability. Airborne ascospores of *M. nawae* are routinely quantified by counting using microscopy. This technique is time-consuming, especially for field sampling for rapid decision making. Monitoring airborne inoculum using spore traps combined with real-time PCR assays for quantification can be rapid, specific, reproducible and reliable. A real-time PCR assay for *M. nawae* quantification (qPCR) was designed and evaluated under laboratory conditions. To validate the technique under field conditions, two Burkard volumetric spore traps were deployed in a 100 m^2 plot. Soil was covered with persimmon leaf litter severely affected by *M. nawae*, and overhead sprinkle irrigation was used to enhance ascospore release. The spore traps were operated during May to July in 2016. Tapes from both spore traps were changed weekly, one was used for microscope counting and the other for qPCR analyses. Ascospore counts were correlated against DNA concentration of *M. nawae* based on Ct qPCR values. Results indicate that monitoring of *M. nawae* ascospores by qPCR may be a more efficient alternative to conventional inoculum counting, based on microscope examination.

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Root colonization of host (*Cucumis sativus*) and non-host (*Solanum lycopersicum*) species by a DsRed-fluorescent strain of the specific pathogen *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. M. DE CARA-GARCÍA[1], C. LECOMTE[2], M. FERNÁNDEZ-PLAZA[1], L. MUELA-JORDÁN[1], A. BOIX-RUIZ[3], C. STEINBERG[2]. [1]*IFAPA Centro La Mojonera, Camino de San Nicolás, 1, 04745, La Mojonera, Spain.* [2]*I.N.R.A. UMR Agroécologie, Rue Sully, 17, 21065, Dijon, France.* [3]*University of Almería. Dept. Agronomía, Ctra. Sacramento s/n., 04120, Almería, Spain. E-mail: franciscom.cara@juntadeandalucia.es*

A monoconidial *Fusarium oxysporum* isolate (codified as *14/1Fo3*), originally collected from sporodochia of a diseased cucumber plant showing root and stem rot, was identified as *F. oxysporum* f. sp. *radicis-cucumerinum*. The isolate was transformed by *Agrobacterium tumefaciens*, by means of 'EHA 105-DsRed2' strain containing the binary vector pAN-DsRed2, carrying red fluorescent insert *DsRed2*, and the *hgh* gene.

One transformant (codified as *Forc3T1*) was selected for its fitness (growth rate, production of chlamydospores and macroconidia), root colonization ability, fluorescence intensity and sporodochium production. *Forc3T1* and *14/1Fo3* isolates were inoculated separately on 2-4 true-leaf cucumber 'Marketer' and tomato 'RAF' plants, by watering each pot with 10^8 microconidia suspended in water. Twenty-four days post inoculation (dpi), all cucumber plants showed rotten stems and roots, and most died, but no tomato plant was symptomatic (100% roots were healthy). Results were identical for both isolates, so tomato responded as a non-host for the transformant, whereas cucumber behaved as a host. In parallel, the root colonization strategy was studied with epifluorescence microscopy. Roots from tomato and cucumber were excised, washed and directly mounted under the microscope, from 1 to 16 dpi. At 3 dpi, appresoria were detected on epidermal cells and at 5 dpi intercellular hyphae were observed for both plant species. However, intracellular invasion of root cells was present on tomato (as early as 5 dpi), but not on cucumber (even at 16 dpi). Many macro- and micro-conidia were recovered from the supernatant obtained after root washing at 16 dpi for both host plant species.

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Rapid isothermal detection of *Grapevine red blotch-associated virus* through recombinase polymerase amplification. R. LI[1], M.F. FUCHS[2], K.L. PERRY[3], T. MEKURIA[4], S. ZHANG[1]. [1]*Agdia, Elkhart, IN, U.S.A.* [2]*Cornell University, Geneva, NY, U.S.A.* [3]*Cornell University, Ithaca, NY, U.S.A..* [4]*Vintage Nurseries, Wasco, CA, U.S.A. E-mail: rugang.li@agdia.com*

Grapevine red blotch-associated virus (GRBaV) is a newly identified DNA virus in the family *Geminiviridae* in North America. GRBaV infects red and white grapevine cultivars, and affects fruit quality by delaying fruit ripening and reducing sugar content at harvest. A rapid, sensitive, and user-friendly test is needed to quickly identify GRBaV-infected grapevines, and facilitate their timely removal from vineyards. An isothermal test (AmplifyRP Acceler8) was developed for GRBaV that can be used in laboratories and vineyards. The test consistently detects GRBaV up to a $1{:}10^8$ dilution of infected grapevine leaf crude extracts diluted in healthy grapevine leaf crude extracts, and up to 11 copies of GRBaV genomic DNA in a matrix of healthy grapevine leaf crude extract. The test has no cross reactivity to host plant tissues and grapevine-infecting pathogens, including *Arabis mosaic virus*, *Grapevine fanleaf virus*, *Grapevine leafroll-associated virus 1*, *Grapevine leafroll-associated virus 2*, *Grapevine leafroll-associated virus 3*, *Grapevine leafroll-associated virus 4* strain 5, *Grapevine fleck virus*, *Tomato ringspot virus*, *Tobacco ringspot virus*, *Xylella fastidiosa*, and *Botrytis cinerea*. The test has been validated using both viral DNA and crude plant extracts as templates.

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Detection of plant pathogenic bacteria by the LAMP based ICGENE mini system. G.R. QUINTERO MACÍAS[1], P. BELLA[2], V. CATARA[3], S. DRAGO[1]. [1]*Enbiotech S.r.l., Via Aquileia, 34. 90144 - Palermo, Italy.* [2]*Dipartimento di Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Ed. 4, 90128 - Palermo, Italy.* [3]*Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, Via S. Sofia 100, 95123 - Catania, Italy. E-mail: patriziabella@unipa.it*

The ICGENE mini system includes ready-to-use kits with reagents and a portable device to perform on-site analyses based on Loop mediated isothermal amplification (LAMP) technology in different fields of applications. This system utilizes rapid DNA extraction from a small quantity of sample, isothermal genetic amplification, detection of the fluorescence emitted from the sample and automatic interpretation of the final result using the instrument ICGENE mini. We developed a diagnostic kit for *Xylella fastidiosa* (Xylella Screen Glow EBT501) that was validated according to EPPO PM 7/98 and PM 7/84, and this is in use in many laboratories. We report the optimization of two additional protocols for the detection and identification of *Erwinia amylovora (Ea)*, which causes fire blight of Rosaceae, and *Xanthomonas campestris* pathovars (*Xc*) infecting cultivated Brassica crops. Both kits were able to identify target strains from different plant species and geographical origins with a sensitivity of approximately 10^2 cells for both bacterial species, and not react with

non-target strains. Spiked samples and naturally infected plants were tested with ICGENE mini, allowing completion of the test in less than 1 h. Diagnosis was also accomplished by isolation on culture media and/or PCR based techniques. Based on laboratory tests, LAMP with the ICGENE mini system could provide a rapid diagnostic presumptive test and direct bacterial colony identification.

Early detection of *Citrus tristeza closterovirus* using remote sensing. F. SANTORO, S. GUALANO, A.M. D'ONGHIA. *CIHEAM - Istituto Agronomico Mediterraneo di Bari, Via Ceglie 23, Valenzano (BA) 70010, Italy. E-mail: fsantoro@iamb.it*

The early detection of *Citrus tristeza virus* (CTV) is crucial for efficient large-scale virus monitoring and the rapid application of control measures. Remote sensing, supported by GIS and spatial analysis methods (automatic tree counting), was evaluated for the identification of CTV-suspected trees on a large scale. Preliminary trials were conducted in agreenhouse and in the field, collecting leaf spectral signatures of CTV-positive and negative plants grafted onto the susceptible rootstock. Spectral reflectance of CTV-positive plants was greater in the visible region and less in the near infrared region. Specific indices (NDVI, mYI, PSRI, NCI, MCARI) were selected for the implementation of a detection algorithm, which was developed for processing GeoEye-1satellite images. The output synthetic image with all combined indices was effective in discriminating CTV-infected and non-ninfected trees in the studied groves. The correlation of CTV infection to different canopy stresses was almost 100% in the severe declining trees, while it reached 75% in highly chlorotic trees. However, 52% of correlation was also reported in mild chlorotic or apparently asymptomatic trees. The developed algorithm was validated by processing a multispectral image from an apparently pathogen-free area. The prediction map obtained showed the suspected infected sites as coloured spots ranging from red (high probability to find CTV infection) to green (low probability to find CTV infection). Three red spots were highlighted in the prediction map, the assessment of which showed a new CTV focus, whereas *Phytophthora* disease was observed in the remaining red spots. The new finding of CTV in a free area revealed the potential of this approach for large scale virus monitoring.

The utility of mtDNA and rDNA for barcoding and phylogeny identification of plant-parasitic nematodes from Longidoridae (Nematoda, Enoplea). J.E. PALOMARES-RIUS[1], C. CANTALAPIEDRA-NAVARRETE[1], A. ARCHIDONA-YUSTE[1], S.A. SUBBOTIN[2,3], P. CASTILLO[1]. *[1]Instituto de Agricultura Sostenible (IAS), Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC), Avda. Menéndez Pidal s/n, 14004, Córdoba, Spain. [2]Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA. [3]Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow, 117071, Russia. E-mail: palomaresje@ias.csic.es*

Traditional identification of plant-parasitic nematode species by morphology and morphometric methods is difficult because of the high morphological variability which can lead to considerable overlapping of many characters and ambiguous nematode identification. It is essential to use several approaches to give accurate species identification (integrative taxonomy). DNA barcoding aids identification of species and advances species discovery. We have unravelled the use of the mitochondrial marker cytochrome c oxidase subunit 1 (*coxI*) for Longidoridae nematode species identification, as barcoding, for determining their molecular diversity and use as phylogenetic marker. Ribosomal markers (ITS region and the D2 and D3 expansion segments of the 28S rRNA gene) have also been explored. This provides molecular markers obtained using voucher specimens identified by integrative taxonomy. The results showed that mitochondrial and ribosomal markers could be used as barcoding markers using several barcoding approaches, with the exception of some species from the *X. americanum*-group. However, some species presented variability in *coxI* that need to be further studied. Analysis of the newly provided sequences, deposited in GenBank, showed some misidentifications, and the use of voucher species and topotype specimens is a priority for this group of nematodes. The use of *coxI* and the D2 and D3 expansion segments of the 28S rRNA gene did not clarify phylogenies at the generus level, but showed important accuracy at the species level.

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Integrative taxonomic approach and molecular phylogeny for identification of dagger and needle nematode species infesting grapevine soils in Portugal. C. GUTIÉRREZ-GUTIÉRREZ[1], M. TEIXEIRA SANTOS[2], M. MOTA[1,3]. *[1]NemaLab/ICAAM, Instituto de Ciências Agrárias e Ambientais Mediterrânicas & Dept. de Biologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal. [2]Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Quinta do Marquês, 2780-159 Oeiras, Portugal. [3]Dept. Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, C. Grande 376, 1749-024 Lisboa, Portugal. E-mail: carlosg@uevora.pt*

"Dagger" (*Xiphinema* spp.) and "needle" (*Longidorus* and *Paralongidorus* spp.) nematodes are economically important parasitic nematode groups in grapevine worldwide. They are polyphagous root ectoparasites causing severe damage to plants by their direct feeding, and in addition some species can transmit plant viruses. *Grapevine fanleaf virus* (*GFLV*) is transmitted by *Xiphinema index*, and is one of the most economically important viral diseases affecting grapevine in many Mediterranean growing regions. Nematode surveys have been conducted from 2015 to 2017 during spring and autumn seasons in the main Portuguese grapevine-growing areas. An integrative taxonomic approach, based on the combination of morphometric and morphological characterizations with molecular analysis using ribosomal DNA (rDNA) sequences from ITS regions and D2–D3 expansion segments of the 28S gene, were used for species delimitation and identification. High biodiversity of longidorid nematode species was found, greater in dagger than needle nematodes. *Xiphinema pachtaicum*, *X. santos* and *X. index* were the most frequently found dagger nematodes in Portuguese vineyards, while *L. vineacola* was the most common needle nematode. Severe nematode infestations were found in grapevine soils in the oldest vineyard regions, highlighting the importance *X. index*. Disease symptoms were observed on aboveground plant parts of the grapevines infected with *X. index*, and these included yellow mosaic pattern in leaves which are characteristic of infections by *GFLV*.

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Epidemiology and modeling

Modelling yield losses, caused by multiple wheat diseases in France. L. WILLOCQUET, S. SAVARY. *AGIR, INRA, Université de Toulouse, INPT, INP- EI PURPAN, Castanet-Tolosan, Centre Inra Occitanie-Toulouse, France. E-mail: laetitia.willocquet@inra.fr*

Yield loss quantification is critical to inform tactical and strategic decisions in plant disease management. Yield loss quantification and modelling entails analysis of relationships between disease intensity, and attainable and actual yields, of a crop grown in a given production situation. Yield losses caused by individual and combined wheat diseases were estimated using a process-based simulation model, WHEATPEST, together with a dataset from a network of experiments on winter wheat in France where disease intensity and actual yields were measured. The disease-free, attainable yield was not measured. The analysis focused on 70 combinations [year × Region × variety × crop management]. These considered five years (2003 to 2008), four French Regions, two varieties (one high yielding and one hardy variety), and two levels of crop management corresponding to two levels of chemical intensification. Simulated overall yield losses from combined diseases ranged from 0 to 4.2 t ha^{-1}, with a mean of 0.80 t ha^{-1} and a standard error of the mean of 0.10 t ha^{-1}. Variety and crop management had significant ($P < 0.05$) effects on yield loss caused by combined diseases. *Septoria tritici* blotch was associated with greatest simulated yield loss, followed by brown rust, Fusarium head blight, yellow rust and powdery mildew. This approach allows estimation of yield losses caused by individual and combined diseases, and can be ap-

plied to other spatial and temporal scales, as well as to other crops and diseases.

Identification of TYLCD-associated begomoviruses and ToLCNDV-ES co-infections in Spain. A. SIMON, L. RUIZ, C. GARCÍA, D. JANSSEN. *Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA), Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain. E-mail: dirk.janssen@juntadeandalucia.es*

In 2014, leaf samples from 50 tomato plants showing symptoms of tomato yellow leaf curl disease (TYLCD),) including leaf curling, chlorosis and vein thickening, were collected from natural infections in commercial greenhouses from southern Spain. None of the sampled tomato cultivars carried resistance genes against begomovirus. The Mld strain of *Tomato yellow leaf curl virus* (TYLCV-Mld, referred to as TYLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *Tomato yellow leaf curl Axarquía virus* (TYLCAxV), and the ES strain of *Tomato leaf curl New Delhi virus* (ToLCNDV-ES) were detected, using species-specific primers and conventional PCR. From the sampled tomato plants, 41% had mixed infections of ToLCNDV-ES and one or more TYLCVD-associated species. The most frequent combination of mixed begomovirus infections was ToLCNDV-ES+TYLCV+TYLCSV, although ToLCNDV-ES+TYLCV+TYLCSV+TYLCAxV was also identified in single tomato plants. Many of the mixed infection plants showed more severe symptoms than plants with single infections, expressed as green-bright yellow mosaic, vein thickening and leaf distortion. Despite of differences in degree of symptom expression, qPCR revealed that the titres of genome DNA-A and DNA-B from ToLCNDV-ES were similar in single and mixed infected tomatoes plants ($P > 0.05$). The infections of ToLCNDV-ES and TYLCV-complex begomoviruses were also independent (Fisher's test, $P > 0.05$). Nevertheless, the frequency of mixed infections of TYLCD-associated begomoviruses and ToLCNDV-ES in tomatoes from southern Spain could pose epidemiological risks, because of genome recombination events which are likely to occur between different begomovirus species.

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Virus diseases affecting chickpea crops in Uzbekistan. S.G. KUMARI[1], Z. ZIYAEV[2], S.D.A. KEMAL[3]. [1]*International Center for Agriculture Research in the Dry Areas (ICARDA), Terbol Station, Beqa'a Valley, Zahla, Lebanon.* [2]*Kashkadarya Scientific Research Institute of Grain Breeding and Seed Production, Beshkent 3km, Karshi, Uzbekistan.* [3]*ICARDA, Rabat, Morocco. E-mail: s.kumari@cgiar.org*

A field survey was conducted during the 2012 and 2013 cropping seasons to monitor occurrence of virus diseases affecting chickpea in the major production areas of Uzbekistan (Tashkent, Sirdarya, Jizzah, Samarkand and Surkhandarya regions). Surveyed fields were randomly selected and types of viruses and their incidence were determined based on symptoms observed. In addition, 15-20 symptomatic samples were collected from each field for laboratory testing. Chickpea samples with symptoms suggestive of virus infection (chlorosis, stunting, necrosis, yellowing, reddening, mosaic/mottling) were collected from 23 (386 samples) during 2012 and 19 fields (288 samples) during 2013. All samples collected were tested by tissue blot immunoassay (TBIA) using 12 specific polyclonal and monoclonal antibodies. Serological tests showed that *Faba bean necrotic yellows virus* (FBNYV) was the most common (detected in 20% of tested samples), followed by *Bean yellow mosaic virus* (BYMV) (6%), *Bean leafroll virus* (BLRV) (5%) and *Chickpea chlorotic stunt virus* (CpCSV) (3%). Molecular characterization (PCR and sequencing) indicated that the viruses which infect chickpea crops in Uzbekistan are BYMV, FBNYV, BLRV, CpCSV, *Beet western yellow virus* (BWYV), *Soybean dwarf virus* (SbDV) and *Cucurbit aphid-borne yellows virus* (CABYV). We conclude that a long term research plan is needed to manage the spread of virus diseases, and to minimize yield losses in areas where virus incidence is high and chickpea crops are important for small holder farmers.

This work was partially supported by CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS).

Ecological succession of pathogenic fungi of pines in Italy associated with climate change. L. GHELARDINI[1,2], P. CAPRETTI[1], L. BOTELLA[3], C. AGLIETTI[1], N. LUCHI[2]. [1]*Department of Agrifood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 28, I-50144, Firenze, Italy.* [2]*Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR), Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Firenze, Italy.* [3]*Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1, 61300 Brno, Czech Republic. E-mail: paolo.capretti@unifi.it.*

Gremmeniella abietina is an ascomycete causing Scleroderris canker on *Pinus* species and conifers in the Northern Hemisphere, including Europe from the Boreal to the Mediterranean regions. The disease occasionally caused severe damage in Europe, and is a constant threat in North America and Japan. The pathogen kills buds, young shoots and foliage of hosts, and bark necroses and branch dieback. Whole crowns may be infected, and plants may die after repeated attacks. Seedlings may die quickly. The pathogen is psychrophilic, favoured by wet and cool weather, recurrent late frost and prolonged snow cover. In Italy, Scleroderris canker was historically observed on young and adult pines in the Alps and the Apennines, where conditions were locally favourable. Fungal populations were genetically differentiated between northern and southern sites, and had different optima and host ranges. We surveyed areas where *G. abietina* had been observed in the past and found that its prevalence decreased over the last 40 years. Especially reduced was the frequency of the thermophilic form of the fungus in southern areas. The pathogen was often replaced by *Diplodia sapinea*, an opportunistic fungus shifting from an endophytic to pathogenic lifestyle in stressed host plants. Replacement of *G. abietina* by *D. sapinea* in the Apennines is likely a bioindicator of current climate change. The incidence of Scleroderris canker has probably decreased in other areas at the southern range edges, and distribution of *G. abietina* will be further reduced, making way for the emergence of other pathogens driven by climate-change related stressors.

Field studies on the primary inoculum and early infections of almond red leaf blotch (caused by *Polystigma amygdalinum*) in Spain. E. ZÚÑIGA[1], J. LUQUE[1], X. MIARNAU[2], O. ARQUERO[3], M. LOVERA[3], A. OLLERO[4], L.F. ROCA[4], A. TRAPERO[4]. [1]*Patologia Vegetal, IRTA, Carretera de Cabrils km 2, 08348 Cabrils, Spain.* [2]*Estació Experimental de Lleida, IRTA, Parc Científic i Tecnològic Agroalimentari de Lleida (PCiTAL), Parc de Gardeny, Edifici Fruitcentre, 25003 Lleida.* [3]*IFAPA "Alameda del Obispo", Avenida Menéndez Pidal s/n, 14080 Córdoba, Spain.* [4]*Departamento de Agronomía (Patología Agroforestal), Universidad de Córdoba. Campus de Rabanales, Edificio C-4, 14071 Córdoba, Spain. E-mail: erick.zuniga@irta.cat*

Red leaf blotch of almond (caused by *Polystigma amygdalinum*), is a common disease in continental climate areas of Spain and other countries in the Mediterranean region. Early symptoms are yellow discoloured blotches on leaves, which turn red and then become dark necroses. The disease usually causes early defoliation of trees that causes decreased fruit production. Little is known about the biology of the pathogen in Spain and worldwide. Co-ordinated research was carried out in southern (Andalusia) and northeastern (Catalonia) Spain, to monitor the dynamics primary *P. amygdalinum* inoculum production, and the period of plant infectivity. Monitoring in Catalonia of ascocarp and ascospore development showed optimum maturation of propagules by mid spring (April-May), which was coincident with high ascospore records obtained from leaf samples in the field. In Andalusia, the primary inoculum potential occurred from February to May, a longer period than in Catalonia. The period of maximum ascospore production was less in both areas, and varied greatly between years and areas. The periodical exposure of almond 'trap' plants to natural infections in the field showed that the infectivity period in Catalonia extended from April to late June, while in Andalusia it occurred from March to May. These preliminary results on the biology of *P. amygdalinum* are a first step in the establishment of an integrated disease control strategy against almond red leaf blotch in Spain, and other almond growing regions.

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Epidemiology and control of *Cucumber green mottle mosaic virus* in Spain. M.A. ELORRIETA[1], L. RUIZ[2], D. JANSSEN[2]. [1]*LABCOLOR, COEXPHAL, C/ Esteban Murillo, 3. Venta El Viso 04746 La Mojonera, Almería, Spain.* [2]*Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA), Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain. E-mail: dirk.janssen@juntadeandalucia.es*

Spain is one of the main producers of cucurbit crops in Europe, and one of the top-ten producers in the world. The tobamovirus *Cucumber green mottle mosaic virus* (CGMMV) was first described in Spain during the early 1990's, and has caused periodic outbreaks since then in cucumber and watermelon in greenhouses in the province of Almeria. To improve CGMMV control, we studied the epidemiology of the virus in the southeast of Spain. Between the years 2013 and 2015, 154 protected crops of cucumber (119), melon (21), watermelon (13) and zucchini (1), located in the provinces of Almeria and Granada, were selected randomly and examined. Leaves of plants were collected for analysis of CGMMV, and detailed information was gathered on the location, the greenhouse features, and the management of crops and diseases. CGMMV infections were detected in 23 greenhouses, predominantly of cucumber (20/119). The presence of CGMMV was not dependent on the use of grafted plantlets, the variety and source of seeds and plantlets, or on the origin of the irrigation water (owned or shared water wells). However, infections did depend heavily on the previous infection history of farms and surroundings. The enquiries revealed that the greenhouse structures and the irrigation water reservoirs were not cleaned periodically. Gloves and disinfectants were rarely used during crop manipulation. Successful control of CGMMV through crop management was positively correlated with soil disinfection by solarization and with crop rotation using non-cucurbit species.

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Temporal persistence and distribution of *Heterobasidion abietinum* in a planted forest of silver fir in Central Italy: a contribution to forest management. L. GHELARDINI[1,2], L.B. DÁLYA[2], C. AGLIETTI[1], P.CAPRETTI[1]. [1] *Department of Agrifood production and Enviromental sciences (DISPAA), University of Florence, Piazzale delle Cascine 18, 50144, Firenze, Italy.* [2] *Department of Forest Protection and Wildlife Management, Mendel University in Brno (ÚOLM). Zemědělská 3, 61300 Brno, Czech Republic. E-mail: luisa.ghelardini@unifi.it*

One of the main problems in mature conifer plantations is related to damage by *Heterobasidion annosum.* Occurrence and persistence of *H. abietinum* were assessed in a planted forest of silver fir (*Abies alba*) in Vallombrosa (Florence, Central Italy) over the past 60 years. The presence of the pathogen in the area has been known since the 18th Century, and is related to management choices. For centuries, silver fir in Vallombrosa was regularly managed with a 100 year growth period and artificial replanting. When a modern Forest Management Plan was first compiled in the 1960s, the occurrence of *Heterobasidion* was reported in the ecological description of all silver fir areas of the forest. Again in 1990, the presence and frequency of *Heterobasidion* in Vallombrosa was investigated in wood samples from Silver fir stumps left after thinning. At the time, *H. abietinum*, identified according to Korhonen's method (paring colonies with testers), was found at over 80% of the intersection points of a square (500 m) sampling grid covering the whole forest, with the greatest frequency (56%) on silver fir stumps. More recently, after a severe wind storm destroyed about 50 ha of the forest in spring 2015, a systematic sampling was carried out, and *Heterobasidion* species were identified with molecular methods. Taken together these studies define distribution of the pathogen over space and time, providing support for design of an informed recovery plan for the Vallombrosa forest.

Ecological succession of pathogenic fungi of pines in Italy associated with climate change. L. GHELARDINI[1,2], P. CAPRETTI[1], L. BOTELLA[3], C. AGLIETTI[1], N. LUCHI[2]. [1]*Department of Agrifood Production and Environmental Sciences, University of Florence. Piazzale delle Cascine 28, I-50144, Firenze, Italy.* [2]*Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR), Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Firenze, Italy.* [3]*Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in*

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Gremmeniella abietina caused Scleroderris canker on *Pinus* species and conifers in the Northern Hemisphere, including Europe from the Boreal to the Mediterranean regions. The disease occasionally caused severe damage in Europe, and is a constant threat in North America and Japan. The pathogen kills buds, young shoots and foliage, causes bark necroses and branch dieback. Whole crowns of trees may be infected, and plants may die after repeated attacks. Seedlings may die quickly. The pathogen is a psychrophilic fungus favoured by wet and cool weather, recurrent late frost and prolonged snow cover. In Italy, Scleroderris canker was historically observed on young and adult pines in the Alps and the Apennines, where conditions were locally favourable. Fungal populations were genetically differentiated between northern and southern sites, and had different optima and host ranges. We surveyed areas where *G. abietina* had been observed in the past, and found that its prevalence decreased over the last 40 years. Especially reduced was the frequency of the thermophilic form of the fungus in southern areas. *Gremmeniella abietina* was often replaced by *Diplodia sapinea*, an opportunistic fungus shifting from endophytic to pathogenic state in stressed host plants. The replacement of by *D. sapinea* in the Apennines is likely to be a bioindicator of current climate change. Incidence of Scleroderris canker has probably decreased in other areas at the southern range edges, and the distribution of *G. abietina* will be further reduced, making way for the emergence of other pathogens driven by climate-change related stressors.

Epidemiology, aetiology and modelling of olive anthracnose. P. TALHINHAS, A. LOUREIRO, A.P. RAMOS, J.P. MELO E ABREU, H. OLIVEIRA. *LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: ptalhinhas@isa.ulisboa.pt*

Olive anthracnose affects olive fruit at maturity, causing yield losses and poor olive oil quality. Specific agroecological circumstances, combining increased average humidity and rainfall during autumn, widespread use of susceptible varieties and abundance of inoculum reservoirs favour high disease incidence and severity. Disease control using agrochemicals is often the only immediate disease management option, although the presence of pesticide residues is problematic in table olives and olive oil. Olive anthracnose is associated with at least six species of *Colletotrichum*, with *C. nymphaeae* being prevalent in some Mediterranean areas and *C. godetiae* in others, while *C. acutatum* s.s. is emerging. *Colletotrichum nymphaeae* and *C. acutatum* s.s. are more virulent than others, although differential interactions between fungal species and olive varieties have been documented. Modelling anthracnose epidemiology is therefore very important, and this should consider the combination of climatic factors, production systems (super-intensive, intensive or traditional orchards, considering also neglected groves and oleaster patches), prevalence of the crop, cultivar preference and predominant pathogen species, at regional scales. Olive anthracnose will be addressed combining epidemiological, aetiological and agroecology-based modelling approaches. This will better characterize the disease, forecast disease risk scenarios, and assist informed decisions regarding disease control.

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Cryptic species and population genetic structure of *Plasmopara viticola* in São Paulo State, Brazil. M.P. CAMARGO[1], C.F. HONG[2], L. AMORIM[1,] H. SCHERM[2]. [1]*Department of Plant Pathology, Luiz de Queiroz College of Agriculture, University of São Paulo, CEP 13418-900, Piracicaba, SP, Brazil.* [2]*Department of Plant Pathology, University of Georgia, Athens, GA 30602, USA. E-mail: meyrielecamargo@usp.br*

Downy mildew (caused by *Plasmopara viticola*) is one of the most important diseases in grape-growing areas worldwide, including Brazil. Little is known about the pathogen population structure in subtropical areas. To examine pathogen diversity, 516 single lesions of *P. viticola* were collected during the 2015/16 growing season from 11 locations in São Paulo State, and from nine grapevine cultivars. To allow recognition of cryptic species (clades), a subsample of 130 isolates were analyzed using cleaved amplified polymorphic sequence (CAPS) markers with two re-

striction enzymes (*AseI* and *HpyCH4V*). In addition, the ITS1 region of 94 isolates was sequenced to substantiate results. Seven previously reported microsatellite markers were used for genotyping all 516 *P. viticola* isolates. Results obtained from CAPS analysis and ITS1 sequencing suggest that the population of *P. viticola* in São Paulo State may a single cryptic species, *P. viticola* clade *aestivalis*. Twenty-three alleles and 55 multilocus genotypes (MLGs) were observed among the 516 isolates. Half of the MLGs observed were clonal, and four dominant MLGs represented 66% of the observed genotypes. Most populations showed significant linkage disequilibrium, and excess of heterozygosity was verified in many loci. Principal coordinate analysis revealed no clusters among populations. No significant isolation by distance was found, suggesting high levels of gene flow. These results demonstrate that epidemics result from multiple clonal infections caused by a few genotypes, and that asexual reproduction predominates for P. *viticola* in São Paulo, Brazil.

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Development and verification of a dynamic model for predicting olive scab development. J. ROMERO[1], L.F. ROCA[1], C. AGUSTI-BRISACH[1], E. GONZALEZ-DOMINGUEZ[2], V. ROSSI[2], A. TRAPERO[1]. [1]*Departamento. de Agronomía, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain.* [2]*Istituto di Entomologia e Patologia vegetale, Università Cattolica S. Cuore, Via E. Parmense 84, 29100 Piacenza, Italy. E-mail: joaquinromrod@gmail.com*

Olive scab, caused by *Venturia oleaginea*, is the main olive leaf disease worldwide. Traditionally, chemical control of this disease was based on a fixed schedule of fungicide applications, mainly using copper products. However, integrated pest management (IPM) should be implemented to rationalize fungicide treatments. A mechanistic model to predict risk of infection and olive scab epidemics was developed, according to the system analyses, and implemented in a computerized system. Hourly data of air temperature, rainfall and relative humidity were used to produce daily olive scab predictions as outputs. Simulations are based on sub-processes of conidial production and dispersal, infection and latent period (i.e., the state variables). Mathematical equations that relate state variables (i.e., the driving variables) were developed using published data on *V. oleaginea*. The model was able to represent the real system, and assisted understanding of olive scab epidemicsin four olive-growing areas, traditionally considered as having different favourable conditions for olive scab development. Model outputs for these areas were generated, agreeing with traditional knowledge. Based on the model outputs, different strategies of fungicide treatments can be suggested in each growing area, reducing the amount of fungicide applied. Weaknesses of the model are discussed, and additional research is advisable. However, this model could be useful for implementing an IPM approach. This is the first olive scab model based on the biological knowledge of the disease. Other disease models will soon be added to complete a decision support system for the main aerial diseases in olive groves.

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Epidemiology of peach powdery mildew (*Podosphaera pannosa*) in Catalonia, Spain: towards a degree-day model to initiate fungicide spray programmes. N. MARIMON[1], J. LUQUE[1], J. MARTÍNEZ- MINAYA[2], D. CONESA[2], A. VICENT[3]. [1]*Patologia Vegetal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Carretera de Cabrils km 2, 08348 Cabrils, Spain.* [2]*Departament d'Estadística i Investigació Operativa, Universitat de València, 46100 Burjassot, Spain.* [3]*Centro de Producción Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Spain. E-mail: neus.marimon@irta.cat*

Powdery mildew of peach (caused by *Podosphaera pannosa*) is a common disease in Spain where these fruit trees are grown. The disease is usually managed by calendar-based fungicide spray programmes, commencing at the petal fall host stage. This study monitored powdery mildew progress in untreated trees, in order to: 1) describe overall disease progress in relation to a degree-day scale starting at 50% blossom; and 2) establish a degree-day threshold for the detection of primary infections and thus initiate a more rational weather-based fungicide programme.

Five trees per experimental plot were chosen in each of seven commercial peach orchards located in Catalonia, NE Spain. Disease monitoring was carried out from March to summer (June-July) 2013 to 2015, by recording the incidence and severity of the disease on fruits. An automatic weather station was located in each plot to record the main environmental data. Accumulated degree-days (ADD) from the blossom biofix were calculated for each orchard. Observations indicated that primary infections were detected at 242.0 ± 13.1 ADD, while last infections were at 483.5 ± 42.2 ADD (mean ± standard error, n = 15). Disease progress followed a clear sigmoidal trend, and Beta-regression equations between disease incidence on fruits and ADD were successfully fitted using Bayesian inference with Integrated Nested Laplace Approximation. The model showed good performance when validated against independent data. This preliminary research is a first step towards a decision support system based on epidemiological modelling for the integrated management of peach powdery mildew in Catalonia.

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Huanglongbing epidemiology in Brazilian orchards. K. PAZOLINI, J.H. ARRUDA, G.A. CHINELATO, A. BERGAMIN FILHO, J. BELASQUE JUNIOR. *Luiz de Queiroz College of Agriculture, University of São Paulo, Av. Pádua Dias, 11 – Piracicaba, Brazil. E-mail: pazolinikelly@gmail.com*

Huanglongbing (HLB) (caused by '*Candidatus* liberibacter spp.') is the main citrus disease worldwide. There are still no viable curative measures or varieties with genetic resistance to HLB. Recommended disease management is the use of healthy seedlings, eradication of symptomatic trees and chemical control of the vector, *Diaphorina citri*. Our aim was to understand the temporal and spatial progress of HLB in an area, with strict management of disease in Brazilian orchards. Temporal (logistic and Gompertz) and spatial (exponential and power law) models were tested, by non-linear regression to orchard data (177 plots for temporal, 12 plots for spatial analyses), on a single farm in São Paulo state. The management of HLB in this property was carried out with four or more inspections per year, for eradication of symptomatic trees and weekly or biweekly sprays with insecticides for vector control. For temporal analyses, the logistic model was adjusted ($P < 0.05$) to 115 of the 177 plots studied (progress rates of 0.2 to 1.5), while the Gompertz model was adjusted to only 29 plots (progress rates from 0.2 to 0.5). For spatial analysis, both models presented a good fit to the 12 plots studied. However, the model inverse power law presented the best residual pattern and greater R^2 (0.91) than the exponential model ($R^2 = 0.88$). The progress of HLB with time was best described by the logistic, and in space by the inverse power model.

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Microbiomes and their roles in plant health

New *Pseudomonas* strains from olive rhizospheres as effective biocontrol agents against *Verticillium dahliae*. C. GÓMEZ-LAMA CABANÁS[1], G. LEGARDA[2], D. RUANO-ROSA[1], P. PIZARRO-TOBÍAS[3], A. VALVERDE CORREDOR[1], J.L. NIQUI[3], J.C. TRIVIÑO[2], A. ROCA[3], J. MERCADO-BLANCO[1]. [1]*Department of Crop Protection, Institute for Sustainable Agriculture (CSIC), Avenida Menéndez Pidal s/n Campus 'Alameda del Obispo', 14004 Córdoba, Spain.* [2]*Bioinformatics Department, Sistemas Genómicos Ltd, Valencia, Spain.* [3]*Bio-Ilíberis Research and Development SL, Granada, Spain. E-mail: cgomezlama@gmail.com*

Previous studies have demonstrated that rhizospheres of nursery-produced olive (*Olea europaea* L.) plants are sources of bacteria with potential as biological control agents (BCA) of Verticillium wilt of olive (VWO), caused by *Verticillium dahliae*. A collection of 189 bacterial isolates from healthy olive (cv. Picual) plants was generated, based on different morphological and biochemical characteristics and *in vitro* antagonistic activity against several olive pathogens. Three strains (PIC25, PIC105 and PICF141) showing the greatest potential as BCAs, particularly against *V. dahliae*, were eventually selected. These were further tested for nutritional requirements and chemical sensitivities. Their effectiveness against VWO

caused by the defoliating pathotype of *V. dahliae* was also demonstrated. Genotypic and phenotypic traits traditionally associated with plant growth promotion and/or biocontrol abilities were evaluated (e.g. phytase, xylanase, and glucanase activities, and siderophore and HCN production). Phylogenetic analysis revealed that the strains belonged to the *Pseudomonas* genus. Strain PICF141 was affiliated to the '*P. mandelii* subgroup', with *P. lini* as the closest species. Strains PIC25 and PIC105 were affiliated to the '*P. aeruginosa* group', *P. indica* being the closest species. Strain PIC105 was identified as *P. indica*, this being the first reort of the species as a potential BCA. Sequencing and *in silico* analyses of the genomes of these strains enabled the identification of traits involved in plant-bacteria interactions. Seed adhesion and root colonization abilities of the novel BCA were also assessed, providing valuable information for the development of future bioformulations based on these rhizobacteria.

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New bacterial antagonists for the biocontrol of fire blight caused by *Erwinia amylovora*. S. AIT BAHADOU[1,3], A. OUIJJA[1], A. KARFACH[2], A. TAHIRI[3]. *[1]Laboratory of Plant Biotechnology and Molecular Biology, Moulay Ismail University, Faculty of Sciences, BP 11201, Ave Zitoune Meknes, Morocco. [2]Laboratory of Microbial Biotechnology, Sidi Mohamed Ben Abdellah University, Faculty of Sciences and Technologies, BP 2202, Route d'Imouzzer FES, Morocco. [3]Department of Plant Protection and Environment of the National School of Agriculture-Meknes, Km10, Rte Haj Kaddour, BP S/40, Meknès 50001 , Morocco. E-mail: s.aitbahadou@edu.umi.ac.ma*

The biocontrol effectiveness of antagonistic bacteria against fire blight (caused by *Erwinia amylovora*) was evaluated under *in vitro* and field conditions. Among 61 bacteria isolated from soil and flowers of fire blight host plants from different Moroccan areas, 20 isolates showed antagonistic activity against the pathogen during agar-diffusion-tests, attached blossoms assays and in a bioassay on immature pear fruits. Effective isolates were identified using biochemical tests and 16S rDNA gene sequencing. These isolates were grouped into the following genera: *Alcaligenes* (ACBC1), *Bacillus* (CPa12, CPa2, HF6, JB2, LMR2, SF14, SF16, SP10, SP13, SP18), *Brevibacterium* (SF3, SF4, SF7, SF15), *Pantoea* (ACBC2, ACBP1, ACBP2), *Pseudomonas* (SP9), *and Serratia* (HC4). The isolates were reported in the NCBI nucleotide sequence database (GenBank) under the accession numbers from KY357285 to KY357304. In a field assay with susceptible apple varieties, spray treatments were carried out with different bacterial antagonists. Their efficacies were evaluated 15 d post-inoculation on blossoms, and ranged from 55 to 95% for 11 strains. Most strains gave efficacies that were better than that obtained with commercial bacterial strains P10c (66%) and QST713 (63%). The strains showed no pathogenicity towards plant tissue (pear fruitlets, pear and apple blossoms, and tobacco leaves), and are, therefore, considered as potential candidates to as microbial biocontrol formulations for fire blight control.

Qualitative and quantitative impacts of *Bactrocera oleae* on the fungal microbiota of ripe olive drupes. D. RUANO-ROSA[1], A. ABDELFATTAH[2], M.G. LI DESTRI NICOSIA[2], S.O. CACCIOLA[3], G.E. AGOSTEO[2], L. SCHENA[2]. *[1]Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. [2]Dipartimento di Agraria, Università Mediterranea di Reggio Calabria, Località Feo di Vito-89122 Reggio Calabria, Italy. [3]Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: ruanodavid@gmail.com*

The olive fly, *Bactrocera oleae*, is a major key pest of olive drupes, greatly affecting quality and quantity of olive oil production. Fungus species associated with olive drupes can also have important impacts on olive production. However, little is currently known about the interaction between olive fly and fungi. Ripe olive drupes of three olive varieties, either with or without olive fly infestations, were collected in southern Italy. These were pitted and total DNA was extracted and analyzed using real-time quantitative PCR (qPCR) and metabarcoding based on Illumina MiSeq sequencing. Both analyses were performed using fungal universal primers targeting the ITS2 region of the rDNA. QPCR analyses enabled the quantification of the total fungal DNA, and revealed

a significant increase of the fungal biomass in all olive fly infested samples. Metabarcoding analyses revealed prevalence of *Ascomycota* (90.2%) followed by *Basidiomycota* (9.4%). Overall, the genera *Aureobasidium*, *Cladosporium*, *Alternaria*, *Colletotrichum* and *Pseudocercospora* were the most abundant, although significant differences were revealed for different varieties and sampling sites. The presence of olive fly ovipositor punctures modified the composition of the fungal microbiota. Although greater fungus diversity was observed in samples without fly, important fungal genera, such as *Aureobasidium* and *Hanseniaspora,* were more abundant (*Aureobasidium*) or exclusively (*Hanseniaspora*) present on infested olives. These yeasts are likely to play important roles in the low quality of olive oil from insect-infested olives by promoting fermentation processes.

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Dynamics of fungus endophytes during different phenological stages of olive trees. F. MARTINS[1,2], J.A. PEREIRA[1], P. BAPTISTA[1]. [1]*CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.* [2] *University of Léon, Department of Engineering and Agricultural Sciences, Av. Portugal, nº 41, 24071 Léon, Spain. E-mail: pbaptista@ipb.pt.*

Endophytic fungi are a diversified group of microorganisms that reside asymptomatically in the tissues of most plant species. Despite their known roles in protecting hosts against several diseases, little is known on the sources of established endophytes and how plants select specific microbial communities to establish associations. We used cultivation-dependent approaches to assess the endophytic fungus communities in olive tree floral buds, inflorescences and fruits, to determine differences in different hos phonological stagesfollow the phenological stages from floral buds to fruits. The fungus endophytes were identified by rDNA sequencing. From the floral bud to flower stage, the frequency of colonization and abundance of endophytes increased progressively up to 2.4-fold; and from flower to fruit decreased up to 5.0-fold. *Biscogniauxia mediterranea* was the most frequent species isolated from the buds and inflorescences (N = 89), whereas at the fruit stage, the most abundant species was *Neofabraea vagabunda* (N = 38). Endophytic fungus communities also differed in composition over the phenological stages, probably due to variations of weather conditions and the chemical nature of the plant organs. *Phomopsis*, *Venturia* and *Coniozyma* were common in floral buds and inflorescences, but disappeared from fruits, being replaced by genera such as *Aspergillus*, *Coriolopsis* and *Eutypella*. Our results indicate that endophytic fungusl communities were distinct and specific to the host phenological stages, raising the question of whether these specific species may induce plant protection against biotic stresses.

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Fungal endophyte communities in olive fruits: effects of maturation index and anthracnose incidence. F. MARTINS[1,2], J.A. PEREIRA[1], P. BAPTISTA[1]. [1]*CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. E-mail: pbaptista@ipb.pt.* [2]*University of Léon, Department of Engineering and Agricultural Sciences, Av. Portugal, nº 41, 24071 Léon, Spain. E-mail: pbaptista@ipb.pt*

Olive anthracnose, caused by different species of *Colletotrichum*, is one of the most economically harmful fruit diseases of olive crop worldwide. In the Trás-os-Montes region (Northeast of Portugal), although the presence of the pathogen has been reported on olive orchards in almost all areas, lower levels of incidence were observed in specific areas. This study evaluated the diversity of endophytic fungi inhabiting fruits of the anthracnose-susceptible cultivar Madural, in olive groves from areas of high and low anthracnose incidence. Differences in the endophytic community composition were assessed. Fungi were isolated from symptomless olive fruits at three different maturation indices (MI). The isolates were identified by rDNA sequencing. Overall, the frequency of colonization and abundance of endo-

phytes were greater in areas with high anthracnose incidence (12.4%; 78) compared with areas with low incidence (7.3%; 46). Despite this, the composition of fungal communities in both areas was very similar. Genera with the greatest abundance were *Trametes* (33%), *Alternaria* (43%) and *Neofabraea* (26%). During fruit maturation, the frequency of endophyte colonization increased up to 16-fold, abundance up to 6-fold, and diversity up to 8-fold. Although endophytic communities of the three MIs overlapped, several genera preferred either olives from MI2 (e.g. *Apodospora*, *Hyalodendriella*, *Pyrenochaeta*), or from MI3 (e.g. *Mollisia*, *Ulocladium*) or MI4 (*Colletotrichum*, *Epicoccum*). In addition to providing insights into fungal endophyte community structures, our survey provided candidates for further evaluation as potential management tools against olive anthracnose.

This research was supported by the Foundation for Science and Technology (FCT, Portugal), and FEDER under Programme PT2020 (UID/AGR/00690/2013). The first author also received a PhD Scholarship (ref. SFRH / BD / 112234/2015) from FCT.

Endophytic and epiphytic fungal communities associated with olive trees differ in antagonistic activity against *Pseudomonas savastanoi* pv. *savastanoi*. T. GOMES[1,2], J. A. PEREIRA[1], T. LINO-NETO[2], P. BAPTISTA[1]. [1]CIMO/ *Polytechnic Institute of Bragança, School of Agriculture, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.* [2]*Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center (CBFP), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: pbaptista@ipb.pt*

Olive knot (OK) caused by the *Pseudomonas savastanoi* pv. savastanoi (Psv) is an important disease, causing severe damage and yield losses in olive trees worldwide. In a previous study, we isolated this bacterium from the phyllospheres of olive trees, together with many fungal species. In these complex communities, microorganisms compete for space and resources, promoting survival of the best-adapted individuals. This has prompted interest in the exploitation of these microorganisms for OK control. In this study, 48 fungal species from the endo- and epiphytic communities of olive twigs were screened for growth inhibition of Psv under *in vitro* conditions. The time course of interspecific interactions (24, 48, 72 and 144 h) was studied on potato dextrose agar and olive leaf + twig extract (OLTE) media, by assessing clear zones of bacterial growth inhibition around fungus colonies. The epiphytic community was the main reservoir for antagonistic fungi. Almost 70% of the tested epiphytes inhibited Psv growth, with *Dothiorella iberica*, *Aspergillus felis* and *Aspergillus brasiliensis* the most prominent species. The proportion of antagonists within endophytic communities was less (46%), with the most efficient being *Epicoccum nigrum* and *Rhinocladiella similis*. Antibacterial activity was observed to be affected ($P < 0.01$) by growth medium and period of interaction. Greater growth inhibition was found with the OLTE culture medium, showing that inhibition of these endophytic and epiphytic fungi was specifically enhanced by the host plant extracts. Most of the fungi tested (up to 64%) from both microenvironments showed greatest antibacterial activity in the first 24 h of interaction, whereas only 16% strongly inhibited Psv after 48h and 19% after 144 h. These results indicate that *D. iberica*, *E. nigrum* and *A. felis* are the best candidates for biocontrol of olive knot, and these should be further evaluated under natural conditions.

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New bacterial antagonists for biocontrol of fire blight, caused by *Erwinia amylovora*. S. AIT BAHADOU[1,3], A. OUIJJA[1], A. KARFACH[2], A. TAHIRI[3]. [1]*Laboratory of Plant Biotechnology and Molecular Biology, Moulay Ismail University, Faculty of Sciences; BP 11201, Ave Zitoune Meknes, Morocco.* [2]*Laboratory of Microbial Biotechnology, Sidi Mohamed Ben Abdellah University, Faculty of Sciences and Technologies; BP 2202, Route d'Imouzzer FES, Morocco.* [3]*Department of Plant Protection and Environment of the National School of Agriculture-Meknes, Km10, Rte Haj Kaddour, BP S/40, Meknès 50001, Morocco. E-mail: s.aitbahadou@edu.umi.ac.ma*

The biocontrol effectiveness of antagonistic bacteria against fire blight (caused by *Erwinia amylovora*) was evaluated under *in vitro* and field conditions. Among 61 bacteria isolated from soil and flowers of fire blight host plants from different Moroccan

areas, 20 isolates showed greatest antagonistic activity against the pathogen in agar diffusion tests, attached blossoms assays and in a bioassay on immature pear fruits. Effective isolates were identified using biochemical tests and 16S rDNA gene sequencing. These isolates were grouped in the following genera: *Alcaligenes (ACBC1)*, *Bacillus* (CPa12, CPa2, HF6, JB2, LMR2, SF14, SF16, SP10, SP13, SP18), *Brevibacterium* (SF3, SF4, SF7, SF15), *Pantoea* (ACBC2, ACBP1, ACBP2), *Pseudomonas* (SP9), and *Serratia* (HC4). The isolates were reported in the NCBI nucleotide sequence database (GenBank) under the accession numbers KY357285 to KY357304. In a field assay with the susceptible varieties of apple, spray treatments were carried out with different genera of bacterial antagonists. Their efficacies were evaluated 15 days post-inoculation on blossoms, and ranged from 54.6 to 95.0% for 11 strains, most of which gave better reductions than that obtained with commercial bacterial strains P10c (66%) and QST713 (63%). The strains showed no pathogenicity towards plant tissues (pear fruitlets, pear and apple blossoms, tobacco leaves), and are candidates for microbial formulations for fire blight control.

Diversity of fungal endophytic community in *Quercus suber* L. and detection of opportunistic phytopathogenic fungi. D. COSTA[1]*, J. CUNHA[1]*, R. M. TAVARES[1], P. BAPTISTA[2], T. LINO-NETO[1]. [1]*BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.* [2]*CIMO/ School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. E-mail: tlneto@bio.uminho.pt*

Cork oak (*Quercus suber*) is of high ecological importance in the Mediterranean region, and has high relevance for the Portuguese economy, due to cork production and processing. The sustainability of cork oak is currently being threatened by reduction of water availability that would increase the occurrence of diseases. Charcoal disease, caused by *Biscogniauxia mediterranea*, leads to death of the cork oak trees. *Diplodia corticola* is involved in various diseases considered responsible for the decline of cork oak in the Mediterranean region. To identify endophytic fungi in cork oak, including opportunistic pathogens, four sites of continental Portugal (Bragança, Gerês, Alcobaça and Grândola), with differences in water availability, were selected for collection of biological material. Fungal endophytes from leaves, stems and roots were evaluated. Roots had more diverse fungal communities than the aboveground organs. Although no disease symptoms were detected on the studied trees, the pathogenic fungi were essentially affecting stems and leaves. In general, greatest endophyt colonization frequency and diversity occurred in Grândola, and least in Alcobaça. From all studied sites, cork oaks from Gerês showed the most distinct community and did not present the pathogens. *Diplodia corticola* only infect trees from southern regions, while *B. mediterranea* also infected trees in Bragança. The exclusive presence of both pathogens in aboveground organs and the absence of visible disease symptoms in all studied cork oaks, encourage the search for adequate biocontrol agents from the endophytic communities for restricting these cork oak diseases.

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Xylella fastidiosa research in Europe

Natural competence and recombination *in vitro* occurs frequently among *Xylella fastidiosa* isolates from subsp. *fastidiosa* and *multiplex*. P.P. KANDEL[1], L. DE LA FUENTE[1]. [1]*Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA, 36849. E-mail: lzd0005@auburn.edu*

Xylella fastidiosa (Xf) is a plant pathogenic bacterium that causes incurable diseases in economically important crops such as grapevine and citrus. For more than a century, Xf-caused diseases were restricted to the Americas, but recent reports in new locations (e.g. Italy, France, Spain), and new hosts (e.g. olive, *Polygala myrtifolia*) exemplifies the ability of this pathogen to adapt to new environmental conditions. Based on genetic diversity studies, inter-subspecific recombination (ISR) was proposed to contribute to host shifts. Natural competence, as a mode of recombination, was shown to occur at high frequen-

cies in model systems mimicking natural habitats; viz. microfluidic chambers with xylem sap. However, little is known about the variability of recombination potential among Xf isolates. Therefore, we compared recombination frequencies of thirteen Xf isolates belonging to two subspecies (*fastidiosa* and *multiplex*), using five different plasmids containing antibiotic resistance markers flanked on either side by Xf homologous regions. Recombination frequency varied greatly among isolates (3.14×10^{-2} to 2.3×10^{-8} recombinants per parental cell), and was not correlated with the sequence identity of the homologous regions. Nevertheless, the ability to recombine was correlated with twitching motility ($r = 0.71$, $P = 0.006$). When combination of marker-tagged, heat-killed donor and live recipient cells from two subspecies were mixed, ISR occurred within a genomic region of ~10kb. This study demonstrates that recombination, and therefore evolutionary potential, differ among Xf isolates, which is a serious threat in those cases where isolates can co-exist in the same environment.

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Photointerpretation of high resolution aerial images for large scale monitoring of the olive quick decline syndrome associated to *Xylella fastidiosa*. S. GUALANO, F. SANTORO, F. VALENTINI, A.M. D'ONGHIA. *CIHEAM - Istituto Agronomico Mediterraneo di Bari, Via Ceglie 23, Valenzano (BA) 70010, Italy. E-mail: donghia@iamb.it*

Xylella fastidiosa (Xf) is the main cause of the olive quick decline syndrome (OQDS), a serious threat for the olive trees in the EU-Mediterranean regions. After the first outbreak in 2013, the rapid identification of Xf on territorial basis was crucial in Apulia, Italy. For this purpose, the photointerpretation of high resolution aerial images was successfully applied to identify OQDS-like trees in a buffer area of Lecce, with 20% correlation between OQDS and ELISA-positive trees. The same technique was evaluated in the buffer/containment zones (apparently Xf-free) of the demarcated area in Apulia. High geometrical resolution aerial images from three regions of interest (ROI), ranging from about 0.5 to 1.6 km^2 each, were processed in the visible (VIS) and near infrared (NIR) in a GIS environment. Analyses were oriented to identification of phototypes, morphologically associated to the OQDS. Results of the recognition process have provided the classification of 637 OQDS-like trees (3.7%) out of 17,220 photointerpreted trees. Following field inspections, 462 trees (73%) showed OQDS while the remaining could not be inspected (pruned trees or inaccessible groves), and few were altered by other factors. All OQDS trees were serologically tested for Xf, which was found in two ROI with different infection rates: 12% (20 infected trees out of 165 OQDS trees) and 3.5% (four infected trees out of 112 OQDS trees). The method was effective for identifying new foci of infection in the buffer and containment zones, orienting inspections in the official monitoring for rapid identification of infected trees and allowing immediate modification of the demarcated areas.

Genetic diversity of *Xylella fastidiosa* assessed in imported ornamental *Coffea arabica* plants. M. BERGSMA-VLAMI*, J.L.J. VAN DE BILT, N.N.A. TJOU-TAM-SIN, C.M. HELDERMAN, P.P.M.A. GORKINK-SMITS, N.M. LANDMAN, J.G.W. VAN NIEUWBURG, E.J. VAN VEEN, M. WESTENBERG. *Dutch National Plant Protection Organization (NPPO-NL), P.O. Box. 9102, 6700 HC Wageningen, the Netherlands. E-mail: m.vlami@nvwa.nl*

The diversity of *Xylella fastidiosa* in imported ornamental *Coffea arabica* plants was assessed through a MLST analysis, and compared with *X. fastidiosa* infecting different host plants worldwide. Different sequence types (STs) of *X. fastidiosa* were found, such as ST 53 and ST 73 (*X. f* subsp. *pauca)* and ST 72 and ST 76 related to *X. f* subsp. *fastidiosa.* Additionally, a novel ST, ST 77 has been assessed, that is related to *X. f* subsp. *fastidiosa,* but shares allelles from at least two different subspecies of *X. fastidiosa.* Isolation of *X. fastidiosa* from infected *C. arabica* plants was successfully performed only after the application of a brief ultrasonication step during extraction. The acquired *X. f* subsp. *pauca* isolates belonged to either ST 53 or ST 73. Data acquired from PACBIO/Illumina next generation sequencing (NGS) on *X. f* subsp. *pauca* isolate PD 7202 (ST 53) demonstrated that, at the chromosomal level, PD 7202 is identical

to CoDiRo-ST53 found in Italy on olive. However, at plasmid level, clear differences have been assessed in individual genes. Virulence studies are currently ongoing after inoculation of *X. f* subsp. *pauca* isolates PD 7202 (ST 53) and PD 7211 (ST 73) on several plant species including *Coffea arabica.* Preliminary results on virulence will be presented.

This study was supported by research grant OS 2015330 project for *X. fastidiosa* of the Ministry of Economic Affairs in the Netherlands and partly by H2020 programme – SFS-09-2016, XF-ACTORS, grant agreement 727987.

Fast and sensitive detection for *Xylella fastidiosa* through recombinase polymerase amplification. R. LI[1], P. RUSSELL[1], S. ZHANG[1], B. DAVENPORT[1], A. EADS[1], K. SCHUETZ[1], S. BERKANI[2], M. AMATO[2]. *[1]Agdia Inc., Elkhart, IN 46514, U.S.A. [2]Agdia-EMEA, 91350 Grigny, France. E-mail: Rugang.li@agdia.com*

Xylella fastidiosa (Xf), living and multiplying in host xylem, is regulated in many countries. Xf originated in the American continent, but in recent years has appeared in Mediterranean countries including Italy, France, and Spain, and is causing grave concern from damage in olive trees of southern Italy and rapid spread to other crops and areas. The genetic diversity indicates that these new introductions are independent of one another. A fast and sensitive detection method is critical to reduce the likelihood of Xf introduction into new areas. Agdia has developed a rapid and sensitive DNA test for specific detection of Xf using the advanced recombinase-polymerase amplification technology (AmplifyRP). The assay performs both as a real-time and an endpoint test, from a single reaction tube at 39°C for 20 min. Reaction template is simply prepared by soaking 50 mg of petiole cross-sections in 0.5 mL AMP1 extraction buffer for 10 min, or by suspending one culture colony in 100 µL AMP1 buffer. The assay reacts to 28 Xf isolates from grapevine, citrus, olive, almond, coffee, oleander, mulberry, American elm, sycamore, oak, blueberry, and blackberry, while consistently detecting 22 and even less copies of spiked Xf genome in soaking extract (1:10, w:v). No reaction background was observed in host tissues, and no cross-reaction was observed to *Xanthomonas*, *Erwinia*, *Pseudomonas*, and *E. coli*. This DNA test provides a reliable tool to fight against Xf spread, as it can be performed directly on site.

This research is supported by Agdia, Inc.

Current situation in France regarding *Xylella fastidiosa*: methods of detection and subspecies characterization, strains and host plant diversity. F. POLIAKOFF[1], B. LEGENDRE[1], V. OLIVIER[1], C. DOUSSET[1], S. PAILLARD[1], D. MOLUSSON[1], A. SAINTE- LUCE[1], V. JUTEAU[1], N. DENANCE[1,2], M.A. JACQUES[2]. *[1]Bacteriology, Virology and GMO Unit - ANSES / Plant Health Laboratory – 49044 Angers, France. [2]IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR4207 QUASAV, 42, rue Georges Morel, - 49071 Beaucouzé, France. E-mail: francoise.poliakoff@anses.fr*

In conjunction with the emergence of *Xylella fastidiosa* (Xf) in Europe, several interceptions of coffee plants contaminated with *Xf* occurred in France. Different XF subspecies and sequence-types (ST) were identified: *fastidiosa* (ST75), *sandyi* (ST72 and ST76) and *pauca* (ST53 and ST74). Since the discovery of a focus of *Polygala myrtifolia* (Pm) in natural settings in 2015 in Corsica and the French Riviera, this pathogen has been detected on thirty plant species with a validated method based on real time PCR (Harper *et al*, 2010) associated with a DNA extraction kit (BioNobile). Characterization of isolates directly on plants, or for strains isolated on modified PWG medium is performed according to multilocus sequence typing (MLST) (http://pubmlst.org/xfastidiosa/). Following EPPO protocol PM 7/24, isolates were mostly allocated to sequence types ST6 and ST7 (subspecies *multiplex)*. Mixed infection by ST6 and ST7 was demonstrated by isolation of both strains from one Pm. Modifications to a proposed amplification protocol revealed infections linked to the subspecies *pauca*, *sandyi*, one recombinant and some mixed infections. The EPPO protocol MLST confirmed identification of four Pm contaminated with subsp *pauca* but not the identification of other contaminants. These contaminations were not observed again in the immediate environment after plant eradication. Subspecies assignation directly from plant material is not always successfully linked to PCR inhibitors depending of host plants. This study confirms the diversity of subspecies of *Xf* in France, but the subspecies *multiplex* was found to greatly predominate.

This research is partly supported by the Project H2020 PONTE.

Flashdiag®XF Kit, a quick field diagnostic tool for detection of *Xylella fastidiosa*. T. VANDEWALLE[1], K. OULDELKABLA[1], C. FABRE[1], G. LOCONSOLE[2], M. MASSON[1]. [1]*Anova-Plus, 4 rue Pierre Fontaine, Genopole Campus 3, Evry 91030, France.* [2]*Department of "Scienze del Suolo, della Pianta e degli Alimenti", University of Bari Aldo Moro, Via Amendola, 165/A 70126 Bari, Italy. E-mail : thomas.vandewalle@anova-plus.com*

Anova-Plus is developing Flashdiag®XF, a diagnostic kit for field use that will detect *Xylella fastidiosa* from a wide spectrum of plant hosts using DNA isothermal amplification. Samples from public collection were obtained for sub-species *X. f. fastidiosa, X. f. pauca and X. f. multiplex*, isolated from different plant hosts (grapevine, coffee, almond, olive or plum). The isothermal amplification method was conceived to test in one reaction all these *X. fastidiosa* sub-species. DNA from healthy plants was used as negative control and absence of cross-reaction was ensured with closely related species (i.e. several *Xanthomonas* species). Flashdiag®XF was designed to be used directly in the field, and is adapted for users without laboratory experience. From a symptomatic plant leaf/petiole, and in less than one hour, the test clearly indicates the presence or absence of *X. fastidiosa* for a given plant sample. In 2017, DNA samples from infected plants of olive, almond, oleander, cherry, *Polygala mirtifolia*, laurel, lavender and rosemary, collected by the University of Bari Aldo Moro, will be tested. In 2018, the kit will be tested with other host species. A field validation will be conducted by the end of 2017 on olive trees in Apulia (Italy), to test the kit in field conditions with a high number of samples. Flashdiag®XF aims to provide a rapid diagnostic leading to quick monitoring of *X. fastidiosa* the pathogen in a field-based, user-friendly format.

This project was supported by Bpifrance (the French Public Investment Bank).

The Android application XylApp for the survey of *Xylella fastidiosa* infections. F. SANTORO, S. GUALANO, G. FAVIA, A.M. D'ONGHIA. *CIHEAM - Istituto Agronomico Mediterraneo di Bari, Via Ceglie 23, Valenzano (BA) 70010, Italy.*

Xylella fastidiosa is an important quarantine bacterium, vector-transmitted, which infects more than 380 plant species worldwide. Following the EU implemention of Decision 789/2015, surveys for *X. fastidiosa* are mandatory in member states, but these suveys time- and human resources- consuming, and require accuracy in field data acquisition and rapid data transmission. Support for inspectors could be provided by handheld devices, such smartphones and tablets. A dedicated application for Android smart devices, named 'XylApp', has been designed and developed for the accuracy in the monitoring activity of *X. fastidiosa* in Apulia, Italy. Improved versions of XylApp have been made to enhance the accuracy and rapid use of survey data, and to support statistical analyses for the epidemiological studies. The version dedicated to inspectors is composed of five independent modules: 'Sample', for data acquisition and geolocalization without map support; 'Browse and Sample', for data acquisition and geolocalization using the regional cartographic grid; 'Find', for finding one or more targets through geographic coordinates; 'Archives', for field data storage and transmission to a remote database; and 'Vademecum', for providing inspectors with a valuable information as a practical guide. An additional module is 'Improve localization', for manual improvement of geolocalization. A light version of the application, composed of three modules (Check, Mail, Learn), was also developed for rapid reporting of suspected symptomatic host plants by stakeholders ($XylApp_{SH}$). XylApp facilitates, optimizes and rationalizes data acquisition, geolocalization, storage and realtime transmission to the a central server of the Plant Protection Service.

Flashdiag®XF Kit, a rapid field diagnostic tool for detection of *Xylella fastidiosa*. T. VANDEWALLE[1], K. OULDELKABLA[1], C. FABRE[1], G. LOCONSOLE[2], M. MASSON[1]. [1]*Anova-Plus, 4 rue Pierre Fontaine, Genopole Campus 3, Evry 91030, France.* [2]*Department of "Scienze del Suolo, della Pianta e degli Alimenti", University of Bari Aldo Moro, Via Amendola, 165/A 70126 Bari, Italy. E-mail: thomas.vandewalle@anova-plus.com*

Anova-Plus is developing Flashdiag®XF, a diagnostic kit for field use, that will detect *Xylella fastidiosa* from a wide spectrum of plant hosts using DNA isothermal amplification. *Xylella fastidiosa* samples from public collections were obtained for sub-species *X. f. fastidiosa, X. f. pauca* and *X. f. multiplex*, isolated from several plant species (grapevine, coffee, almond, ol-

ive and plum). The isothermal amplification method has been conceived to test, in one reaction, all the above sub-species. DNA from healthy plants was been used as negative control, and absence of cross-reaction has been ensured with closely related species (i.e. several *Xanthomonas* species). Flashdiag®XF has been designed for field use, and is adapted for users without laboratory experience. From a symptomatic plant leaf/petiole and in less than 1 h, the test will clearly indicate the presence or absence of *X. fastidiosa* for a given plant sample. In 2017, DNA samples from infected plants of olive, almond, oleander, cherry, *Polygala mirtifolia*, laurel, lavender and rosemary, collected by the University of Bari Aldo Moro, will be tested. In 2018, the kit will be tested on other host species. Field validation will be conducted by the end of 2017 on olive trees in Apulia (Italy), to test the kit in field conditions with a high number of samples. Flashdiag®XF aims to provide rapid diagnosis leading to efficient monitoring of *X. fastidiosa* in a field-based, user-friendly format.

This project was supported by the French Public Investment Bank (Bpifrance).

The emergence of *Xylella fastidiosa* in the Balearic Islands, Spain, is associated with several subspecies and sequence types of the bacterium. D. OLMO[1], M. MONTES-BORREGO[2], A. NIETO[1], F. ADROVER[1], A. URBANO[3], O. BEIDAS[4], A. JUAN[4], E. MARCO[5], M.M. LÓPEZ[5], I. NAVARRO[5], A. MONTERDE[5], J.A. NAVAS-CORTÉS[2], B.B. LANDA[2]. [1]*Serveis de Millora Agrària. Govern Balear. Eusebi Estada 145. 07009, Palma de Mallorca, Spain.* [2]*Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain.* [3]*TRAGSA, Empresa de transformación Agraria, Delegación de Baleares. Pasaje Cala Figuera, 6. 07009, Palma.* [4] *Servicio de Agricultura. Conselleria de Medi Ambient, Agricultura i Pesca. C/Reina Constança, 4. 07006 Palma.* [5]*Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada-Náquera km 4,5, Moncada 46113, Valencia, Spain*

Xylella fastidiosa is a quarantine organism in the European Union (EU), that was first detected in Europe in Italy in 2013 where it is associated to a severe epidemic on olive trees. The bacterium has also been detected in France (2015) and Germany (2016). Due to the recent outbreaks and to different interceptions, mainly on ornamental coffee plants, the EU has implemented annual surveys in its member states to prevent new introductions or the spread of this harmful organism. During official surveys in late autumn 2016 in Mallorca Island, Spain, the bacterium was first detected in a garden centre near the locality of Manacor. Since then a total of 189 positive samples in 11 different host species have been found in different disease foci in the islands of Mallorca (124), Menorca (16) and Ibiza (49). Sequence analysis of the RNA polymerase sigma 70 factor sequence and multilocus sequence analysis (MLST)/typing revealed the presence of *X. fastidiosa* subsp. *fastidiosa* ST1 and *X. fastidiosa* subsp. *multiplex* ST6* (a new ST closest to ST6) and ST7 in Mallorca island, *X. fastidiosa* subsp. *multiplex* ST6* in Menorca island, and *X. fastidiosa* subsp. *pauca* ST80 (a new ST) in Ibiza island. *Polygala myrtifolia* was found to be infected by all subspecies and ST types. These results suggest that the emergence of *X. fastidiosa* in the Balearic Islands is likely due to several introduction events of diverse strains and different subspecies. Eradication measures were taken in the garden centre according to the Spanish contingency plan and EU legislation. Following the Commission Decision 2015/789/EU of establishing a 10 km radius delimiting buffer zone for each infection focus, 80% of the territory of Mallorca 50% of Menorca, and 90% of Ibiza are considered as demarcated areas. The best strategies to control the different outbreaks are under study.

This study was supported by funding from the European Union's Horizon 2020 research and innovation programme, under grant agreements No. 635646 POnTE (Pest Organisms Threatening Europe) and No. 727987 XF-ACTORS (Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy).

Fast and sensitive detection for *Xylella fastidiosa* through recombinase polymerase amplification. R. LI[1], P. RUSSELL[1], S. ZHANG[1], B. DAVENPORT[1], A. EADS[1], K. SCHUETZ[1], S. BERKANI[2], M. AMATO[2]. [1]*Agdia Inc., Elkhart, IN 46514, U.S.A.* [2]*Agdia-EMEA, 91350 Grigny, France. E-mail: Rugang.li@agdia.com*

Xylella fastidiosa (Xf), living and multiplying in host xylem systems, is regulated in many countries. Xf originates from the American continent. In recent years the pathogen has appeared in Mediterranean

countries, including Italy, France, and Spain, and is causing grave concern through damage in olive trees of southern Italy and rapid spread to other crops and areas. The genetic diversity of Xf indicates that these new introductions are independent of one another. Therefore, a fast and sensitive detection method is required to reduce the likelihood of Xf introduction into new areas. Agdia has developed a rapid and sensitive DNA test for specific detection of Xf, using advanced recombinase-polymerase amplification technology (AmplifyRP). The assay performs both as a real-time and an endpoint test, from a single reaction tube held at 39°C for 20 min. Reaction template is simply prepared by soaking 50 mg of plant petiole cross-sections in 0.5 mL AMP1 extraction buffer for 10 min, by suspending one culture colony in 100 µL AMP1 buffer. The assay reacts to 28 Xf isolates, from grapevine, citrus, olive, almond, coffee, oleander, mulberry, American elm, sycamore, oak, blueberry, and blackberry, while consistently detecting 22 (and even less) copies of spiked Xf genome in soaking extract (1:10, W/V). No reaction background was observed in host tissues. No cross-reaction was observed to *Xanthomonas*, *Erwinia*, *Pseudomonas*, and *E. coli*. This test provides users with a reliable tool to assist against Xf spread as it can be performed directly on site.

This research is supported by Agdia, Inc.

Isolation , genetic characterization and phenotipic profiling of *Xylella fastidiosa* strains from Costa Rica. N. RODRÍGUEZ-MURILLO, I. ABDALLAH-QUIROS, A. BADILLA-LOBO, G. GONZÁLEZ-ESPINOZA, C. CHACÓN-DÍAZ. *Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San Pedro 2060, Costa Rica. E-mail: carlos.chacondiaz@ucr.ac.cr.*

Xylella fastidiosa is endemic in Costa Rica. In the last decade this pathogen has been detected and isolated from more than 20 different economically important crops and ornamentals, extending the geographic range of detection of the bacterium. However, although *X. fastidiosa* has great potential to cause disease, and is widespread througout Costa Rica, the symptoms related to infected plants are usually mild or infections are asymptomatic. In recent years, the presence of *X. fastidiosa* in Europe has had important social and economic consequences, and also in plant exporting countries such as Costa Rica. From previous reports it is known that *X. fastidiosa* strains isolated from Costa Rica have broader genetic variability than strais in other countries. There is genetic similarity among ST53 isolates from Costa Rica and the CoDiRo strains from affected olives in Italy. The parallel study *of X. fastidiosa* circulating strains from Costa Rica can contribute to outline of specific traits of the European *X. fastidiosa* strains. We isolated and characterized *X. fastidiosa* strains from different hosts to broaden genetic and phenotipic information on our circulating strains. We have isolated *X. fastidiosa* ST33, ST21 and ST61 strains from coffee and ST33 from guava, and these sequence types are related to *X. fastidiosa* subspecies *fastidiosa*. Complementary to genetic profiling, we are phenotipically characterizing our strains through biochemical and fatty acid profiling and through biofilm formation assays. Our goal is to standarize a series of *In vitro* assays that could eventually be used in reference and research units for *X. fastidiosa* profiling.

This research was supported by the European Union's Horizon 2020 research and innovation programme, under grant agreements No. 635646: POnTE (Pest Organisms Threatening Europe) and No. 727987: XF-ACTORS (*Xylella fastidiosa* active containment through a multidisciplinary oriented research strategy).

A new molecular LAMP tool for *Xylella fastidiosa* early detection. C. AGLIETTI[1], L. GHELARDINI[1,2], P. CAPRETTI[1], A. SANTINI[2], N. LUCHI[2]. *[1]Department of Agrifood production and Enviromental Sciences (DISPAA), University of Florence, Piazzale delle Cascine 18, 50144, Firenze, Italy. [2]Institute for Sustainable Plant Protection- National Research Council (IPSP-CNR), Via Madonna del piano 10, 50019, Sesto fiorentino (Firenze), Italy. E-mail: chiara.aglietti@unifi.it*

Xylella fastidiosa is a Gram-negative bacterium that causes considerable economic damage by xylem occludion in over 200 different plant hosts. This pathogen was confined to America until 2013, when it was found in Italy (Apulia) and thought to be responsible of olive quick decline syndrome. The pathogen was also reported in Europe on oleander (*Nerium oleander*) and on *Polygala myrtifolia.* As an invasive pathogen, its spread might cause severe environmental and economic damage. An effective control

plan is necessary to contain *X. fastidiosa* impacts, and this requires specific and sensitive diagnostic tools. PCR-based methods are favoured for their sensitivity and specificity, but these require laboratory facilities. Advantages might be gained from moving testing closer to sampling sites. A diagnostic assay based on loop mediated isothermal amplification (LAMP) was developed to detect *X. fastidiosa*. This assay, optimized on the portable instrument Genie II (Optigene, UK) and based on RimM target region, can recognize the pathogen DNA with high levels of specificity, identifying only *X. fastidiosa*, and sensitivity, detecting DNA as little as 0.128 pg/μL, equalling results obtained with the compared *X. fastidiosa* qPCR assay. The LAMP method used for detecting *X. fastidiosa* on symptomatic and asymptomatic samples could assist checking of imported and exported live plants, limiting the uncontrolled spread of this pathogen. Simplicity, sensitivity and specificity, high speed (only 30 min) and minimum required equipment make the assay ideal for field applications, helping routine plant testing in cities and forests.

Wood, root and foliar diseases in fruit and forest crops in the Mediterranean region

Pine wilt disease: insights into the biology of *Bursaphelenchus xylophilus*-associated *Serratia*. C.S.L. VICENTE[1], K. HASEGAWA[2], M. MOTA[1]. [1]*ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.* [2]*Department of Environmental Biology, Chubu University, Kasugai, Japan. E-mail: cvicente@uevora.pt*

Pine wilt disease (PWD) is caused by the parasitic nematode *Bursaphelenchus xylophilus* (pinewood nematode; PWN), which infects mainly *Pinus* species with the aid of an insect-vector, *Monochamus* sp.. Bacteria isolated from *B. xylophilus* are being considered as a fourth element in this disease complex. Their precise roles of these organisms in this interaction are unclear, as both beneficial and pathogenic bacteria have been found associated with PWD. Previously, we have shown the high oxidative stress tolerance of the PWN-associated bacteria *Serratia* sp. LCN16 and *Serratia marcescens* PWN146, and their beneficial effects towards the nematode under harsh oxidative stress conditions. Here, we present a detailed analysis of the genome sequences of these two PWN-associated bacteria and provide new insights into their biology and contributions to PWD and the PWN. *Serratia* sp. LCN16 is phylogenetically most closely related to the phytosphere group of *Serratia*, and shares many features with endophytes (plant-associated bacteria). These include genes coding for plant polymer degrading enzymes, iron uptake/transport, siderophore and phytohormone synthesis, aromatic compound degradation and detoxification enzymes. *Serratia marcescens* PWN146 can also withstand and colonize the plant environment, without having any deleterious effects towards *B. xylophilus* nor to the nematode model *C. elegans*. PWN146 has the potential to interfere with plant metabolism via hormonal pathways or nutritional acquisition (i.e. iron), and to be competitive against other bacteria and fungi, through resource acquisition or production of antimicrobial compounds.

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Comparative study of *Pseudomonas syringae* pv. syringae strains isolated from mango trees distributed worldwide with over 25 years apart. F. APRILE, J.A. GUTIERREZ-BARRANQUERO, F.M. CAZORLA, A. DE VICENTE. *Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: aprile@uma.es*

Mango (*Mangifera indica* L.) is one of the most important world fruit crops. In 1992, the disease bacterial apical necrosis (BAN) of mango was described for the first time in southern Spain. BAN is caused by *Pseudomonas syringae* pv. syringae (Pss), and is mainly associated with Mediterranean climate. The disease has been described in other mango-producing areas with similar weather (Portugal, Italy, Israel, Egypt, Florida and northeast Australia). Different Pss isolates from mango have been studied for years, to decipher their virulence and epiphytic fitness mechanisms. Genes associated with these biological characteristics have been described: *mbo* operon involved in the mangotoxin production, *cop*ABCD or *cus*CBA

operons involved in copper resistance, and the production of cellulose by *wss* genes. Phylogenetic studies have revealed a differentiated phylotype of the Pss strains isolated from mango characterized by mangotoxin production. This study analysed epidemiology and evolution of different Pss isolates from mango from different Spain, Portugal, Italy, Israel and Australia, isolated in 2000 (UMA lab collection), and recently obtained isolates (2016 and 2017). A comparative genomic analysis representative strains of each collection will be carried out to unravel the evolutionary processes which have occurred during the last 25 years.

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Survey of *Cylindrocarpon*-like anamorphs in Spanish forest nurseries. B. MORA-SALA[1], A. CABRAL[2], M. LEÓN[1], C. AGUSTÍ-BRISACH[3], J. ARMENGOL[1],, P. ABAD-CAMPOS[1]. *[1]Instituto Agroforestal Mediterráneo, Universidat Politècnica de València, Camino de Vera s/n, 46022-Valencia, Spain. [2]Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal. [3]Departamento de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain. E-mail: beamosa@upvnet.upv.es.*

Cylindrocarpon-like anamorphs infect herbaceous and woody plants, mainly in agricultural situations, but also in forests. This study characterized, by DNA analysis, a wide collection of *Cylindrocarpon*-like isolates recovered from roots of a broad range of forest hosts showing decline symptoms in nurseries. From 2009 to 2012, 18 Spanish forest nurseries were surveyed and a total of 103 *Cylindrocarpon*-like isolates were obtained. The isolates were identified based on sequencing a fragment of the histone H3 gene (HIS), which was amplified by PCR with the primer pair CylH3F and CylH3R. Some isolates were additionally sequenced for the Internal Transcribed Spacer (ITS) region, and partial β-tubulin (TUB) and translation elongation factor 1-α (TEF) genes, to better resolve their phylogenetic positions. Thirteen species of *Cylindrodendrum*, *Dactylonectria, Ilyonectria* and *Neonectria* were identified from damaged roots of 15 different hosts. The species *C. alicantinum*, *D. macrodidyma*, *D. novozelandica*, *D. pauciseptata*, *D. pinicola, D. torresensis*, *I. capensis, I. cyclaminicola, I. liriodendri, I. pseudodestructans*, *I. robusta*, *I. rufa* and *Neonectria* sp. were identified. In addition, two new *Dactylonectria* and one new *Ilyonectria* species were described. This study is the first characterization of a wide collection of *Cylindrocarpon*–like anamorphs obtained from forests plants, and demonstrates the prevalence of this fungal group associated with seedlings of diverse hosts showing decline symptoms in Spanish forest nurseries.

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Simulation of the potential infectivity range of *Phytophthora cinnamomi* under climate change. M.C. CABALLERO, I.M. PÉREZ-RAMOS, L. MATÍAS, M. SERRANO. *Instituto de Recursos Naturales y Agrobiología de Sevilla, Avenida Reina Mercedes, 10, 41012, Seville, Spain. E-mail: maria.serrano@irnas.csic.es*

Quercus open-woodlands, dehesas in Spain, are one of the most important ecosystems of the Mediterranean Basin, but their sustainability and persistence could be seriously affected by global change and exotic pathogen introductions. This study examines the interactive effects of climate change and land-use (over-grazing) changes on production of sporangia by *Phytophthora cinnamomi* in high risk area of trees suffering from root rot disease. An experiment of reduced rainfall and increased temperature (simulating the future climate conditions predicted by climate change models) was set up in three dehesas systems facing different grazing intensities (low, medium or high) in open areas and below *Q. ilex* trees, during September 2016. A total of 48 replicates were established, with six replicates per site, location and climate treatment. Five months later (January 2017), soils obtained under trees were more stimulatory to sporangium production than the soils from open areas, regardless of the grazing intensity, due to release of root exudates. Preliminary results showed that the number of *P. cinnamomi* sporangia producee with the soil obtained from the low grazing intensity dehesa was greater than in the other two intensities, which did not differ. Differences in pathogen response

among the climate change treatments have not yet observed, but differences were recorded for plant composition related to treatments and sites. These differences increased during spring. Climate change and grazing effects on plant communities and their relationships with sporangium production with time will be presented.

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Increasing diversity of vegetative compatibility types in *Cryphonectria parasitica* in the Eastern Black Sea region of Turkey and its relation to sexual reproduction. E. MANGİL, O. ERİNCİK. *Adnan Menderes University, Faculty of Agriculture, The Department of Plant Protection, 09100, Aydın, Turkey. E-mail:oerincik@adu.edu.tr.*

This study aimed to determine the vegetative compatibility (vc) type diversity of *Cryphonectria parasitica* in the Eastern Black Sea Region of Turkey, and the role of sexual reproduction in this diversity. Vc types of 344 *C. parasitica* isolates collected from Artvin, Trabzon and Rize provinces in 2016 were identified by growing pairs of isolates on media. Mating types were detected using a PCR with specific primers. Single ascospore isolates were obtained from perithecia of 21 field cankers and at least 25 isolates per perithecium were subjected to vc type assay. There is large vc type diversity in the region. Among 344 isolates, 293 were compatible with the European vc testers, EU-1 (68%), EU-17 (6.7%), EU-12 (6%), and EU-3 (4%), whereas 51 isolates were not compatible with any of the European testers. The unidentified vc types were in six groups, designated TU-1 (6.4%), TU-2 (2.6%), TU-3 (1.2%), TU-4 (1.7%) and TU-5 (0.9%). MAT-1 and MAT-2 comprised, respectively, 37.4% and 55.3%, respectively. Thirteen isolates were heterokaryotic carrying both mating alleles. Perithecia were found in 130 bark samples, which indicates widespread occurrence of sexual reproduction. Number of vc types within the group of single ascospore isolates from single perithecia ranged from 1 to 13. Diversity of vc types increases in Turkey, and this increase can be partially related to the recombination of vegetative incompability genes through sexual reproduction.

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Phenotypic, molecular and pathogenic characterization of *Phlyctema vagabunda*, the cause of olive leprosy. J. ROMERO[1], M.C. RAYA[1], L.F. ROCA[1], C. AGUSTI-BRISACH[1], J. MORAL[1,2], A. TRAPERO[1]. *[1]Departamento. de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain. [2]Department of Plant Pathology, University of California, Davis, Kearney Agricultural Research and Extension Center, 9240 South Riverbend Ave., Parlier 93648, CA, USA. E-mail: ag1trcaa@uco.es*

Olive leprosy, caused by the fungus *Phlyctema vagabunda,* is a classic fruit rot disease widespread in the Mediterranean basin. From 2009-2013, new disease symptoms consisting of small circular necrotic leaf lesions, coin branch canker and shoot dieback were observed in Spanish and Portuguese olive orchards showing intense defoliation. *Neofabraea*-like fungal colonies were consistently isolated from symptomatic leaves and shoots. Representative isolates from affected leaves, shoots and fruits were characterized by morphology of colonies and conidia, optimal growth temperatures and comparison of DNA sequence data from four regions: ITS, tub-2, MIT and rpb2. Pathogenicity tests were performed on apple and olive fruits, and on branches and leaves of olive trees. Maximum mycelial growth rate ranged between 0.54 and 0.73 mm d^{-1}. Morphology of conidia produced on apple fruit and phylogenetic analysis showed homogeneity among fungal isolates, which were identified as *Phlyctema vagabunda*. On fruits, influence of wounding, ripening and cultivar resistance were studied, with cv. Blanqueta being the most susceptible cultivar. On branches, mycelial plug inoculation reproduced olive leprosy symptoms and caused shoot dieback. On leaves, Koch´s postulates were fulfilled and the pathogen caused characteristic necrotic spots and plant defoliation. Wounds had a key role on olive leprosy development. This is the first time that *Ph. vagabunda* is described as a causal agent of leaf spot and defoliation in olive trees. The integration of mechanized practices in olive crop management could be the cause of re-emergence of this disease.

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The importance of identifying the vegetative compatibility types of chesnut blight (*Cryphonectria parasitica*) at local level; case study in a chesnut stand in El Bierzo (León). A. LORENZANA[1], D. RODRÍGUEZ[1], S. MAYO[2], M.P. CAMPELO[1], F. CASTEDO-DORADO[1]. [1]*Departamento de Ingeniería y Ciencias Agrarias, Escuela Superior y Técnica de Ingeniería Agraria, Universidad de León, Campus de Ponferrada, Avda. de Portugal s/n, 24401 Ponferrada, León, Spain.* [2]*Grupo de Investigación de Ingeniería y Agricultura Sostenible, Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad, Universidad de León, Avda. de Portugal, 41, 24071 León, Spain. E-mail: alorv@unileon.es*

The most used treatment for control of chestnut blight (caused by *Cryphonectria parasitica*) is based on the use of hypovirulent fungal strains. The success of this method depends on the knowledge of the vegetative compatibility (vc) types present in the stand of chesnut trees. Our research highlights the importance of establishing the vc types at local levels, through the case study of a stand of 250 ha located in Oencia (El Bierzo, León province). The aims were: (i) to determine the prevalence of chesnut blight canker in the stand; (ii) to identify vc types existing; and (iii) to compare these vc types with the most common European vc types in El Bierzo, according to the literature. A systematic sampling was carried out in 60 plots in which samples of bark with symptoms of the disease were collected. The results indicated that more than 70% of the trees and 100% of the sampled plots were affected. Furthermore, four vc types were identified in the stand, a large numbers considering that recent studies found five vc types throughout the province of León and nine vc types in Galicia. Of the fourvc types found, only two were compatible with the European vc testers EUI and EUII. The high diversity found could be due to genetic recombinations caused by previous infections by hypovirulent strains.

Diversity of subtypes of *Cryphonectria hypovirus 1* in the chestnut areas of Turkey where hypovirulence is present. O. ERİNCİK, S. AÇIKGÖZ, S. HOSSEİNALİZADEH, S. YORGANCI, M.T. DÖKEN, *Adnan Menderes University, Faculty of Agriculture, The Department of Plant Protection 09100, Aydın, Turkey. E-mail:oerincik@adu.edu.tr*

Biological control, based on the use of hypovirulent strains of *Cryphonectria parasitica*, is one the most effective methods for managment of chestnut blight. Success in biological control on the subtype of *Cryphonectria hypovirus 1* (CHV1) infecting hypovirulent strains. This study aimed to determine the diversity of the subtypes of CHV1 in Turkey. In 2014 and 2015, *C. parasitica* isolates were obtained from hypovirulent-type cankers from 14 provinces of the Marmara and Black Sea Regions, where hypovirulence is present. Among the 215 double-stranded RNA (dsRNA) positive isolates, 92 CHV1-infected *C. parasitica* isolates were sampled to use in subtype determination. The dsRNA of the virus was extracted and reverse transcription (RT) PCR product was obtained, using the primer pair hvep-1 and EP721-4. This amplifies a polymophic DNA fragment from the open reading frame A region of the viral genome. Nucleotide sequence and phylogenetic analyses of the PCR products showed evidence of low diversity of subtype in CHV1 throughout the sampling area. The two subtypes of CHV1, Subtype I and Subtype F2, were found. Subtype I comprised of 78% of the isolates (76) and was dominant and found in 11 provinces. Subtype F2 accounted for 12% of the isolates (16) and was found in six provinces and restricted mostly to the Eastern Black Sea Region.

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Investigation of mycovirus double-stranded RNA in *Phomopsis viticola* isolates from grapevine in the Aegean Region of Turkey. S. ACIKGOZ, S. HOSSEINALIZADEH, O. ERINCIK. *Adnan Menderes University, Faculty Of Agriculture, Dep. of Plant Protection. E-mail: oerincik@adu.edu.tr*

Certain dsRNA viruses found in fungi have been associated with hypovirulence, and they are recommended as biological control agents in the management of several plant diseases caused by fungi. The

most successful example this strategy is the use of mycoviruses for management of chestnut blight. One of the important fungal diseases of grapevine that leads to economic damage in the Aegean Region, is dead arm (Phomopsis cane and leaf spot), caused by *Phomopsis viticola*. This study determined the presence of dsRNA in *P. viticola* isolates from grapevines in Turkey. Eighty samples were collected in 2016 from grapevine dark fissure-like lesions on canes and leaf spot symptoms, in the Manisa-Salihli and İzmir provinces of Aegean Region. A total of 75 *P. viticola* isolates were obtained. Nucleic acids were extracted from freeze-dried fungal mycelia and dsRNA was separated by cellulose CF-11 chromatography. The dsRNA electrophoretic pattern of 18-20 kb was detected in eight *P. viticola* isolates, on agarose gel. The diagnosis of this new mycoviral dsRNA in *P. viticola* has not yet been made, and it is not known whether this mycovirus is associated with hypovirulence. In future studie, the dsRNAs found be diagnosed by full genome sequence analysis. Vrulence tests will be conducted on potted grapevine plants to determine the relationship between the mycoviral dsRNA and *P. viticola* hypovirulence.

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Characterization of *Fusarium oxysporum* isolated from a young vineyard affected by grapevine decline. T. CINELLI[1], P. REVEGLIA[2], C. COMPARINI[1], M. NOCENTINI[1], A. EVIDENTE[2], L. MUGNAI[1]. [1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy.* [2]*Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy. E-mail: tamara.cinelli@unifi.it*

A young vineyard (2 years old) of cv. Pinot Gris, located in Veneto, North-Eastern Italy, showed two large areas of declining grapevines. The affected vines were stunted or dead. Sixteen plants were sampled from the two areas following a defined sampling scheme. The explanted vines showed large numbers of aerial roots, while the root systems of 87% of the vines were severely damaged. The majority of the few roots of the affected plants were fully necrotic or showed internal necrotic tissues. The necroses extended into the rootstocks and, in some, also into the scions. Fourteen of the 16 plants were mostly colonized by a single species, which was isolated from 70 to 90% of the woody tissues of the roots, of the rootstock and of the cultivar, and occasional *Cylindrocarpon*-like isolates were also obtained. For identification, multigene phylogenetic analyses were carried out. The internal transcribed spacer (ITS1-5.8S-ITS2) region and parts of the translation elongation factor 1-α (TEF1) and β-tubulin (TUB) genes of four isolates were sequenced. Nucleotide sequences were compared with those in the NCBI databases, showing a 100% identity with those belonging to *Fusarium oxysporum*. Since this is a species known for the production of phytotoxic metabolites that could have roles in symptom induction, EtOAc-pH6 and EtOAc-pH2 extracts from culture filtrates were tested on tobacco leaves. Both the extracts showed toxicity on tobacco leaves, inducing necrosis of the tissues. The phytotoxic compounds produced are currently being purified, and chemically and biologically characterized.

Grapevine trunk diseases: the relevance of disinfection of propagation material. L. MUGNAI[1], T. CINELLI[1], C. COMPARINI[1], M. NOCENTINI[1], E. BATTISTON[1], M. BENANCHI[1], F. OSTI[2], T. NEMCIK[3], S. DI MARCO[2]. [1]*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy.* [2]*Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy;* [3]*510 Las Lomas Road, Sonoma, CA 95476, USA. E-mail: laura.mugnai@unifi.it*

Grapevine trunk diseases (GTDs) are a major threat for viticulture, in all grape-growing countries. The main diseases affecting vineyards in Europe are the Esca complex: grapevine leaf stripe disease, black wood streaking, Petri disease and white rot. Cankers caused by Botryosphaeriaceae are also found with increasing frequency, associated with the death of grapevine cordons and spurs. Nursery production has a major role in producing plants strong enough to withstand aggressive wood pathogens, once they are planted in the field. At the same time, they must be as free as possible from pathogen infections at early life stages. Many years of trials have been carried out to evaluate strategies for reducing early nursery infections, comparing different new with established methods. Plant material infections by *Phaeomoniella*

chlamydospora, Phaeoacremonium minimum, and species of Botryosphaeriaceae were assessed in either non-inoculated or artificially inoculated graftings, treated with different products. Promising results were obtained in the control/limitation of GTDs pathogen infections by treatment with innovative, low impact products (e.g. electrolysed water, ozone) and biological control methods. The benefits and relevance of superior quality planting stock are only realized when subsequent agricultural activities follow well-planned and balanced vineyard management practices.

Occurence of *Hop stunt viroid* (HSVd) in Turkish pistachio trees. S.C. BALSAK, N. BUZKAN, M.Z. AY and M. GÜRBÜZ. [1]*Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaraş Sütçü Imam, 46060 Kahramanmaraş, Turkey. E-mail: nbuzkan@gmail.com*

Turkey is one of the greatest world producers of pistachio (*Pistacia vera*) after Iran and the USA. Plantations are generally in semi-arid areas of the southern part of Turkey. Recently, *Hop stunt viroid* (HSVd) (*Hostuviroid, Pospiviridae*) infection was reported from Tunisia, although information of diseases associated with viruses and viroids is scarce. HSVd has a wide host range including trees, shrubs and herbaceous plants. In Turkey, HpSVd has been detected in grapevine, plum, peach, apricot, sweet cherry and almond, by RT-PCR, but without molecular characterization. We investigated HpSVd in a pistachio tree collection in Turkey. In July 2016, leaf and shoot samples were collected from 50 pistachio trees with virus-like symptoms, from the research and experimental orchard of the University of Kahramanmaraş Sütçü Imam in the Kahramanmaras province of eastern Mediterranean. RT-PCR detection of HSVd was performed with dsRNAs using VP-19 and VP-20 primers. One sample of positive PCR was directly sequenced in two directions and was aligned with isolates from GenBank using CLUSTALX 1.8. Blast analysis of the Turkish HSVd pistachio isolate showed 99% nucleotide similarity with an HSVd isolate from Japan (Accesion number: X00009). A phylogenetic tree was constructed with 17 HSVd isolates from various hosts, using the neighbour-joining method. The Turkish HSVd isolate from pistachio trees aligned with an HSVd-grapevine isolate from Turkey. To our knowledge, this is the first report of HSVd in pistachio trees in Turkey. No symptom association was made with HSVd in pistachio trees.

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Comparative study of *Pseudomonas syringae* pv. syringae strains isolated from mango trees distributed worldwide and separated by 25 years. F. APRILE, J.A. GUTIERREZ-BARRANQUERO, F.M. CAZORLA, A. DE VICENTE. *Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: aprile@uma.es*

Mango (*Mangifera indica*) is one of the most important fruit crops in the world. In 1992, the disease bacterial apical necrosis (BAN) was described for the first time on mango in southern Spain. BAN is caused by *Pseudomonas syringae* pv. syringae (Pss), and is mainly associated with Mediterranean climate. BAN has also been described in other mango producing areas with similar weather (Portugal, Italy, Israel, Egypt, Florida and northeast Australia). Different Pss isolates from mango have been studied, to decipher their virulence and epiphytic fitness mechanisms. Different genes associated with these biological characteristics have been described: *mbo* operon involved in the mangotoxin production, *cop*ABCD or *cus*CBA operons involved in copper resistance, and *wss* genes involve with cellulose production. Phylogenetic studies have revealed the presence of a differentiated phylotype of Pss strains isolated from mango, and characterized by mangotoxin production. This study included epidemiological and evolutionary analyses of different Pss isolates from mango from different growing areas (Spain, Portugal, Italy, Israel, Australia), isolated by 2000 (UMA lab collection), and new isolates obtained in 2016 and 2017. We will perform a selection of the most representative strains of each collection, to carry out a comparative genomic analysis to unravel the evolutionary processes which have taken place through more 25 years.

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Characterization of *Colletotrichum acutatum* isolates causing almond anthracnose in Spain. A. LÓPEZ-MORAL[1], M.C. RAYA-ORTEGA[1], C. AGUSTÍ-BRISACH[1], L.F. ROCA[1], M. LOVERA[2], F. LUQUE[1], O. ARQUERO[2], A. TRAPERO[1]. [1]*Departamento de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain.* [2]*Departamento de Fruticultura Mediterránea, IFAPA, Alameda del obispo, 14004 Córdoba, Spain. E-mail: trapero@uco.es*

Almond anthracnose, caused by *Colletotrichum* spp., is a serious and emerging disease in the major almond-growing areas worldwide. All isolates causing almond anthracnose have been assigned to the *C. acutatum s.l.* complex, in which only *C. fioriniae* and *C. godetiae* have been associated with the disease. This study characterized *Colletotrichum* isolates recovered from almond fruits affected by anthracnose from ten commercial orchards located in Andalusia, between 2014 and 2016. Additionally, two *Colletotrichum* isolates causing olive anthracnose were also included for comparison. Morphological characters, mainly colony colour and conidial shape, were useful to separate the isolates within fungal groups or species. Pathogenicity tests were conducted on detached fruits from almond, olive and apple. Results showed differences in virulence and some degree of pathogenic specialization among isolates. Molecular characterization using six genomic regions was essential to clarify the identification of *Colletotrichum* isolates tested. Olive isolates were identified as *C. godetiae* and *C. nymphaeae*, which had been identified before in Andalusian olive orchards. For isolates from almond, two phylogenetic species were identified: *C. godetiae* (grey colony subpopulation), which is well known in other countries; and *C. acutatum sensu stricto*, (pink colony subpopulation), which was more virulent and did not match with *C. fioriniae*, the pink colony subpopulation described in other countries. This is the first report of a new *Colletotrichum* species causing almond anthracnose within the *C. acutatum s.l.* complex.

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Phenotypic, molecular and pathogenic characterization of *Phlyctema vagabunda*, causal agent of olive leprosy. J. ROMERO[1], M.C. RAYA[1], L.F. ROCA[1], C. AGUSTI-BRISACH[1], J. MORAL[1,2], A. TRAPERO[1]. [1]*Departamento. de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain.* [2]*Department of Plant Pathology, University of California, Davis, Kearney Agricultural Research and Extension Center, 9240 South Riverbend Ave., Parlier 93648, CA, USA. E-mail: ag1trcaa@uco.es*

Olive leprosy, caused by *Phlyctema vagabunda,* is a classic fruit rot disease widespread in the Mediterranean region. From 2009-2013, new disease symptoms consisting of small circular necrotic leaf lesions, coin branch canker and shoot dieback were observed in Spanish and Portuguese olive orchards showing intense defoliation. *Neofabraea*-like fungal colonies were consistently isolated from symptomatic leaves and shoots. Representative isolates from affected leaves, shoots and fruits were characterized using morphology of colonies and conidia, optimal growth temperature and comparison of DNA sequence data from four regions: ITS, tub-2, MIT and rpb2. Pathogenicity tests were also performed on apple and olive fruits, and on branches and leaves of olive trees. Maximum mean mycelium growth rate ranged from 0.54 to 0.73 mm d^{-1}. Morphology of conidia produced on apple fruit and phylogenetic analysis showed homogeneity among fungal isolates, which were identified as *Phlyctema vagabunda*. On fruits, influence of wounding, ripening and cultivar resistance was studied, with cv. Blanqueta being the most susceptible cultivar. On branches, mycelial-plug inoculation reproduced olive leprosy symptoms and caused shoot dieback. On leaves, Koch´s postulates were fulfilled, and the pathogen caused characteristic necrotic spots and plant defoliation. Wounds had a key role on olive leprosy development. This is the first description of *Ph. vagabunda* as a causal agent of leaf spot and defoliation in olive trees. The integration of mechanized practices in olive crop management could be the cause of the disease re-emergence.

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Unravelling the beta diversity of plant-parasitic nematodes associated with cultivated olive in southern Spain. A. ARCHIDONA-YUSTE[1], T. WIEGAND[2], P. CASTILLO[1], J.A. NAVAS-CORTÉS[1]. [1]*Instituto de Agricultura Sostenible, CSIC, Avenida Menéndez Pidal s/n, 14004-Córdoba, Spain.* [2]*Helmoltz Centre for Environmental Research-UFZ, Leipzig, Germany. E-mail: aarchidona@ias.csic.es*

Olive trees host many plant-parasitic nematodes (PPNs). Understanding the factors that maintain biodiversity in communities depends on identification of diversity. We investigated the effects of environmental conditions, soil properties, agronomic management practices and spatial structure, on the variation of species community composition (β-diversity) and species richness of PPNs infesting rhizosphere soil from 376 commercial olive orchards widely distributed in Andalusia, southern Spain. We identified 128 species of PPNs with different feeding behaviours. Constrained ordination analysis showed that all explanatory variables together accounted for approx. 13% of the variation of community composition and 30% of species richness. These low values showed that spatial variability in the distribution of plant-parasitic nematodes is generally very stochastic. Also, with redundancy analysis and variation partitioning, we determined the relative importance of environmental conditions, soil properties, agronomic management and spatial structure, as well as different tendencies among species composition and richness. Environment (6% of community composition variance), soil (35%), agronomic management (7%) and spatial structure (18%) explained variance from the total explained variance of community composition. For species richness, environment explained 0% of variance, soil 5%, agronomic management 14%, and spatial structure 34%. Overall, the diversity of PPNs species infesting soils from cultivated olive is mainly influenced by land properties and spatial habitat, and to a lesser extent by environmental conditions and agronomic management.

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A new selective growth medium for *Phaeoacremonium aleophilum*, a first colonizer in grapevine trunk disease. G. CARRO-HUERGA[1], J.A. RUBIO[2], E. BARAJAS[2], S. MAYO[1], A. RODRÍGUEZ-GONZÁLEZ[1], V. SUAREZ-VILLANUEVA[1], O. GONZÁLEZ-LÓPEZ[1], S. GUTIÉRREZ[3], P.A. CASQUERO[1]. [1]*Research Group of Engineering and Sustainable Agriculture, Natural Resources Institute, Universidad deLeón, León, Spain.* [2]*Unidad de Cultivos Leñosos y Horticolas ITACYL, Junta de Castilla y León. Ctra. Burgos km 119, Finca Zamadueñas 47071, Valladolid, Spain.* [3]*Research Group of Engineering and Sustainable Agriculture, Area of Microbiology, University School of Agricultural Engineers, Universidad de León, Ponferrada, Spain. E-mails: gcarh@unileon.es; pacasl@unileon.es*

Phaeoacremonium aleophilum is one of the first colonizers in grapevine trunk disease, and is the main pathogen isolated in Castile-Leon vineyards. The general growth media PDA and MEA have been used for growing this pathogen, but its growth rate is very slow compared to other grapevine trunk pathogens. A new growth medium, containing vine sawdust, dextrose and agar (VSDA), has been assayed, and compared with PDA (potato, dextrose and agar). Using compounds from vines could improve pathogen growth rates, and provide information on which compounds have roles in development of disease. *Phaeoacremonium aleophilum* strain Y-38-05-03-a from ITACyL was taken from 14-d-old mycelium. This strain was put onto VSDA and PDA in Petri plates, and the plates were each marked with two perpendicular crosses. The experiment was repeated, using four replicates of each time. The plates were incubated at 28ºC in a phytotron for 15 d, and colony growth (mean colony diameters) was then measured. In VSDA, mean *P. aleophilum* colony diameter was 2.49 (±0.25) (typical error 0.09), and on PDA was significantly less ($P < 0.05$) (1.25 ±0.17) (typical error 0.06), as indicated by Tukey LSD tests. After this positive first assay, other parameters fungal will be evaluated on VSDA, including spore production, growth of other *Phaeoacremonium aleophilum* strains, and other growth media.