

## Abstract

**Abstracts of invited, oral and poster papers presented at the 16th Congress of the Mediterranean Phytopathological Union, April 4–8, 2022, in Limassol, Cyprus**

The 16th Congress of the Mediterranean Phytopathological Union (MPU2022) took place in Limassol, Cyprus from 4–8 April, 2022. Plant pathologists from the region and beyond celebrated the 60th anniversary of the Union and the 62 years of publication of *Phytopathologia Mediterranea* as a top level plant pathology journal.

MPU2022 entitled “Safeguarding Mediterranean Plant Health” promoted dissemination of the latest scientific advances, and facilitated dialogue and collaboration between researchers interested in all aspects of Phytopathology. This conference also addressed wider perspectives in scientific fields not previously explored at MPU Congresses, including abiotic stresses, biopesticides, forest pathology, smart agriculture and gender equality in research, funding, teaching and professional development.

Papers presented at the Congress included 83 oral presentations, including 15 keynote presentations from leading scientists, and 63 poster presentations, covering all areas of plant pathology and plant stress research, from 130 participants. Five special sessions in cutting edge subjects were organised. A special session was organised jointly with the Arab Society of Plant Protection. All these scientific contributions are part of this issue.

**KEYNOTE LECTURES AND INVITED TALKS**

**Euphresco: a platform to link research and policy in the Mediterranean area and beyond.** B. GIOVANI, I. AL-JBOORY, M. CHOUIBANI, N. HORN, L. MUGNAI and A.M. D'ONGHIA. *Euphresco network for phytosanitary research coordination and funding. E-mail: bgiovani@euphresco.net*

Mediterranean agriculture, forests and other environments are threatened by numerous quarantine and emerging pests. The negative impacts caused by these pests are expected to increase due to acceleration of global trade and climate change, that respectively favour movement of these organisms over long distances and facilitate their adaptation to and establishment in new environments. In the face of these challenges, the Mediterranean region is particularly vulnerable, due to the shortcomings in national quarantine systems, limited expertise and phytosanitary infrastructures, and the lack of funds for research activities in support of statutory plant health. The strengthening of research in the field of plant health is one of the main challenges that countries

in the Mediterranean region have to address. The diversity of priorities, both for pests and infrastructures and skills, has reduced the impacts of national efforts, but plant health challenges require rethinking of the organization of research activities in all countries and their coordination, to increase efficiency and impacts. Coordination at the Mediterranean level will reduce fragmentation of actions; it will promote convergence of national programmes; it will build critical mass. Several Mediterranean organizations and initiatives have joined forces to improve international collaboration in, and coordination of, research efforts on plant health and plant protection for Mediterranean countries. The various activities undertaken to date, and the results of discussions with Mediterranean country representatives, will be presented.

**CIHEAM policy in plant health to enhance food security in the Mediterranean region.** A.M. D'ONGHIA. *Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy. E-mail: donghia@iamb.it*

Most pests and diseases affecting Mediterranean crops are seriously compromising food security, and the sustainability of rural populations in several countries in the Mediterranean region. The importance of Plant Health has been clearly highlighted by the United Nations, that declared 2020 as the International Year of Plant Health. CIHEAM, an intergovernmental Organization of 13 Member States, has a long experience in plant health, which is one of the main pillars of the CIHEAM Strategic Agenda 2025 – adopted in 2016 by the Ministries of Agriculture of CIHEAM Member States. This is approached through: training of researchers, officers, professionals; applied research linking local scientists with the international research communities; participatory governance that encourages discussions and interactions among scientific, institutional and private stakeholders; and cooperational development of programmes enhancing country capacity building and raising of awareness. The CIHEAM Institute of Bari, in particular, has more than 30 years of experience in plant health, implementing and financing numerous international research initiatives in the Mediterranean region and neighbouring countries (e.g. Iran, Sultanate of Oman, Iraq). It is also engaged in initiatives funded by the Italian Cooperation, addressing plant health in several countries (in the Mediterranean, Balkan, African, Near eastern and Middle eastern regions). These initiatives have delivered institutional capacity building and technical assistance to many officials of national and local entities. Furthermore, in line with the statutory mission of CIHEAM Bari (higher education, research and development cooperation), since 1985 the Institute has delivered specialized and post-graduate diplomas on Integrated Pest Management to thousands of trainees, from over 50 countries. Particular attention of CIHEAM is on early surveillance, detection and control of transboundary pests and diseases which may seriously threaten crops, environments and sustainability of rural populations (e.g. *Xylella fastidiosa*). The achieved results have demonstrated that it is possible to ensure movement towards sustainable production if quarantine measures are strengthened and comprehensive phytosanitary management is applied. CIHEAM initiatives are therefore focusing on harmonizing national rules with the EU technical/ phytosanitary/legal standards, in order to safeguard agriculture and promote improvement in domestic and export trading of safer food in conformity with Governments' strategic plans.

**Plant biology and climate emergency.** D. INZE. VIB-UGent Center for Plant Systems Biology, Belgium. E-mail: [dirk.inze@psb.vib-ugent.be](mailto:dirk.inze@psb.vib-ugent.be)

The world is experiencing an unprecedented climate crisis that requires urgent action at all fronts. Plant biology offers great opportunities for adapting to climate change and to mitigate accumulation of greenhouse gasses. Higher temperatures and longer drought episodes will greatly reduce crop productivity in many areas, including the Mediterranean basin. Understanding how plants adjust their growth and development in response of environmental cues such as drought is the first step towards breeding and/or engineering stress tolerant crops. This paper illustrated the challenges of achieving this ambitious goal, by research on drought responses in maize. The molecular and phenotypic comparison of drought response of maize plants cultivated in growth chamber, greenhouse and field conditions highlighted the importance of applying an experimental 'lab to field to lab' approach. Furthermore, drought responses involve many interacting genes. Genome editing offers new perspectives to tackle complex multi-genic traits such as drought and to engineer drought tolerant crops. Plants have evolved to efficiently use CO<sub>2</sub> for growth and development. Selection of plants with improved CO<sub>2</sub> sequestration capabilities above ground and below ground is likely to become a valuable tool to combat climate change. These plants should also be resilient to other environmental stresses, and show a low dependency on fertilizers. Various approaches to develop such plants for climate emergency were outlined.

**Gender in plant health and pathology: Exploring the research agenda.** M. VAN DER BURG. *Gender Studies, Social Sciences, Wageningen University, NL*. E-mail: [margreet.vandenburg@wur.nl](mailto:margreet.vandenburg@wur.nl)

This presentation takes the audience on a short journey to explore gender in the plant health and pathology research agenda. First explained is the importance of the EU efforts to promote gender integration in research organisations in both their operational functioning (culture, recruitment and career, work-life balance, and governance) as well as their research and educational content. Since 2022 the EU made having a Gender Equality Plan (GEP) conditional to EU funding. Second, it is illustrated with historical and contemporary examples how gender differences are deeply engrained in plant health and pathology. These examples clearly under-

line the awareness of the societal importance of this research. Though, it also has traditionally overlooked its bias in campaigns which unconsciously reflected and reinforced stereotypes of gender behaviour and white supremacy; it also did not anticipate in preventing harmful social impacts. Third, a transgression from sole component approaches to socially connected system approaches (e.g., pest and weed integrated management) are providing opportunities to include social context and impacts into the research design and research circle. It helps to reckon with that wherever interventions on farm or natural resources environment are suggested, all other parts in the system from the micro to the macro level will be influenced. This certainly includes the people who are directly or indirectly affected, often differently according to gender or other social axes of inequality. To contribute to equality is definitely mandatory to address social impact and people as actors to anticipate on contributing to inequality and transform research accordingly.

This presentation was financially supported by the *Gender-SMART* project, EU-Horizon 2020-no 824546

**Molecular host-microbe interactions in plants: from microbes to host innate immunity.** P.F. SARRIS. *Department of Biology, University of Crete, 714 09 Heraklion, Crete, Greece; Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece; Biosciences, University of Exeter, Exeter, United Kingdom. E-mail: p.sarris@imbb.forth.gr*

In their long history of interspecific interaction, plants and invading microorganisms – beneficial or pathogenic – are constantly involved in complex co-evolutionary dynamics. Several plant-associated microorganisms have acquired sophisticated colonization mechanisms to manipulate the physiological processes of their hosts. These mechanisms also include specialised proteins, known as “effectors”, that are utilized by microbes to promote colonization. However, their molecular function(s) and their targets in host cells, are largely unknown for many of these effectors. This is a major question in Molecular Plant-Microbe Interactions (MPMI) research. However, effector activities/functions can be used as tools to identify important components of plant innate immunity and physiology, that could lead to innovative strategies for crop improvement. Plants have also evolved well-organized and complex innate immunity systems, to recognize pathogen invasion. The recognition occurs through perception of microbial

structural patterns, and the secreted effector proteins, by specialized transmembrane or intracellular receptors of host cells. The microbial effectors are mainly recognised, in resistant hosts, by members of the (Nucleotide-binding Leucine-rich Repeat (NLR) receptors. The elucidation of plant NLR molecular functions will assist future development of novel and/or synthetic immune receptors with new recognition capacities in crop plants.

**Enabling genome editing for European agriculture: will scientific evidence prevail?** D. INZE. *VIB-UGent Center for Plant Systems Biology, Belgium. E-mail: dirk.inze@psb.vib-ugent.be*

Agriculture and food production must become more sustainable in a world facing growing population under changing climate conditions and environmental degradation. All possible approaches, including improved plant breeding technologies, are essential to address these challenges. To develop new crop varieties, scientists and plant breeders need to have access to the array of breeding tools. The most recent addition is precision breeding, also known as genome editing, with a preference for CRISPR. This technology allows scientists and breeders to develop desired crop varieties in rapid, relatively simple and directed ways compared to older breeding technologies. However, there are legal and procedural uncertainties regarding the status of genome edited crops in Europe. While CRISPR technology is being increasingly adopted, the current regulatory framework remains outdated. Moreover, the European Court of Justice (ECJ) ruling from 2018 (C-528/16) brought increased confusion, because of the interpretation that crops obtained by precision breeding are subject to the GMO regulatory provisions. This regulatory burden even applies to crops with small, specific CRISPR-mediated DNA alterations, that can also occur spontaneously in nature. The ruling leads to inconsistency in the legislation, because conventional mutagenesis - which results in thousands of random DNA alterations - is exempt from the GMO regulatory provisions. The legislation no longer correctly reflects the current state of scientific knowledge. Subjecting genome-edited crops to the current EU GMO regulation will also delay development of climate-resilient crops, hinder progress in sustainable agriculture, reduce the EU competitiveness and hamper global trade. The ruling hampers cultivation of genome edited crops with beneficial traits for human or environmental health, as improved nutritional composition, increased digestibility, reduced contents of anti-nutritional components, reduced allergenicity

or requiring reduced production inputs. The scientific community in Europe responded unitedly, and published a position paper and an open statement to call upon the European Commission and the European Parliament. With an increasing number of signatories, currently 129, European research institutions and organizations, from 21 Member States and the United Kingdom, the European Sustainable Agriculture through Genome Editing EU-SAGE network was launched. This aims to provide information about genome editing, and promote development of European and EU member state policies that enable the use of genome editing for sustainable agriculture and food production. An update of the ongoing discussions with the European Commission was presented. Hopefully, scientific evidence will prevail.

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

Plants host different fungi and bacteria in their roots, stems, leaves, flowers, fruits and seeds. Most of these microbes are derived from the soil and rhizosphere, as recognized since the 19th century, but other sources like the anthosphere, carposphere, phyllosphere, lamiophere and caulosphere can also provide endophytes inside plant tissues. Other microbes can also be derived from animals or from plants growing near each other. Thorough understanding of the communities, routes and colonization niches of endophytes in the phytobiome has led to a better knowledge on how to use specific microbes for stimulating plant growth and increase host health, and to increase understanding of how plants shape their microbiomes. Most of the research has been done on fungi or bacteria, but recent studies further show that some bacteria can internally colonize beneficial fungi, and increase benefits to plants, leading further to a strategy of using multipartite interactions to boost plant protection.

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

*Xylella fastidiosa* (Xf) was first reported in 2016 in Majorca in the Balearic Islands, and in 2017 in Alicante province, Mainland Spain. Currently, three Xf subspecies and four STs have been detected in the Balearic Islands (subsp. *fastidiosa* ST1, subsp. *multiplex* ST7 and ST81, and subsp. *pauca* ST80). In contrast, only Xf subsp. *multiplex* ST6 has been detected in the Valencian Community, where (as at November 2021) the Demarcated Area covered an extension of >136,200 ha, of which >1,100 ha, 12,500 orchards and 90,000 trees were eradicated. The main research initiatives in Spain were outlined, which aimed at understanding Xf epidemics and mitigating its impact in Spain and Europe. These initiatives have contributed to filling knowledge gaps on Xf in Europe, gathering fundamental information on several aspects including: i) Characterizing the genetic population structures of Xf in the different EU outbreaks, including Spain, and linking the genetics of the bacterium with its pathways of entry; ii) testing and developing new diagnostic tools based on molecular and proximal- and remote-sensing approaches; iii) searching for new control tools targeting Xf in hosts or searching for host resistance; and iv) understanding the epidemiology and modelling disease development and developing risk analyses. These results have contributed knowledge to inform Spanish and EU policy related to Xf management at different spatial scales (from regional to EU levels) and socio-economic contexts.

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**The sanitary crisis caused by *Xylella fastidiosa*, a plant pathogenic bacterium recently discovered in Europe, with a focus on the situation in France.** M-A JACQUES, E DUPAS, and S CESBRON. *University of Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France. E-mail: marie-agnes.jacques@inrae.fr*

Emergence of plant pathogenic bacteria have important effects when they cause diseases with major socio-economic consequences or that threaten valuable plant production and the environment. This is the case for diseases caused by *Xylella fastidiosa* recently discovered in Europe. Ability to anticipate, prevent and mitigate this type of emerging plant pathogen depends on increased understanding of current pathogen distribution over space and time, invasion routes, conditions

favouring their emergence, and population reservoirs. Development and testing of several detection/identification methods (multiplex qPCR assays, nested MLST, metagenomics) has allowed reconstruction of the invasion scenario of *X. fastidiosa* subsp. *multiplex* ST6 and ST7 in France. These studies have relied on genome sequence analyses and tip-dating to date the divergence of French *X. fastidiosa* subsp. *multiplex* strains from their American relatives. A MultiLocus Variable Number of Tandem repeat Analysis (MLVA) on infected plant samples was developed to take advantage of both a large sample collection and the capacity of these markers to monitor recent evolutionary events. The divergence time between French strains and their American relatives suggests that previous introductions have remained unnoticed, probably because of the unspecific symptoms affecting diverse ornamental and native species in a diversified landscape. Bayesian methodologies were used to infer the number of introductions and the most probable scenario of population evolution and spread in the French regions of Corsica and PACA.

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

**Fungicide resistance in *Botrytis cinerea* populations from protected crops in the Mediterranean basin: Current status and implications with its management, fungicide sensitivity and genetic diversity of *Botrytis cinerea* populations from conventional and organic tomato and strawberry fields in Cyprus and Greece**

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*Botrytis cinerea* is one of the most destructive pathogens infecting numerous crops, including several important crops cultivated in the Mediterranean basin such greenhouse grown vegetables or strawberries. Resistance of the pathogen to fungicides used for disease management is widespread throughout the Mediterranean basin in crops heavily treated, including those cultivated in greenhouses, and resistance is associated with reduction in product performance and control failures. The current status was reviewed of resistance to major botryticide groups, including such as SDHIs, QoIs, hydroxylanilides or anilinopyrimidines, in *Botrytis* spp. populations, and detailed information was presented on methodologies

for resistance detection, variability of mutations conferring resistance to fungicides, their effects on the fungal sensitivity to the different active ingredients, and the fitness of resistant strains. Implications of cross-resistance pattern complexity and fitness cost for the resistant mutants were discussed in relation to resistance management in greenhouse grown vegetables, taking into account the recent introduction of new active ingredients into the agricultural practice, such as new SDHI molecules. Future prospects and challenges were presented, with emphasis on the roles of alternative control methods, such as the integration of Biological Control Agents (BCAs) with conventional fungicides, for reducing fungicide selection pressure on fungal populations, and thus minimizing the risk for resistance development.

**From Myco-key to myco-twin: mycotoxin management along food/feed chains.** A.F. LOGRIECO. *Institute of Sciences of Food Production, Italian National Research Council (ISPA-CNR), Via Amendola 122/O, 70126, Bari, Italy. E-mail: antonio.logrieco@ispa.cnr.it*

Fungal diseases are important contributors to the occurrence and severity of mycotoxin contamination of crop plants. Phenotypic and metabolic plasticity has enabled mycotoxigenic fungi (MF) to colonize many agriculturally important crops, and to adapt to different environmental conditions. New mycotoxin-commodity combinations provide evidence for the ability of fungi to adapt to changing conditions, and the emergence of genotypes with enhanced aggressiveness towards plants and/or altered mycotoxin production profiles. Among diseases caused by MF, the most important are from species complexes. Examples are the Fusarium ear rot of maize, Fusarium head blight (e.g. of wheat, barley, and oat), black point of wheat kernels caused by the *Alternaria alternata* species complex and related species; and various rots caused by Aspergilli. Mycotoxins in plant products and in processed food and feed have significant economic impacts and pose serious problems for animal and human health. Good pre-harvest management practices are important for minimizing risks of mycotoxin accumulation in the crops before harvest. These practices can involve crop rotation, tillage, appropriate fertilization, and fungicide or biological controls, host variety selection, timely planting and harvests, and control of insects which facilitate infections by toxigenic fungi. It is also important to prevent post-harvest contamination, and develop practical and effective post-harvest procedures to reduce mycotoxins in food supply chains,

and provide alternative and safe use options for contaminated products. An update review was presented on integrated management of pre- and post-harvest practices aiming to minimize risks of mycotoxin contamination along production chains, and main effective solutions developed by EU projects MycoKey (<http://www.mycoketwin.eu/>) and MycoTwin (<https://www.mycotwin.eu/project>).

**Fungal pathogens of wood: are they threats to Mediterranean fruit crops?** V. GUARNACCIA. *Dept. Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Largo Braccini 2, 10095 Grugliasco (TO), Italy. E-mail: vladimiro.guarnaccia@unito.it*

Intensification, diversification and globalization cause collateral effects on agriculture. The risks of disease dissemination via trade and the movement of goods and people are increasing. Climate change is also affecting plant health by modifying interactions between host plants, pathogens and the environment. Fruit tree plantations are increasingly threatened by fungal diseases. How fruit trees share fungal pathogens with woody plants not considered as trees has also been observed. Primary and opportunistic pathogens can cause cankers, twig blight and wood rotting. *Diaporthe* and *Neofusicoccum* spp. are examples of pathogens able to infect broad ranges of fruit trees. There are also numerous latent pathogens such as *Diatrypaceae* or *Cytospora* and *Neocosmospora*, and many others. Recent knowledge of fungus/host combinations was presented, with emphasis on connections between species identification development of modern diagnostic tools. Cultivar diversity, propagation material health and rootstock selection, also should be investigated in relation to the pathogens, to provide support for producers of the economically important agriculture sector.

**Plant and seed priming for improved growth and abiotic stress protection under a changing climate.** V. FOTOPOULOS. *Cyprus University of Technology. E-mail: vassilis.fotopoulos@cut.ac.cy*

Increased frequency of extreme environmental events resulting from global climatic changes influence plant growth and development. Examination of plant-to-plant communication in nature has revealed the development of unique strategies from plants for responding to abiotic stresses, including priming for improved defence responses. The process of priming involves prior expo-

sure to biotic or abiotic stress factors making plants more tolerant to future exposures. Priming can also be achieved by applying natural or synthetic compounds, which act as signalling transducers 'activating' host defence systems. An up-to-date overview was presented describing research carried out at the Cyprus University of Technology, using priming agents towards induced acclimation of plants to environmental challenges. Recent findings were also presented on evaluation of chemical compounds and nanoparticles that potentially have growth promoting properties in plants. These studies were related to existing expertise and previous observations in priming against stress.

**Cypress Canker: a model pathosystem for study of fungal invasions.** M. GARBELOTTO<sup>1</sup>, G. DELLA ROCCA<sup>2</sup>, C. EYRE<sup>1</sup>, T. OSMUNDSON<sup>1</sup> and R. DANTI<sup>2</sup>. <sup>1</sup>*Department of ESPM, 54 Mulford Hall, University of California, Berkeley, CA 94720, United States of America.* <sup>2</sup>*IPSP, National Research Council CNR, Via Madonna del Piano 10, 50127 Sesto Fiorentino, FI, Italy. E-mail: matteog@berkeley.edu*

Understanding of invasions by plant pathogens lags behind that of other biological invasions. Cypress Canker Disease (CCD), caused by *Seiridium cardinale* (Sc), is an excellent pathosystem to provide increased understanding of fungal invasions. By using a population genetics approach, Sc has been shown to be endemic to the interior of California, where the pathogen occurs exclusively on exotic, off-site or artificially-bred cypress species. The early 1920s outbreak in the interior of California was responsible for introductions of the pathogen into Italy and New Zealand. Coalescent analyses showed that the Italian population of Sc played a major role in the diffusion of the pathogen throughout the Mediterranean. Phenotypic traits in Mediterranean invasive Sc populations indicate that small conidium size, high phenotypic plasticity and high sporulation potential are key traits for a successful invasion, while high virulence and high growth rate are not necessary. Comparative analyses indicate that geographically isolated Sc populations are now genetically and phenotypically distinct, and inoculating a range of California Sc genotypes on Italian cypress has shown that resistance to CCD can be eroded by Californian genotypes. A recent study has shown that this has occurred within the last 20 years. The Mediterranean and California populations, respectively, were sources for new introductions into New Zealand and Morocco, indicating that additional introductions are possible. These data show that invasive populations can-

not be regarded analogous to the source populations that generated them.

**Innovative smart technologies for agricultural production and plant health.** T. CAFFI. *DIPROVES - Sustainable Crop and Food Protection Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, I-29122 Piacenza, Italy. E-mail: tito.caffi@unicatt.it*

Pests are defined by FAO and IPPC as “any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products”. They can cause severe enormous crop losses with damage that can occur in the field, from sowing to harvesting, and also in the product storage. Secure food supply for future generations requires environmentally safe and sustainable production. A goal of the modern agriculture is to reduce intensive fertilizer and pesticide use and decrease heavy exploitation of natural resources (water, soil, energy). Precision agriculture (PA) includes agricultural practices that focus on specific areas of a field at particular times. The main goal of PA can be summarized in doing “the right thing, at the right time, in the right place”). With recent scientific advancements, technological innovations and legislative tools, it is now possible to achieve these strategic goals and to increase agricultural sustainability. Use of Smart Technologies can contribute to enhance plant health, reduce hunger and poverty, protect the environment, and boost economic development, goals highlighted in 2020, the International Year of Plant Health. The actual availability of innovative tools and data management techniques, also leading to big data management and analysis requirement, allow consideration of integrated systems that provide phytosanitary monitoring for pilot crops. This is effective, rapid, objective, repeatable in the most varied environmental contexts, and suitable to provide appropriate support to the various phytosanitary control needs in particular regions or areas. It is possible to integrate this monitoring into modern decision support systems information, protocols, and guidance, to allow trained personnel to carry out surveys and data collection. This can be achieved through information, alerts and guidelines (photographic or video supported) provided by the systems. This approach could lead to complementary monitoring and enhancement to the forecast models, which will allow to optimization of the information and alerts provided to operators, guaranteeing clear pictures of the phytosanitary and physiological states of host plants and crops.

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

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

This research received funding from the European Union's Horizon 2020 research and innovation programmes, under grant agreements No. 773718 (OPTIMA) and No. 778219 (OchraVine Control)

**Applications of remote sensing and information technology in the surveillance of quarantine diseases of fruit tree crops.** A.M. D'ONGHIA. *Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Via Ceglie, 9 - 70010 Valenzano (BA), Italy. E-mail: donghia@iamb.it*

Early and accurate detection systems for quarantine diseases of fruit trees are essential for efficient large-scale surveillance, rapid implementation of control measures and evaluation of their effects. New technologies can also provide solutions in plant health, facilitating operators to implement efficient, accurate, timely and cost-effective real-time and large-scale surveillance programmes. Large amounts of data are required (e.g. climatic, geographical, satellite imaging, diagnostic), accurately captured at distance and on the ground, using applications, sensors, forecasting models, and specific diagnostic methods. Remote sensing (RS) can help to identify infections on large scales, even when symptoms are not yet visible. There are many platforms and sensors designed for the acquisition of remote sensing data, including from satellites, aircraft, and, more recently, Unmanned Aircraft Systems (UAS, drones). The availability of high-resolution time series of images, such as Sentinel-1 and Sentinel-2 introduced with the European Copernicus programme, has facilitated a scale-step in the use of Earth Observation (EO) data for agricultural and phytosanitary applications. In addition to RS, Information Technology (IT) provides other tools for plant protection programmes, allowing accurate storage, retrieval, transmission and manipulation of monitoring data. IT tools can be used for this purpose, including diagnostic devices, Applications (accurate on-site data acquisition), and DSS. An overview on RS and IT systems was provided, with particular reference to those already available and applied in official pathogen monitoring programmes (Citrus tristeza virus, *Xylella fastidiosa* in Apulia region, Italy) These systems include: (i) an automatic procedure for tree counting, using GeoEye-1 multispectral images; (ii) prediction maps using WorldView-2 satellite images in GIS environments for early detection of CTV-suspected trees; (iii) photointerpretation of high-resolution aerial images for identification of OQDS trees in the demarcated area of *X. fastidiosa*; (iv) applications for accurate field data acquisition by NPPOs (e.g. Apps for *Xylella fastidiosa*, XylApp, XylApp<sup>EU</sup>, XylApp<sup>NENA</sup>) and citizens (XylApp<sup>citizens</sup>); and (v) integrated DSS made of databases, forecasting models and users interfaces.

## COMMUNICATIONS

### Session organized by the Arab Society for Plant Protection

**Pathogenic viability of wheat rust diseases in the southern and eastern Mediterranean region: current**

**status, challenges, and regional collaboration.** K. NAZARI. ICARDA, Izmir, Turkey. E-mail K.Nazari@cgiar.org

Wheat yellow, stem and leaf rust diseases are continued threats to wheat production in Central West Asia and North Africa (CWANA), and the Mediterranean region. The rust pathogens are capable of rapidly developing new virulence to resistance genes, following the “boom and bust” disease cycle. Considering the transboundary nature of the rust pathogens, regional monitoring of these pathogens and information exchange are key requirements in disease resistance mitigation strategies. In response to a call for regional rust research collaboration, the Turkey-ICARDA Regional Cereal Rust Research Center (RCRRC) was established in Izmir in 2013. The Center has coordinated regional rust surveillance and Trap Nurseries, and since 2018, regional race analysis of yellow rust and stem rust was carried out using a Cereal Rust Biosafety Laboratory at RCRRC. A regional precision wheat rust phenotyping platform was also established within the RCRRC, which has provided precise field phenotyping data for more than 60,000 wheat accessions from the international and national breeding programmes. The RCRRC has received more than more than 1000 yellow rust and Stem rust samples from the Middle east and Mediterranean regions and East Africa since 2018. Using differential varieties and molecular marker approaches, it was evident that most of the the samples from these regions have common virulence structures with the European races, indicating similarity of the races and free movements of the pathogens. Regional race analysis and Trap Nurseries, and regional collaboration for precision phenotyping capacity were described.

**Epidemiology and management of legume and cereal viruses in Arab and Mediterranean regions.** S.G. KUMARI<sup>1</sup>, A. NAJAR<sup>2</sup>, N. ASAAD<sup>3</sup> and A. RAHMAN MOUKAHEL<sup>1</sup>. <sup>1</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Terbol Station, Zahle, Lebanon. <sup>2</sup>National Agricultural Research Institute of Tunisia (INRAT), Ariana, Tunisia. <sup>3</sup>General Commission for Scientific Agricultural Research (GCSAR), Al-Ghab, Hama, Syria. E-mail: s.kumari@cgiar.org

In the Arab and Mediterranean regions, cool-season food legumes (chickpea, faba bean, lentil and pea) are infected naturally by many viruses, and the number of viruses involved continues to increase. However, at any specific location only a few of these pathogens are of economic concern. Yield losses resulting from virus

attack vary widely, from little, as in the case of *Broad bean mottle virus* in Tunisia and Morocco, to complete crop failure when conditions permit widespread virus infection at the vulnerable early plant growth stage, as with *Faba bean necrotic yellows virus* in Egypt and Syria. Cereal crops (wheat, barley and oat) in Arab and Mediterranean regions are also affected by viruses, mainly *Barley yellow dwarf virus-PAV*, and this virus is reported to occur at epidemic levels in only Algeria, Morocco and Tunisia. Epidemic spread of most viral diseases was always associated with high vector populations and activity. Although virus disease management can be achieved through the combined effects of several approaches, development of resistant genotypes is one of the most promising control components. Experience over the last few decades has clearly showed that no single method of virus disease control reduces yield losses in legume crops. Some progress was made on the disease management of some legume and cereal viruses using combinations of healthy seed, host resistance, cultural practices (such as adjustments of planting date and plant density, and roguing of infected plants early in the season) and chemical control virus vectors.

**Phytoplasma and virus diseases of fruit crops in East Mediterranean countries.** E. CHOUEIRI. *Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, P.O. Box 287, Zahlé, Lebanon. E-mail: echoueiri@lari.gov.lb*

Crops of stone and pome fruits and grapes are important in the eastern Mediterranean region. Quality and quantity losses in these crops due to virus and phytoplasma infections are reported from these countries. Almond witches' broom associated with '*Candidatus Phytoplasma phoenicium*' is responsible for death of >100,000 almond and peach trees in Lebanon. In Egypt, serious economic losses due to infections with European stone fruit yellows (ESFY) on apricots and peaches have been reported. In Turkey, ESFY was mostly detected in symptomatic apricot and plum. In Jordan, aster yellows phytoplasma (16SrI) affecting peach trees was reported, in addition to recent incidence of '*Candidatus Phytoplasma solani*' infecting plum. On pome fruits, pear decline induced by *Ca P. pyri* has been observed on pear cultivars in Lebanon and in pear and quince orchards in Turkey. Some apple varieties were infected with apple proliferation disease in Turkey. *Ca. Phytoplasma solani*, the causal agent of bois noir in grapevine has been recorded in Lebanon, Syria, Turkey and Jordan associated with typical grapevine yellows, whereas *Ca. Phy-*

*toplasma omanense*' was detected in a grapevine sample from cultivar Syrah in Lebanon. Many viruses were recorded on fruit trees such as *Prunus necrotic ringspot virus*, *Prune dwarf virus*, *Apple mosaic virus* in several countries, while *American plum line pattern virus* and *plum pox virus* were detected only in some countries. On grapes, grapevine leafroll associated viruses, vitiviruses and nepoviruses (GLRaV-1, GLRaV-2, GLRaV-3, GVA, GVB, GFLV) were the most common virus pathogens.

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

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

financial, technical and scientific measures against *Xf* is necessary in each country.

This research was financially supported by the Food and Agriculture Organization of the United Nations (FAO) Regional Office for the Near East and North Africa Region.

**Fusarium head blight and crown rot of wheat in Algeria and other southern Mediterranean countries: distribution, identification and pathogenicity of associated species.** H. BOUREGHDA. *Laboratory of Phytopathology and Molecular Biology, Department of Botany, The National Higher School of Agronomy (ENSA), El Harrach, Algiers, Algeria. E-mail: hou.bouregghda@gmail.com*

Fusarium head blight (FHB) and crown rot (CR) of wheat are serious diseases which may affect yields and grain contamination by mycotoxins. FHB occurs when prolonged wet weather coincides with host anthesis. CR is a chronic problem in dry climatic conditions and where continuous wheat cropping is adopted. In the southern Mediterranean countries, climatic conditions are conducive for both diseases, that can coexist. In Algeria, CR is more widespread because wheat is commonly grown in arid and semi-arid regions, whereas FHB is restricted to humid and sub-humid areas where CR is also present. In Tunisia, FHB was reported only in the sub-humid and semi-arid upper areas. Based on published data, in Algeria, *Fusarium culmorum* was reported as the dominant species associated with both diseases, with *F. pseudograminearum* as the second causal agent. In Tunisia, *F. culmorum* was the main pathogen causing CR, but for FHB, the dominant species was *Microdochium nivale* followed by *F. culmorum*. In Morocco and Egypt, *F. culmorum* and *Bipolaris sorokiniana* were reported as major species associated with CR. In addition, *Rhizoctonia oryzae* was also associated with CR and *F. graminearum* with FHB in Egypt. Pathogenicity assessments have shown that in Algeria, *F. culmorum* was the most aggressive on wheat seedlings and heads, while *F. pseudograminearum* was the most aggressive on host crowns. In Tunisia, *F. culmorum* and *F. pseudograminearum* were the most aggressive on crowns, and in Egypt *F. culmorum* was the most aggressive pathogen.

**Evaluation of the susceptibility of improved and landrace durum wheat genotypes to *Zymoseptoria tritici* under nitrogen supply.** M. HASSINE and S. AYADI. *University of Carthage, National Agronomic Institute of*

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Appropriate nitrogen nutrition is a limiting factor for durum wheat production. This nutrient is important in agriculture practices and causes significant environmental and production costs. The fungal pathogen *Zymoseptoria tritici* is responsible for significant yield losses on durum wheat (*Triticum turgidum* ssp. *durum*) in Tunisia. The present study was conducted to evaluate effects of different nitrogen rates on development of Septoria leaf blotch (STB) and durum wheat yield components. Different levels of nitrogen (0, 50, 75, 150, 200 and 250 kg N ha<sup>-1</sup>) were applied under field conditions, to evaluate the degree of susceptibility of the improved wheat varieties Karim and Salim, and the landrace genotypes Chili and Mahmoudi. Disease severity increased from 9% without nitrogen supply to 40% under the high rate (250 kg N ha<sup>-1</sup>). The improved genotypes were more sensitive to STB (Karim 35% and Salim 51%) than the landrace genotypes (Mahmoudi 17% and Chili 12%). Decreases in thousand kernel weights (TKW) were measured with the increasing disease severity and nitrogen rates, from means of 48.58 g from 0N to 41.66 g from 250 kg N ha<sup>-1</sup>. These results provide new insights into the implications and efficiency of the use of different sources of nitrogen fertilizers in field performance of durum wheat and disease control.

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

**Determination of antibiotic residues in the endemic spurge honey (*Euphorbia resinifera* O. Berg) from Morocco, using biochip multi array technology and LC-MS/MS.** R. BENJAMAA<sup>1</sup>, A.K. ESSAMADI<sup>1</sup>, A. MOUJANNI<sup>2</sup>, B. NASSER<sup>1</sup>. <sup>1</sup>Hassan First University of Settat, Faculty of Sciences and Technologies, Laboratory of Biochemistry, Neurosciences, Natural Resources and Environment. <sup>2</sup>National Office of Food Safety (ONSSA), Avenue Hadj Ahmed Cherkaoui, Agdal, Rabat, Maroc. E-mail: essamadi@uhp.ac.ma

Antibiotic residues in honey present risk to the health of consumers, because they could be sources of allergic reactions and can lead to obtaining bacterial resistant strains to antibiotics after consumption of honey. Screening methods are the first step in controlling antibiotic residues in foods. These procedures can detect the presence of an antibiotic or group of antibiotics,

and usually provide qualitative results. A second step includes testing of positive samples for quantification, mostly by quantitative confirmation methods such as high performance liquid chromatography associated with a mass detector (HPLC-MS/MS). In this study, a total of 37 *Euphorbia resinifera* honey samples were analysed, using a screening test: Evidence Investigator™, an immuno-enzymatic method for detection of 27 antibiotic residues; followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for confirmation of suspect samples. In all tested samples, no antibiotic residues were detected, except for one showing Trimethoprim at 6.48 µg kg<sup>-1</sup>.

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

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

Pine wilt disease (PWD), caused by the nematode *Bursaphelenchus xylophilus* (pinewood nematode (PWN)), is a threat to conifer forests in Europe and Asia. *Pinus pinaster* is one of the most affected hosts due to its high susceptibility to PWN. Heritable resistance has been reported in *P. pinaster* trees, opening the possibility for host selection and breeding for this trait. To uncover the mechanisms underlying resistance, transcriptional responses were analysed after inoculation with PWN to plants showing resistant and susceptible phenotypes. Strong reprogramming of gene expression was demonstrated, particularly in resistant plants. Differential gene expression analysis indicated roles for lignin synthesis

and jasmonic acid defence pathways in resistant phenotypes, confirmed by biochemical analyses of cell wall composition and hormone pathways. Additionally, secondary metabolism, oxidative stress response and resistance genes were also probably relevant to overcome PWD. Approximately 100 miRNAs were responsive to PWN inoculation, some of which had predicted targets associated with roles in the jasmonate-response pathway, ROS detoxification and terpenoid biosynthesis. Several *P. pinaster* genes putatively targeted by PWN miRNAs were also identified, which was supported by degradome analysis. Targets for *P. pinaster* miRNAs were also predicted in PWN, suggesting a role for trans-kingdom miRNA transfer and gene silencing, both in PWN parasitism and in *P. pinaster* resistance to PWD. These results provide novel insights into transcriptional and post-transcriptional regulatory mechanisms which may be useful for the development of new strategies to protect *P. pinaster* forests from PWD.

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

*Verticillium dahliae* is a cosmopolitan soilborne pathogen with a wide host range, leading to major agricultural losses. Isolates of the pathogen are categorized as defoliating (D) and non-defoliating (ND) pathotypes, with the former being highly virulent causing distinct defoliation in cotton, okra and olive. To differentiate isolates, a new molecular marker based on the necrosis and ethylene inducing *VdNEP* gene has been developed. Southern blots probed with *VdNEP* showed fragment polymorphisms between isolates of the two pathotypes. To isolate regions flanking the gene, inverse PCR was performed on genomic DNA from D and ND reference strains of *V. dahliae*. Sequencing detected differences between the

two pathotypes in the 3' untranslated region of *VdNEP*. Based on these findings, specific primers were designed for PCR detection. The primers were evaluated by screening of a collection of *V. dahliae* isolates from olive and cotton from Greece, as well as a limited number of isolates from different parts of the world. Primer efficiency was compared with already available markers. For the D isolates, pathogenicity experiments were conducted with cotton plants to verify the molecular findings. The results indicated that the new primers are a robust and reliable tool for the differentiation of pathotypes, leading to subsequent increased chances for successful management of the pathogen.

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**Biological control of *Aspergillus carbonarius* and *Botrytis cinerea* in grapevine berries, and transcriptomic changes of genes encoding pathogenesis-related (PR) proteins.** D. GKIZI<sup>1</sup>, E. POULAKI<sup>2</sup> and S.E. TJAMOS<sup>2</sup>.

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*Botrytis cinerea* and *Aspergillus carbonarius* are among the most important widespread pathogens causing bunch rot of grapevine berries, resulting in significant economic losses. Additionally, *A. carbonarius*, although a secondary invader, is the main source of ochratoxin A in grapes. The plant protective activity of the biological control agents, *Bacillus velezensis* (previously *Paenibacillus alvei*) K165, *Blastobotrys* sp. FP12 and *Arthrobacter* sp. FP15 was examined against *B. cinerea* and *A. carbonarius* on grapes. Preliminary dual culture experiments showed reductions of fungus growth by K165, while FP15 restricted only the growth of *A. carbonarius*, and FP12 was ineffective. *Ex vivo* experiments on grape berries showed that K165, FP12 and FP15 reduced *A. carbonarius* rot severity by, respectively, 81%, 57% and 37%, compared to controls. Growth of *B. cinerea* in berries was reduced only by K165 (by 75%). Gene expression analyses of pathogenesis-related proteins PR2, PR3, PR4 and PR5, using real-time PCR, indicated activation of multiple defence responses involved in the biocontrol activity of the assessed biocontrol agents.

**Comparative proteome analysis provides new insights into the complex responses of *Citrus aurantium* grafted with *C. sinensis* and infected with Citrus tristeza virus.** M. TRINDADE<sup>1,§</sup>, S.A. DANDLEN<sup>2,§</sup>, L. ANJOS<sup>1</sup>, A. DUARTE<sup>2</sup>, D.M. POWER<sup>1</sup>, N.T. MARQUES<sup>3</sup>.

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Sour orange (*Citrus aurantium* L.; CA) has low susceptibility to Citrus tristeza virus (CTV). When sweet orange is grafted onto CA and is infected with a quick decline CTV isolate, a quick decline syndrome may develop. This syndrome is related to obliteration and necrosis of the sieve tubes in rootstocks close to the bud unions, which culminate in dieback symptoms. To investigate the molecular response of CA to CTV, CA stem tissues below bud unions were analysed by SWATH-MS in 2-year-old plants, infected or uninfected with the quick decline isolate T36, in the following conditions: i) CA grafted with CA infected or not; or ii) CA grafted with sweet orange [*C. sinensis* 'Valencia Late' (VL)] infected or not. A Library of 2689 proteins was generated for CA. Differentially expressed proteins (DEPs, for a  $P < 0.05$  and  $FD = 1.5x$ ) between CA infected and uninfected were 352, from which 31 were modified in common with VL infected and uninfected. Enriched terms (KEGG pathways) of modified proteins common to CA grafted with uninfected or infected CA and CA grafted with uninfected or infected VL, were mainly assigned to glycolysis and glyoxylate and dicarboxylate metabolism. Specific modified terms in CA grafted with VL, infected and uninfected, were mainly in alpha-linolenic acid metabolism, tyrosine and carbon fixation metabolism. In summary, VL grafted scions promote considerable modifications in the stem CA proteomes. Furthermore, CTV infection differently affects the CA stem proteome when the grafted scion is CA or VL.

***Bacillus velezensis* K165 mediated resistance against *Verticillium dahliae*, *Botrytis cinerea* and *Hyaloperonospora arabidopsidis*, and the role of histone acetyltransferases in biocontrol.** D. GKIZI<sup>1</sup>, M. MALAI<sup>2</sup>, K.

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

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**Enriched epigenetic marks at Pm-0 locus genes prime courgette and induce SAR responses against powdery mildew.** T. MARGARITOPOULOU<sup>1</sup>, D. KIZIS<sup>1</sup>, D. KOTOPOULIS<sup>1</sup>, I.E. PAPADAKIS<sup>2</sup>, C. ANAGNOSTOPOULOS<sup>3</sup>, E. BAIRA<sup>3</sup>, A. TERMENTZI<sup>3</sup>, A.-E. VICHOU<sup>1</sup>, C. LEIFERT<sup>4,5</sup> and E. MARKELLOU<sup>1</sup>. <sup>1</sup>Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, Athens, 14561, Greece. <sup>2</sup>Faculty of Crop Science, Agricultural University of Athens, Athens, 11855, Greece. <sup>3</sup>Scientific Directorate of Pesticides' Assessment

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Powdery mildew, caused by *Podosphaera xanthii*, is an important disease of cultivated *Cucurbita* species. Recently, the appearance of highly aggressive *P. xanthii* strains has led to powdery mildew outbreaks even in resistant crops, making disease management difficult. Plant breeders rely on host genetic characteristics for powdery mildew control. Analysis of commercially available intermediate resistance courgette varieties, using cytological, molecular, and biochemical approaches, showed that the plants were under a primed state and an induced Systemic Acquired Resistance status. Plants exhibited enhanced callose production, upregulation of Salicylic Acid (SA) defence signalling pathway genes and accumulation of SA and defence metabolites. The intermediate resistant varieties also showed an altered epigenetic landscape in histone marks that affect transcriptional activation. Courgette plants were shown to have enriched H3K4me3 marks on SALICYLIC ACID-BINDING PROTEIN 2 (SABP2) and YODA (YDA) genes of the Pm-0 interval introgression, a genomic region that confers resistance in Cucurbits against *P. xanthii*. The open chromatin state of SABP2 and YDA genes was consistent with gene differential expression, induced SA pathway, altered stomata characteristics and activated SAR responses. These results indicate that the Pm-0 SABP2 and YDA genes modulate resistance against *P. xanthii* by setting plants in a primed state, and that the epigenetic background of courgette varieties has an important regulatory role in defence and induced SAR responses. This background could be further explored for production of varieties with enhanced resistance to the pathogen.

## Invasive pathogens and emerging diseases

**Potential insect vectors of *Xylella fastidiosa* in Morocco: the case of spittlebug.** N. HADDAD<sup>1,2</sup>, I. MRABTI<sup>1,2</sup>, M. AFECHTAL<sup>1</sup>, K. EL HANDI<sup>3</sup>, R. BENKIRANE<sup>2</sup> and M.C. SMAILI<sup>1</sup>. <sup>1</sup>National Institute for Agricultural Research (INRA), Regional Center for Agricultural Research of Kenitra, Box: 257, Kenitra, Morocco. <sup>2</sup>Faculty of Sciences, Laboratory of Plant, Animal, and Agro-Industry Productions, University Ibn Toufail, Kenitra, Morocco. <sup>3</sup>Laboratoire de Biotechnologie Végétale et Valorisation des Bio-Ressources, Faculté des Sciences,

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*Xylella fastidiosa* is a potential risk to the Moroccan flora. Thorough understanding of the distribution and ecology of potential vectors in wine-producing systems in Morocco is important for development of successful control of this pathogen. A sweeping net was used to collect insects (2604 specimens), and to assess *X. fastidiosa* occurrence, and to establish its potential insect vectors during 2019 and 2020. Five potential vectors were recorded, and these were mainly located in the northern part of Morocco. The vectors were; *Philaenus tessellatus*, *P. maghresinus*, *Philaenus* sp., *Neophilaenus campestris* and *N. lineatus*. *Philaenus tessellatus* was the most important spittlebug recorded, with uneven occurrence throughout the country. *Philaenus spumarius* was not found in the surveys, which indicates that it has been replaced by *P. tessellatus*. Therefore, *P. tessellatus* is probably the main potential insect vector of *X. fastidiosa* in Morocco.

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

Temperature is a key factor affecting *Xylella fastidiosa* (*Xf*) biology and epidemiology. Knowledge of the response of *Xf* to temperature is needed to assess the potential establishment of *Xf* strains occurring in the European outbreaks of the pathogen, and to develop regionalised risk models. Furthermore, as this is a xylem-inhabiting microorganism, it is relevant to determine whether air temperature, used to develop these models, is related to that in xylem vessels of host plants. This study aimed to determine the effects of temperature on *in vitro* cell cultures of *Xf* strains, and to determine xylem vessel temperature dynamics in an olive orchard. growth, biofilm formation and survival were determined of 37 *Xf* strains representative of five subspecies and 13 STs from a wide geographic origin, and host plants were evaluated in a temperature range from 4 to 40°C. Several thermocouples were installed within plant branches and trunks at 1 and 4 cm depths, to measure air, soil and xylem temperature at 10-min intervals for 31 months. Results indicated that *Xf* strains showed differential responses to temperature. When grouped by subspecies, the widest optimal growth range was estimated for *Xf*

subsp. *fastidiosa* (19 to 33°C) and subsp. *multiplex* (20 to 31°C), while *Xf* subsp. *pauca* strains had lower optimal ranges (19 to 27°C). Similarly, extreme temperatures differentially affected cell survival. Temperatures between 4 to 10°C did not affect cell survival, while incubation at 36 and 40°C were lethal. The relationship between air and xylem or soil temperatures estimated by regression models indicated buffer effects of trunk tissues and soil, especially for maximum temperatures occurring during summer.

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

*Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4) is an important soilborne pathogen of banana, causing mortality to Cavendish group bananas. The pathogen was first detected in South East Asia, spread to the greater Mekong subregion, Australia and was disseminated to India, Pakistan, Oman, Turkey and Mozambique (Africa), and recently was identified in Colombia and Peru. TR4 was discovered in the Middle East (Jordan and Lebanon in 2014, and Israel in 2016). In Israel, typical TR4 symptoms of leaf-yellowing and wilting, accompanied by internal vascular discolorations of rhizomes and pseudostems, were observed in mature 'Grand Naine' Cavendish plants from Shfeya, the Carmel coastal plain and locations along the eastern shores of Lake Galilee. TR4 representative isolates from symptomatic plants were tested for pathogenicity, and identification was confirmed by PCR. Sequenced genomes of five representative TR4 isolates (two from Israel, and one each from Jordan, the Philippines, and Indonesia), and 11 additional isolates from other countries, were compared by single nucleotide polymorphisms (SNPs) analysis, to determine the origin of the Israeli isolates. SNP detection and phylogeographical analyses indicated that the Middle Eastern isolates are closely related, implying that Jordan was the origin of the pathogen in Israel, while isolates from Colombia were related to a representative isolate from

Indonesia. Host range, susceptibility/tolerance of germplasm to TR4, and epidemiological studies and survival of the pathogen are currently being investigated.

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

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

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**A new disease complex threatening fig (*Ficus carica* L.) in Southern Italy.** W. HABIB<sup>1</sup>, CAVALIERI<sup>1,2</sup>, M. CAR-

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Symptoms of severe decline of fig (*Ficus carica*) have been recently detected in Salento area of the Apulia region (southeastern Italy). Symptoms begin with leaf chlorosis and evolve into necroses, twig defoliation and death of lateral branches. Cankers are often observed, associated with extensive wood discoloration under the dead bark and together with bark beetle galleries. In September 2021, wood sections and adult insect specimens were collected from symptomatic trees in several orchards. Bark beetles were morphologically and molecularly identified as *Cryphalus dilutus* (*Curculionidae*, *Scolytinae*). Fungal isolations were performed on PDA amended with 0.5 g L<sup>-1</sup> streptomycin sulfate, from 200 wood fragments and 52 *C. dilutus* adults. Three groups of potential pathogens were recovered from the plant tissues and insects, including *Botryosphaeriaceae* (38.5%), *Fusarium solani* species complex FSSC (36.9%), and Ophiostomatoid fungi (6.7%). Mixed infections with at least two pathogens were common (67%). A set of 47 single spore isolates were identified using multilocus phylogenetic analyses based on the sequences of three informative genes. Preliminary results showed that almost 50% of the FSSC isolates belong to the newly described species *Neocosmospora perseae* causing trunk cankers on avocado in Sicily. At least three species of *Botryosphaeriaceae* (*Neofusicoccum parvum*, *Lasiodiplodia theobromae*, *Botryosphaeria dothidea*) and two genera of Ophiostomatoid fungi (*Graphium* and *Ceratocystis*) were also identified. Phylogenetic analyses and pathogenicity tests are ongoing to determine the role of each fungus in the aetiology of the observed decline syndrome, and verify the potential role of the *C. dilutus* as a pathogen vector.

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

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The health of olive groves is under threat due to an increase of diseases caused by vascular pathogens, including *Xylella fastidiosa* and soil-borne *Verticillium dahliae*. Both pathogens may compromise olive production due to their capacity to reduce olive plant growth by colonizing and blocking host xylem vessels causing desiccation of leaves and branches and ultimately death of trees. Knowledge of the xylem-inhabiting microbiota is important to provide understanding of the resistance response observed in some olive cultivars under high inoculum pressure, and to select potential biocontrol microorganisms. Xylem-inhabiting bacterial communities were investigated in stem and root tissues of “Grapolo” olive trees at five field locations (two in the state of Sao Paulo and three in Minas Gerais State) in Brazil. Changes occurring on trees infected and non-infected by *Xylella fastidiosa* were also compared. Sequencing data resulted in a total of 925 bacteria ASVs, distributed in 15 phyla and 421 genera. Proteobacteria was the most abundant bacterial phylum (91.4%) followed by Firmicutes (4.2%), whereas *Pseudomonas* (31.2%) and *Methylobacterium* (24.2%) were the dominant genera. Microbial diversity was firstly determined by field location, followed by ecological niche and in a lesser extent by *X. fastidiosa* infection. Network analysis identified 32 keystone species with positive and negative associations with *X. fastidiosa*. 1174-901-12 and *Pseudomonas* showed the greatest number of negative ASVs associated with *X. fastidiosa*, whereas *Acidiphilium* and *Corynebacterium* co-occurred with the pathogen. This research increases understanding of the contribution of the plant microbiome to host health status, to promote and maintain sustainable olive agroecosystems.

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**Etiology and management of Phytophthora crown and root rot of pistachio in California.** F.P. TROUILLAS, A.I. HERNANDEZ, M.T. NOURI, R.J. FRIAS and T.B. BOURRET. *Department of Plant Pathology, University of California, Davis, 95616 Davis, CA, USA. E-mail: [flotrouillas@ucanr.edu](mailto:flotrouillas@ucanr.edu)*

Pistachio is a widely cultivated nut crop in California, with approx. 115,000 ha of bearing pistachio trees. Several orchards have been identified with declining trees

leading to substantial tree losses. Symptoms included trees with poor vigour, yellowing and wilting of leaves, crown rot and profuse gumming on the lower portions of trunks. Thirty-seven *Phytophthora*-like isolates were obtained from crown rot tissues of rootstocks of grafted pistachio trees, and were characterized using multi-locus phylogeny comprising ITS rDNA, beta-tubulin and mt cox1 sequence data. This identified *Phytophthora niederhauserii*, *P. mediterranea* and *P. taxon* walnut associated with declining pistachio trees. Pathogenicity studies in potted UCBI rootstocks confirmed that all three *Phytophthora* species can cause crown and root rot of pistachio, thus fulfilling Koch's postulates. The widespread occurrence of *Phytophthora* crown rot in recently planted pistachio orchards and the susceptibility of UCBI rootstocks suggest this disease is an emerging threat to the Californian industry. Experiments have been conducted to determine the relative tolerance of UCBI (*P. atlantica* × *P. integerrima*), PGI (*P. integerrima*) and Platinum (*P. integerrima* × *P. atlantica*) commercial rootstocks to *Phytophthora*, and identify tolerant/resistant rootstocks that can be used to sustainably managed this soil-borne disease. Experiments using mycelium plugs for stem inoculation, or inoculated rice grain or zoospores for soil/root inoculations, of the various commercial rootstocks have indicated that Platinum is the most tolerant rootstock to crown rot diseases, compared to PGI and clonal UCBI rootstocks.

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**First report of Glomerella leaf spot in South Tyrolean (Italy) apple orchards.** E. DELTEDESCO<sup>1</sup>, J. CRISTANELL<sup>2</sup> and S. OETTL<sup>1</sup>. <sup>1</sup>Research Centre Laimburg, Laimburg 6, 39040 Auer/Ora (BZ), Italy. <sup>2</sup>South Tyrolean Extension Service for Fruit and Wine Growing, Andreas-Hofer-Str. 9/1, 39011 Lana (BZ), Italy. E-mail: [evi.deltedesco@laimburg.it](mailto:evi.deltedesco@laimburg.it)

*Glomerella* leaf spot (GLS) of apple is an emerging disease, caused by several *Colletotrichum* spp. GLS was restricted to apple growing areas with humid and subtropical climates, but had not been reported from European apple growing areas. Extreme weather conditions, including heavy rainfall and warm temperatures at the end of August 2020, led to an unknown symptomatology in South Tyrolean (Italy) apple orchards. This included leaves with necrotic lesions, proceeding rapidly to extended chloroses. Affected leaves dropped prematurely and resulted in complete defoliation after

approx. 1 month. A few days after the appearance of the first leaf lesions, brownish spots, often surrounded by purple halos, started to develop on fruit, progressing to affect up to 100% of individual crops. Fungal isolates were recovered from symptomatic leaves and fruit spots, and morphological analysis identified these isolates as *Colletotrichum*. A multi-locus sequence analysis based on the ITS region and fragments of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT) and beta-tubulin (TUB2) genes, assigned all isolates to *Colletotrichum fructicola*. Pathogenicity assays on apples were carried out, and Koch's postulates were confirmed for this pathogen. Climatic conditions, such as increasing air temperatures and frequency and intensity of precipitation, may lead to a further spread of this pathogen, resulting in notable yield losses in commercially managed orchards. Targeted plant protection procedures and containment strategies should be implemented to control and prevent the spread of this disease.

***Sclerotinia sclerotiorum*: a new pathogen of sugar beet in the USA.** M.F.R. KHAN<sup>1,2</sup> and M.Z.R. BHUIYAN<sup>1</sup>.

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*Sclerotinia sclerotiorum* (Lib.) DeBary adversely affects a wide range of crops. This fungus may exist as mycelia and survive as resting sclerotia. The pathogen was reported for the first time on sugar beet (*Beta vulgaris*) in North Dakota, USA in 2019, and was confirmed in several beet producing states areas in 2020, causing foliar blight and root rot. Radial growth of mycelium and development of sclerotia was evaluated on PDA media. Co-cultivation experiments with commercial sugar beet seeds with mycelium plugs and sclerotia of the pathogen were carried out to determine the pathogenicity of inocula. Inoculated seeds showed partial emergence and/or complete seedling death, whereas non-inoculated controls produced well-developed seedlings. The sensitivity of *S. sclerotiorum* was determined on PDA amended with 0, 0.01, 0.1, 1, or 10 ppm of Proline® (prothioconazole, DMI), Priaxor® (pyraclostrobin, QoI + fluxapyroxad, SDHI), or triphenyltin hydroxide. Priaxor® gave the greatest reductions of radial mycelium growth. Commercial varieties from three seed companies were evaluated for sensitivity to *S. sclerotiorum*. All the varieties were susceptible to the pathogen, indicating no resistance to the pathogen. Presence of this new pathogen is important for the

sugar beet industry which is economically important in sugar beet producing states. Field research will be implemented to develop strategies for managing disease caused by *S. sclerotiorum*.

**Biocontrol, natural compounds and plant defence stimulants**

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

Powdery mildew (PM), caused by *Podosphaera xanthii*, is an important courgette disease, causing high yield losses. The diseases and is mainly controlled using fungicides, biocontrol agents, botanicals, and sulphur applications in conventional and organic courgetti production. Giant knotweed (*Reynoutria sachalinensis*; RS) extract is a known elicitor of plant defences, but its mode of action remains unknown. This study investigated the mechanisms of foliar RS applications and how these affect PM severity and crop performance when used alone or in combination with genetic resistance. Physiological, cellular, and molecular approaches showed that RS can have lasting effects on PM progress on courgette leaves, by enhancing plant defence responses at different levels. RS foliar treatments reduced PM severity on

both an intermediate resistance (IR) and a susceptible (S) host genotype. RS induced the formation of callose papillae, hydrogen peroxide accumulation, and expression of the Salicylic Acid (SA)-regulated *NPR1*, *PR1*, *PR2* and *PAL* genes, which correlated with callose deposits and ROS production. Increased SA production was also detected, along with increased p-coumaric and caffeic acid concentrations. These results show that RS elicits plant defences through the SA pathway. To gain molecular level insights, transcriptomic (Illumina sequencing), proteomic (nanoHPLC-HRMS/MS) and metabolomic (UHPLC-HRMS/MS) analyses were carried out on the S genotype inoculated with *P. xanthii* and treated with *R. sachalinensis* prior to inoculation. Functional classifications of the DEGs showed enriched GO-terms, including hormone-mediated responses, oxidation-reduction processes, lipid biosynthesis and metabolism, and defence responses. Glycerophospholipids were also detected, which are chemical signals facilitating signalling during defence responses.

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

Excessive use of chemical plant protection products has caused significant adverse effects on the environment. In agriculture, Use of selective beneficial bacterial biological control agents (BCAs) in agriculture can effectively support plant protection. Due to their characteristic compositions, medicinal plants from the Mediterranean basin are potential sources of efficient endophytic bacterial BCA candidates. Bacterial endophytes occur within host plant without causing visible pathogenic symptoms. Endophytic *Bacillus* isolated from the native medicinal

plants *Calendula officinalis* and *Hypericum hircinum* showed strong antagonism to phytopathogenic fungi, including *Botrytis cinerea* and *Colletotrichum acutatum* under *in vitro* and *ex vivo* conditions. All the *Bacillus* strains studied successfully colonized plant tissues and competed with the phytopathogenic fungi for nutrients and space. An integrative approach was applied, coupling genome mining and metabolic profiling to indicate potential of these endophytes as BCAs against pathogenic fungi. Chemical analyses of the bacterium extracts indicated that these *Bacillus* endophytes secreted several bioactive secondary metabolites, and the bacteria were investigated for fungal growth suppression, either as individual strains or as bacterium mixtures. Formulation of compatible bacterial strains resulted in some mixtures with strong BCA ability.

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

Effect of the volatile organic compounds (VOCs) emitted by *Paenibacillus alvei* K165 grown on Luria Broth (LB) medium were assessed against *Verticillium dahliae*, under *in vitro* and *in planta* conditions. GC/MS analysis showed that K165 grown on LB produced several volatile compounds, including 2,3 butanediol and tropone. Pure 2,3 butanediol and tropone inhibited the growth of *in vitro* *V. dahliae* by, respectively, 10 and 20 %. The *in planta* VOC emitting effect of the K165 against *V. dahliae* was examined in a dual compartment device, where the plants were placed in soil in the upper part of the device and the bacterial cells are applied in the lower part, ensuring spatial separation of plant roots from K165 cells. K165 was inoculated on LB medium in the lower part of the device. The endophytic presence of *V. dahliae* was examined in the plants of the different treatments at 3 and 7 dpi, by Real Time PCR analyses. The K165 treated plants were less colonized by *V. dahliae* compared to controls, at both sampling time points. In agreement with the Real Time PCR results, disease

assessments showed that the symptoms, mainly wilting, were less in the plants exposed to the K165 VOCs compared to controls.

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**Valorization of *Gelidium sesquipedale* residue in the control of *Ascochyta* blight of chickpea.** H. ERRATTI<sup>1,2,3</sup>, S. LEBBAR<sup>3</sup>, K. DARI<sup>1</sup>, L. HILALI<sup>1</sup> and S. KRIMI BENCHEQROUN<sup>2</sup>. <sup>1</sup>University Hassan 1st, Faculty of Science and Techniques, Laboratory of Agro Alimentary & Health, P.O. Box 577, Settat, Morocco. <sup>2</sup>National Institute of Agriculture Research (INRA, CRRA-Settat), P.O. Box 589, Settat, Morocco. <sup>3</sup>Setexam Company for the Study and Exploitation of Algae and Maritime Products, Kenitra - 14000 Morocco. E-mail: sanee.krimibencheqroun@inra.ma

The use of biological products as alternatives to chemical fungicides has become important due to potentially adverse health and environmental impacts. The red alga *Gelidium sesquipedale* is mainly used for extraction of Agar-Agar. However, large amounts of by-product residues are also produced and are unused. Bioactive compounds in *G. sesquipedale* residues were evaluated for their antifungal activity against *Ascochyta rabiei* which causes *Ascochyta* blight of chickpea. The alga residues extraction were carried out using water, dichloromethane, or 1:1 (v:v) dichloromethane:ethanol as solvents. Antifungal activity against the pathogen was first tested *in vitro* on PDA medium amended with different extracts, using seven concentrations from 0 to 8 mg mL<sup>-1</sup>. Phytotoxicity of these products was also evaluated on chickpea seeds. *In vivo* experiments were carried out in a greenhouse to evaluate the efficacy of aqueous residue extracts (as seed or foliar treatments) for control of *Ascochyta* blight. The chemical fungicide azoxystrobin (at 250g L<sup>-1</sup>) was used for comparison. Aqueous residue extract at 8 mg L<sup>-1</sup> was the most effective at inhibiting mycelium growth of *A. rabiei* by 80%. No phytotoxic effects of aqueous extract was observed on chickpea seed germination at all tested concentrations. Applications of aqueous residue extract as foliar treatments controlled the disease, reducing disease severity by 73%, similar to azoxystrobin. However, no effect was observed from the seed treatments. Aqueous extract from *G. sesquipedale* residues, used as foliar treatments, could be further investigated as a potential a biological antifungal product.

***Trichoderma atroviride* SC1: a biocontrol solution for pathogens of grapevine and other important crops.** M. KAIIFA, A. NESLER, J. GOOSSENS and A. VERMAETE. Bi-PA nv (Biological Products for Agriculture), Technologielaan, 7. 1840 Londerzeel, Belgium. E-mail: maria.kaiifa@bi-pa.com

Biological Products for Agriculture (Bi-PA nv) is working to develop and register biological plant protection products. A success for the company is Vintec®, a bio-fungicide based on active conidia of *Trichoderma atroviride* strain SC1. Vintec® was initially developed and registered for protection of grapevines against trunk diseases (Esca, *Eutypa lata*). The product is applied preventively on pruning wounds. The mode of action is mainly based on prevention of pathogen colonization of pruning wounds, through creation of barrier against the pathogens and competition for space and nutrients. After registration of Vintec®, Bi-PA nv has continued to develop the product. Vintec® has obtained label extensions for the use against *Botrytis cinerea* on grapes and tomatoes, and against the fungal pathogens of stone fruit, *Coryneum beijerinckii*, *Monilinia laxa* and *Taphrina deformans*. Protection of tomato plants against *Botrytis cinerea* was confirmed by efficacy trials in protected conditions in the Netherlands, Italy and Spain. Greatest efficacy resulted from Vintec® applied at 5 to 10 g 100 L<sup>-1</sup>. Efficacy was greater than, or comparable to, the reference products, when applied as preventive applications. For protection of stone fruits against major pathogens, Vintec® performed well in efficacy trials in open field conditions in Italy, Spain, Greece and Portugal. Optimal dose was 200 g ha<sup>-1</sup>, which gave greater than or comparable efficacy to reference fungicide products.

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

The biocontrol agent *Clonostachys rosea* is soil borne, and is known for its antagonistic abilities against numerous plant pathogenic fungi, nematodes and insects. This

fungus has not been investigated for antagonism to grapevine trunk disease pathogens. This study characterized the biocontrol capabilities of *C. rosea* against the pathogens causing GTDs. Confrontation tests were carried out with five *C. rosea* isolates and six GTD pathogens. The greatest growth inhibition was against the *Phaeomoniella chlamydospora* (50% reduction) and *Eutypa lata* (30%). Mycoparasitism was observed against *Botryosphaeria* and *Phomopsis* species. Elicitor and biomass production and the sporulation of the *C. rosea* isolates were also compared. The 19b1 of *C. rosea* was selected for *in planta* assessments. Cabernet sauvignon cuttings were grown under greenhouse conditions, and inoculated with the pathogens *P. chlamydospora*, *E. lata* and *Botryosphaeria dothidea*. Plants were further grown in untreated soil or a soil amended with  $10^4$  spores  $g^{-1}$  of isolate 19b1. After 3 months incubation, symptom development was assessed. Lesion lengths caused by *P. chlamydospora* and *E. lata* were decreased in the presence of the isolate, while virulence of *B. dothidea* was unaffected. The biocontrol fungus was frequently re-isolated from the cutting bases, colony forming units were increased from  $10^4$  to  $10^5$   $g^{-1}$  of treated soil. These results indicate that *C. rosea* can be used as a biocontrol agent against GTDs.

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**Evaluation of biological and synthetic plant protection products for the management of downy mildew in grapevines.** C.K. KAVROUMATZI, M.K. ILIADI, M. VARVERI, D. AKRIVOPOULOU and D.I. TSITSIGIANNIS. *Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece. E-mail: dimtsi@aau.gr*

Downy mildew, caused by *Plasmopara viticola*, is the most serious threat to grapevines in most viticulture regions where rainfall occurs regularly during the late spring and summer. The pathogen can destroy a vineyard if no control measures are taken. The main strategies to restrict the pathogen have been based on fungicides that result in soil accumulation and potential environmental hazards. Large amounts of fungicides must be applied many times through each production season to keep the pathogen under control. Present strategies for grapevine downy mildew control mainly use preventive copper or mancozeb treatments, from the beginning of periods when plants are susceptible

to infections. The efficacy of different plant protection products (PPPs) was assessed in grapevine leaves after artificial infection with *P. viticola* zoospores. Nine commercial bio-PPPs and five synthetic PPPs were tested against the pathogen, using susceptible host cultivars Moscato and Agiorgitiko. The most effective bio-PPPs for both cultivars were Amylo-X®, Remedier®, Triamum® and Vacciplant®, but these were not as effective as copper formulations. All the tested synthetic PPPs controlled the disease. The most efficient was Bion-MX® which combines the biostimulant acibenzolar-S-methyl and a chemical metalaxyl.

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

Fungal cankers are ubiquitous in perennial crops grown for fruit and nut production in regions with a Mediterranean climate. In almond, these diseases decrease yields and severely limit the productive lifespan of orchards. The fungal pathogens infect almond trees through pruning wounds, as pruning is a commonly used to establish tree architecture and invigorate fruitwood. Biological control agents (BCAs) have gained increased interests for pruning wound protection because of increasing restrictions for pesticide use. The present study evaluated the biocontrol potential of commercial and experimental BCAs against common fungal canker pathogens of almond. Initial screening of nine BCAs in dual cultures with seven pathogens allowed the selection of the four most promising BCAs for further testing of their antagonistic activities *in planta*. The four BCAs were evaluated against a subset of four pathogens (*Cytospora plurivora*, *Eutypa lata*, *Neofusicoccum parvum* and *Neoscytalidium dimidiatum*). This showed that some BCAs reduced almond pruning wound infections by canker pathogens. The four BCAs were further evaluated in the field in two almond orchards planted with two distinct cultivars, Sonora and Nonpareil. Based on 2 years of field data, two BCAs provided levels of disease control similar to those reached with thiophanate-methyl. These BCAs are promising candidates for use in integrated pest management strategies for almond production.

**Mineral oils against powdery mildew: paraffin oil induces resistance in grapevine against *Erysiphe necator*, and is applicable in disease management.**

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

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**Towards Nutrition-Sensitive Agriculture: an evaluation of biocontrol effects, nutritional value, and**

**ecological impacts of bacterial inoculants.** G.-I-R. SHAZHAD<sup>1</sup>, A. PASSERA<sup>1</sup>, V. VACCHINI<sup>3</sup>, G. COCETTA<sup>1</sup>, A.A. ARPANAHI<sup>1,2</sup>, P. CASATI<sup>1</sup>, A. FERRANTE<sup>1</sup> and L. PIAZZA<sup>3</sup>. <sup>1</sup>Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy, Università degli Studi di Milano, Milan, Italy. <sup>2</sup>Present address: Soil Sciences Department, Agriculture Faculty, Lorestan University, Khorramabad, Iran. <sup>3</sup>Department of Environmental Science and Policy – Production, Landscape, Agroenergy, Università degli Studi di Milano, Milan, Italy. E-mail: gul.shahzad@unimi.it; paola.casati@unimi.it

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

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## Integrated disease management

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

*Botrytis cinerea* Pers. affects more than 200 plant species, and causes significant economic losses to sugar beet industries in regions where beet roots are stored for up to 9 months. The pathogen is typically present as a characteristic gray mold on roots, causing increased respiration rates that reduce recoverable sucrose by up to 60 to 80%. There are many fungicides that are extensively used for sugar beet production, but relatively few have been evaluated for control of storage rot of sugar beet. Penthiopyrad, a new SDHI fungicide labelled for sugar beet, was evaluated for reducing the activity of *B. cinerea* on sugar beet roots in storage conditions. Harvested roots were washed, dried and sprayed or dipped in different concentrations of the fungicide. Harvested sugar beet roots were washed, dried, and sprayed and dipped in different concentrations (10, 20, 40 or 80 mL L<sup>-1</sup>), followed by inoculation with mycelium plugs of *B. cinerea*. The treated beets were then kept at 4°C and their respiration rates were measured at 0, 30, 60, 90 and 120 days post-inoculation. Penthiopyrad at 20 mL L<sup>-1</sup> or greater reduced respiration rate (CO<sub>2</sub>) in treated beets, and has potential for use to reduce storage losses caused by *B. cinerea* on sugar beet in long term storage.

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

Grapevine powdery mildew (GPM) caused by *Erysiphe necator* is usually controlled by sterol demethylation inhibitor (DMI) fungicides, but long-term use of these agents has resulted in resistance in GPM to these fungicides. A common marker of resistance is the A495T nucleotide substitution in the CYP51 gene. The GPM populations in Europe can be divided into two groups, which may differ in their seasonality and fungicide resistance. We sampled GPM in Hungary to describe genetic composition of these populations, and investigate the prevalence of the A495T-marker, and the relationships between its occurrence, fungicide treatments, sampling sites and years, and *E. necator* genetic groups. Sampling was conducted in six wine regions for 3 years. Fragments of four genes were sequenced. Occurrence of A495T was determined by sequencing or real-time PCR, and the probability of A495T occurrence was analysed with a generalized linear model. Fourteen haplotypes of *E. necator* were obtained, of which eight were previously unknown. These included haplotypes which represented SNPs characteristic to both genetic groups, and might represent recombinants. A495T was detected in all wine regions, in ≈16% of the samples. Differences in occurrence of A495T were found among several wine region and cultivar combinations, and between study years. Occurrence of A495T was not significantly different between treated and untreated sites, neither between seasons nor between genetic groups. These results indicate that in Hungary, *E. necator* populations consist of diverse haplotypes, including recombinants. These frequently harbour the A495T mutation, occurrence of which is mainly influenced by wine region and cultivar, and the sampling year.

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**Development of cost-effective methods for detection of the DMI fungicide resistance marker A495T of the grapevine powdery mildew fungus *Erysiphe necator*.** M.Z. NÉMETH<sup>1</sup>, A. PINTYE<sup>1</sup>, O. MOLNÁR<sup>1</sup>, F. MATOLCSI<sup>1,2</sup>, Á.N. HORVÁTH<sup>1</sup>, ZS. SPITZMÜLLER<sup>3</sup>, K.Z. VÁCZY<sup>3</sup> and G.M. KOVÁCS<sup>1,2</sup>. <sup>1</sup>Plant Protection Institute, Centre for Agricultural Research, ELKH, Herman Ottó út 15., 1022 Budapest, Hungary. <sup>2</sup>Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/C, 1117 Budapest,

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

This research was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences, and by the Széchenyi 2020 Programme, the European Regional Development Fund and the Hungarian Government (GINOP-2.3.2-15-2016-00061) and partly by the ELTE Thematic Excellence Program 2020 (TKP2020-IKA-05) of the National Research, Development and Innovation Office.

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

Botrytis bunch rot (BBR) is an important disease affecting grapevines, which requires adequate control, often based on routine application of fungicides at the end of flowering (A), pre- bunch closure (B), veraison (C), and before harvest (D). This simple, calendar-based scheduling of fungicides is at odds with the complexity of the *Botrytis cinerea* life cycle and existence of different infec-

tion pathways. A mechanistic model has shown that early-season development of *B. cinerea* determines the severity of BBR on ripe bunches, because of latent infection of young berries and saprophytic colonisation of bunch floral debris (or bunch trash). A meta-analysis of 116 studies confirmed that BBR control at growth stage A is more effective than at B, with control at B being useful only under high disease pressure when the full A, B, C and D strategy is needed for effective BBR control. The combination of control at A, C and D provides good and additive control of the early-season infection pathways and the multiple infection events during berry ripening. Following an integrated BBR management approach, early-season use of alternative products (e.g., biological control agents and botanicals) based on model predictions provides effective BBR management in vineyards.

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

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

ered to evaluate the model's accuracy and robustness in a wide range of conditions.

**Evaluation of epiphytic grape yeasts for the control of *Aspergillus carbonarius* and ochratoxins in grapes.**

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Yeasts are considered ideal biological control agents, as they are able to survive in a wide range of environmental conditions, grow rapidly with simple nutritional requirements, can colonize plant surfaces even under prolonged dry conditions, and do not secrete toxic substances for humans. These fungi have been widely used against plant pathogens at pre- or post-harvest levels. This study aimed to discover effective endemic yeasts as biocontrol agents against the black rot of grapes, caused by *Aspergillus carbonarius*. This pathogen adversely affects the organoleptic characteristics of wine, and produces carcinogenic ochratoxins. Grapevine yeasts were assessed from the collection of Laboratory of Phytopathology (AUA), as well as new isolates from different grapevine varieties and regions in Greece. Several strains were tested *in vitro* for inhibition of conidium production by the ochratoxigenic strain *A. carbonarius* Ac-29 on Yeast Malt Agar. Antagonism and ochratoxin inhibition bioassays were also carried out on detached grape berries. Yeasts with high antagonistic properties against *A. carbonarius* were selected for field trials on the Greek white grape varieties Savatiano and Asyrtiko. Results of these experiments were presented. Discovery of endemic yeast strains that inhibit ochratoxin production and are acclimated and adapted to local physical and biological environments may lead to development of novel biocontrol products.

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**Modelling potential climatic suitability for olive vascular diseases in southern Spain.**

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Andalusia in Southern Spain is the main world olive oil producing region, 1.6 million ha of olive trees. Verticillium wilt (VW) caused by soil-borne *Verticillium dahliae* (Vd) and the olive quick decline syndrome, caused by *Xylella fastidiosa* (Xf) are currently the main threats for olive production. Vd occurs widely in Southern Spain, causing severe yield losses in some areas. In contrast, Xf is not known to be present in this region, but represents a threat since this pathogen has been detected in Spain. This study carried out a risk analysis that may help to prevent the spread of Vd to avoid the establishment of Xf in Andalusia. Species distribution modelling was used to determine relationships between sample location for the two pathogens with associated environmental variables, and estimate the ecological requirements for particular species. Bio5 and Bio15 were identified as the main climatic factors that determine the potential distribution of Vd in Andalusia, with the areas along the Guadalquivir River showing the greatest suitability, which agrees with the current known Vd distribution in the region. The model also identified extensive areas with moderate to high suitability in regions where olive plantations are currently expanding. Suitable areas for Xf are mainly characterized by warm temperatures during summer-dry periods, mild winters, and a well-defined rainy seasons, with the eastern part of Andalusia being at greatest risk. The developed models would allow better management of VW by identifying the risk levels for established and new plantations, and for prioritizing surveillance programmes for Xf, based on the level of risk from this pathogen.

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**Strategic management of fungicide resistant *Cercospora beticola* in sugar beet using a holistic management strategy in the climate change era.**

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*Cercospora beticola* causes Cercospora leaf spot (CLS), a damaging foliar disease of sugar beet grown in warm and humid regions. Since the 1970s, growers have become dependent on fungicides for effective control of

CLS. Fentin acetate and triphenyltin hydroxide, benzimidazoles, demethylation inhibitors (DMIs), and quinone outside inhibitors (QoIs) have been used with varying levels of success for control of *C. beticola*. The pathogen produces large numbers of conidia and multiple generations during each host growing season, and has developed resistance to most of the fungicides used for its control. In 2016, growers in the United States of America lost >\$200 million because of QoI resistance and a CLS epidemic. Few fungicides currently provide effective control of *C. beticola*, when used alone, and there are very few effective alternating partners. Recently developed varieties (CR+) with improved resistance to *C. beticola* had better resistance to the pathogen than the best currently approved varieties. Fungicides were applied on a calendar basis, and only when required when symptoms and favourable environmental conditions occurred. CR+ varieties with and without fungicide applications resulted in similar or increased higher recoverable sucrose compared to similarly treated older varieties. Strategies including incorporating infected crop debris, crop rotation, planting away from previously infected fields, use of CR+ varieties, and judicious use of fungicide mixtures will be necessary for sustainable and economic production of sugar beet.

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

Red leaf blotch (RLB) of almond, caused by *Polystigma amygdalinum*, is the main foliar disease affecting almond orchards in the Mediterranean Basin. The pathogen overwinters in leaf litter, and ascospores are released from perithecia in spring to infect new almond leaves. RLB is characterized by a long incubation period, and fungicide sprays should be scheduled during the period of ascospore release. Airborne *P. amygdalinum* ascospores were monitored from 2019 to 2021 (February to September each year), by placing a 7-day volumetric spore trap (Hirst type) in two RLB-affected almond orchards in Catalonia. Exposed tapes were collected weekly, and were analysed with real-time qPCR to quantify *P. amygdalinum* trapped ascospores on a daily basis. Hierarchical Bayesian beta regression models were

developed to fit the dynamics of accumulated ascospore catches to several environmental variables for both locations. The best model included accumulated degree-days (ADDs) and ADDs considering both rainfall and vapour pressure deficit (ADDwet) as fixed factors, and year as the random factor. For this model, the linear regression of the median posterior predictive distribution against observed values accounted for 78% of the total variance, with a mean absolute error of 0.1415 and a root mean square error of 0.1799. A Decision Support System using this model is currently under development to schedule fungicide sprays and optimize control of almond RLB.

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

Plant pathology studies have mainly focused on the one host/one pathogen paradigm, but pathosystems can also involve co-infections by several pathogen species. Co-infections may produce symptoms dissimilar to infections by each pathogen alone, and may increase host damage. This study described the synergistic effects of co-infection by *Cucumber green mottle mosaic virus* (CGMMV) and *Pythium* spp. Late-wilting has increased in cucumber greenhouses during CGMMV outbreaks. As wilting occurs in defined patches accompanied by root rot, we hypothesized that the disease is caused by co-infections of soilborne pathogen/s and CGMMV. A field survey showed that 69% of the wilting plants were colonized simultaneously by *Pythium* spp. and CGMMV, whereas only 20% of the wilting plants were colonized only by *Pythium* spp. and 6.6% were infected with CGMMV. Artificial inoculations of cucumber plants

showed that co-infection with *P. spinosum* and CGMMV gave a synergistic wilting effect and reduced host growth parameters. This synergism was detected under a wide range of (optimal and suboptimal) temperatures, and *P. spinosum*, which mostly prevails in mild temperatures, caused high mortality at an extended temperature range and even at 32°C. This study has demonstrated the complexity of pathosystems involving co-infections by two pathogens, and encourages a broader perspective of the complexity of agricultural diseases so appropriate disease management can be applied.

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#### **A mechanistic weather-driven model for *Ascochyta rabiei* infection and disease development in chickpea.**

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

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### **Microbiomes and their role in plant health**

**Rhizosphere-enriched microbes as a pool to design synthetic communities with beneficial effects on plant fitness and health.** M.-D. TSOLAKIDOU<sup>1</sup>, I.A. STRINGLIS<sup>2</sup>, N. FANEGA-SLEZIAK<sup>1</sup>, S. PAPAGEORGIOU<sup>1</sup>, A. TSALAKOU<sup>1</sup> and I.S. PANTELIDES<sup>1</sup>. <sup>1</sup>*Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Lemesos, Cyprus.* <sup>2</sup>*Plant-Microbe Interactions, Department of Biology, Science4Life, Utrecht University, Utrecht, the Netherlands. E-mail: iakovos.pantelides@cut.ac.cy*

Suppressive composts are an environmentally friendly approach to combat soilborne plant pathogens and improve plant growth. In a previous study, the microbial nature of the suppressiveness of a compost was demonstrated. The compost-derived microbial communities enriched in the rhizosphere of plants were identified and characterized for *in vitro* antifungal activity against soilborne fungal pathogens, and for their potential to change plant growth parameters. In the present study, two simplified microbial synthetic communities (SynComs) were designed with different composition, using representative bacteria from the rhizosphere community, to provide evidence of their beneficial effects on plant fitness and health. SynCom1, consisting of bacterial strains reflecting their relative abundance in the rhizosphere community, displayed a negative effect on *Arabidopsis in vitro*, but promoted tomato growth in pot experiments. SynCom2, consisting only of *Bacillus* strains, did not affect *Arabidopsis* growth but increased tomato growth and suppressed disease symptoms caused by *Fusarium oxysporum* f. sp. *lycopersici*. These results demonstrate that the composition of complex microbial communities can affect plant phenotypes, and these effects can be host-dependent. Identification and characterization of their traits could facilitate design of novel synthetic microbial communities, that could be used as inoculants with defined and controllable properties, conferring consistent beneficial effects towards plants.

**Detailed survey of fungal communities of four vineyards containing different cultivars, located in the**

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

Grapevine cultivars are differentially vulnerable to infections caused by fungal trunk disease (TD) pathogens. This study examined the total mycoflora (including TD pathogens) in four vineyards containing different cultivars located in the Palava region, the most famous wine-growing region in the Czech Republic. Ten plants each of cultivar Blaufränkisch, Palava, Pinot Noir and Welschriesling, showing symptoms of TD, were collected in each vineyard in July 2019. Fungal communities were examined using traditional isolation on artificial media and high-throughput amplicon sequencing (HTAS) of the internal transcribed spacer (ITS2) region. HTAS increased the resolution of the fungal community analysis and revealed a highly diverse and complex mycoflora from grapevine wood, compared to the classical isolation approach. According to symptom severity evaluated in 2020 and 2021, Pinot Noir was the most sensitive cultivar to TD pathogens. This observation correlated with the results of fungal detection assays. The most prevalent fungi detected by the classical approach were *Diaporthe* and *Phaeoacremonium*, and this was comparable with the HTAS results. This study provides important and practically useful insights into fungal communities among the four cultivars, and is the first to examine fungal communities on grapevine plants by using MiniSeq, 2-channel sequencer combined with classical isolation. The study also provides the most comprehensive survey of TD pathogens in Czech vineyards, and of grapevine tolerance against TD pathogens in the Palava region.

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

Grapevine is vulnerable to numerous diseases including grapevine trunk disease (GTD), which is a threat in wine industries, causing serious losses due to the premature vine decline and yield losses. Several studies have examined causes of GTD, but key questions on emergence and severity of the disease remain unanswered, including possible differences in plant pathogenic fungal communities of asymptomatic and symptomatic grapevines. Fungal DNA metabarcoding data were generated from bark, perennial wood, and soil samples from symptomatic and asymptomatic grapevines from three terroirs. Larger compositional differences in plant pathogenic fungi were found among different plant parts within, than among, grapevine plants. GTD-related fungi were dominant in perennial woody tissues, while non-GTD pathogens were found in soil. Asymptomatic plants did not differ from symptomatic plants. These results indicate that fungi generally associated with Esca disease belong to the core grapevine microbiome, and are likely to be commensal endophytes and/or latent saprotrophs, some of which can act as opportunistic pathogens on stressed plants. In addition, compositional differences were found among sampling sites, particularly in soil, which suggest influence of local edaphic and mesoclimatic factors on communities of plant pathogenic fungi. Differences among terroirs in plant pathogenic fungal communities were also detected in grapevine woody parts, which indicates that environmental factors are important for the development of Esca disease. Further research is required to determine effects of abiotic conditions on fungal compositional dynamics in Esca-affected plants.

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emy of Sciences) to KZV, and the Széchenyi 2020 Programme GINOP-2.3.2-15-2016-00061 (the European Regional Development Fund and the Hungarian Government).

### Fungus-bacterium interactions in grapevine wood: consequences for plant health

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Viticulture is confronted with heavy economic losses caused by Grapevine Trunk Diseases (GTDs). Fungi have been described as the main causes of GTDs, but bacterium diversity is very high in grapevine wood. The function of these wood-inhabiting bacteria is relatively unknown, with only limited established knowledge of their interactions with grapevines or their wood-inhabiting fungi. Using different bacterial strains isolated from wood of Bordeaux grapevines, and three major GTD pathogens, bacterial-fungal interactions were shown to range from synergism to antagonism, depending on the species of bacteria and fungi involved. Screening of bacterial strains against *Neofusicoccum parvum*, *Phaeomonella chlamydospore* (*Pch*) and *Fomitiporia mediterranea*, showed that some strains inhibited the growth of these fungi, and reduced severity of the wood necroses caused by *N. parvum* and *Pch* in young grapevines. In contrast, synergistic relationships were observed for other bacterial strains and *N. parvum* or *F. mediterranea*. Some strains independently destroyed grapevine wood components (cellulose and hemicellulose) and fungus ability to degrade wood structures was strongly influenced by bacteria inhabiting wood. A newly described bacterium *Paenibacillus xylinteritus* displayed synergism with *F. mediterranea*, and promoted wood degradation compared to that caused by *F. mediterranea* alone. These results raise question the functional diversity of the bacterial communities colonizing grapevine wood, and their direct or indirect involvement in GTDs.

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

Grapevine is associated with diverse endophytic fungal communities affecting the health status and productivity of the host plants. Previous research has shown that several *Alternaria* spp. are common endophytes inhabiting different grapevine varieties, and these fungi have been studied primarily because of their challenging taxonomy and abilities to produce a variety of secondary metabolites. However, data are limited on well-identified species in Hungarian vineyards, and their secondary metabolites. Molecular identification was determined for endophytic *Alternaria* species colonizing above-ground grapevine tissues, and the metabolite profiles of the *Alternaria* isolates were assessed. Based on the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, the *Alternaria* species in *Alternaria* sect. *Alternaria* predominated in isolates from asymptomatic leaves and clusters. Since species-level discrimination within the *Alternaria* sect. *Alternaria* is not adequate using only the ITS region, the isolates were subjected to combined analyses of five additional genomic loci (RPB2, ALTA1, endoPG, OPA10-2 and KOG1058). The metabolite profiling was carried out using ultra-high performance liquid chromatography (UHPLC)-high-resolution mass spectrometry (HRMS). This indicated that the endophytic fungi from healthy grapevine shoots were of two distinct lineages, the *A. alternata* and *A. arborescens* species complexes, and that numerous compounds characteristic to the genus occur in the different *Alternaria* lineages.

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ences (D.G. Knapp, G. Tóth, K.Z. Váczy), and the Bolyai+ New National Excellence Program of the Ministry for Innovation and Technology (D.G. Knapp, G. Tóth, K.Z. Váczy).

**Fungal diversity in necrotic wood of *Prunus* trees in Germany.** U. DAMM<sup>1</sup> and S. BIEN<sup>2</sup>. <sup>1</sup>Senckenberg Museum of Natural History Görlitz, PF 300 154, 02806 Görlitz, Germany. <sup>2</sup>Department of Forest Protection, Northwest German Forest Research Institute, 37079 Göttingen, Germany. E-mail: ulrike.damm@senckenberg.de

Wood diseases of fruit trees have far-reaching ecological and economic consequences. Lack of knowledge of the diversity and taxonomy of wood disease fungi impedes effective and targeted control measures in cases of sudden disease outbreaks. A survey was conducted in orchards of *Prunus avium*, *P. cerasus* and *P. domestica* in three important fruit production areas in Germany. More than 1000 fungus isolates were obtained from symptomatic host wood, and at least 172 species (mostly *Ascomycota*) were identified, based on ITS and LSU sequence data. *Aposphaeria corallinolutea* (*Dothideomycetes*) and *Pallidophorina paarla* (*Leotiomyces*) were most frequently detected, from all three host species and from all sampling regions. Based on the number of nucleotide differences to reliable reference sequences, species were identified with high certainty and 20 species with low certainty. The first group included 14 taxa that were analysed in-depth within this project, of which eight species (*Arboricolonus simplex*, *Collophorina badensis*, *C. germanica*, *C. neorubra*, *Cadophora prunicola*, *Ca. ramosa*, *Minutiella pruni-avium*, *M. simplex* and *Proliferodiscus ingens*) and two genera (*Arboricolonus* and *Pallidophorina*) were newly described. A further 69 taxa could not be assigned to any species, and were regarded as potential new species. Reduced conidiogenous cells and a yeast-like phases were frequently observed (e.g. in *P. paarla*, and *Collophorina* and *Minutiella* species), and are likely to adaptations to living within host wood.

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## Mycotoxins: prevention and control

***Fusarium tricinctum* species complex members are emerging pathogens in several crops: the case of apple.** M.T. SENATORE<sup>1</sup>, R. SOLDESTI<sup>1</sup>, M. CALI<sup>1</sup>, E. CAPPELLETTI<sup>1</sup>, M. SÜLYÖK<sup>2</sup> and A. PRODI<sup>1</sup>. <sup>1</sup>Department

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Members of the *Fusarium tricinctum* species complex (FTSC) are polyphagous and widespread pathogens, although some are considered of secondary importance on several crops. However, their presence has increased in crops such as wheat, barley, ryegrass, redcurrant, box and apple, causing yield losses, quality reduction and mycotoxin contamination. FTSC members have been reported as emerging pathogens on apple fruits and wood. This study analysed FTSC fungi isolated from Italian apple wood compared with isolates from different crops. The abilities of the FTSC isolates to produce mycotoxins such as Enniatins and Beauvericin (ENN and BEA) was also assessed, through molecular detection of *Esyln* gene, and this was confirmed in all isolates examined. Phylogenetic analysis based on the *TEF1a* gene was conducted, and this assigned the analysed isolates into four phylopecies: FTSC 2 (*F. acuminatum*), FTSC 3 (*F. tricinctum*), FTSC 4 (*F. avenaceum*) and FTSC 14. One isolate did not cluster with any of the reference sequences representing a potential novel species in the population. Pathogenicity and mycotoxin production of *F. acuminatum* and *F. avenaceum* strains was evaluated by artificial fruit inoculation on the apple cultivars Golden Delicious and Fuji. Although patulin has been the main mycotoxin problem for apple production, with *Fusarium* threatening wet apple cores, the problem is extended to emerging mycotoxins such as ENNs and BEA.

**Biocontrol by atoxigenic *Aspergillus* strains and *Trichoderma* spp.** C. ALTOMARE and A. LOGRIECO. Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, Via Amendola 122/O, 70126 Bari, Italy. E-mail: claudio.altomare@ispa.cnr.it

Aflatoxins are produced *Aspergillus* from infections of agricultural crops and spoilage of stored food and feeds. These compounds are carcinogenic, mutagenic and hepatotoxic, and are found in cereals, oilseeds, spices, tree nuts, maize, peanuts, pistachios, dried fruit and figs, and their derived products. Studies of genetic and aflatoxigenic diversity in *A. flavus* have led to development of a biological control strategy based on competitive exclusion, which relies on the inundative introduction of

non-toxigenic strains, that displace native aflatoxigenic genotypes. This has been effective for reducing aflatoxin contamination by more than 90% in maize, peanuts, pistachios and cottonseed. However this strategy limits and drawbacks. Non-aflatoxigenic *A. flavus* strains may not be non-pathogenic, and can crop cause diseases and yield losses. Capability to produce aflatoxins may be transferred to progeny of non-toxigenic strains by mating with aflatoxigenic strains. Other toxic metabolites beside aflatoxins may be still produced by non-aflatoxigenic *A. flavus* strains. The capability of members the biocontrol genus *Trichoderma* to control *A. flavus* and reduce aflatoxins biosynthesis has been recently highlighted, is raising increasing interest. Using *Trichoderma* spp. for biocontrol of aflatoxigenic fungi may overcome some limits, and provide advantages compared to use of non-aflatoxigenic *A. flavus* strains. As broad-spectrum biocontrol agents, *Trichoderma* can also protect plants from other pathogens other than *A. flavus*. *Trichoderma* strains also have indirect favourable effects of enhancement of plant resilience to drought stress and prevention of insect pest damage, which are factors that facilitate aflatoxin occurrence, particularly in climate change and global warming scenarios. An overview was presented of current technologies based on non-aflatoxigenic strains, and of prospects for the use of *Trichoderma* spp. for biological control of *A. flavus* and mitigation of aflatoxins risk.

## Wood diseases in fruit crops

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

Grapevine trunk diseases (GTDs) are major concerns for grape industries. In Cyprus, there has been no comprehensive study of the pathogen population structure and aetiology of this important disease complex. During 2017, 182 fields were surveyed in the main grape-producing areas of the country (Limassol and Paphos). Wood pieces from three to five selected vines exhibiting typical GTDs symptoms per field, were excised for fungal isolations. Samples were collected from 24 grapevine cultivars, although 62% were from the two most prevalent, indigenous wine cultivars Mavro and Xinisteri. More than 600 samples were processed and approx. 750 fungal strains were isolated. Molecular identification based

on ITS sequences identified more than 60 species or genera. GTD-related pathogenic species genera identified were *Phaeomoniella*, *Phaeoacremonium*, *Neofusicoccum*, *Botryosphaeria*, *Diplodia*, *Eutypa*, *Eutypella*, *Diaporthe*, *Cryptovalsa*, *Cytospora*, *Neofabraeae*, *Seimatosporium*, *Kalmusia*, and *Paraconiothyrium*. Phylogenetic analyses of these, using species-specific markers, identified *P. chlamydospora* (14.5% of isolates), *Phaeoacremonium minimum* (4.4%), *Diplodia seriata* (4%), *Botryosphaeria dothidea* (3.5%), and *Eutypa lata* (3.2%) as the predominant GTD-related species. Isolates of *Alternaria*, *Cladosporium*, *Fusarium*, *Neosetophoma*, *Ulocladium*, *Paecilomyces*, and *Penicillium*, commonly associated with grapevine microbiomes, were also detected. Pathogenicity tests were carried out using wood inoculation of young potted grapevine plants. Symptoms were recorded and re-isolations were confirmed, based on morphological characteristics, thus fulfilling Koch's postulates. This is the first systematic study of GTDs in Cyprus.

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**In vitro effects of cell density on development and metabolism of *Phaeomoniella chlamydospora*, a pathogen causing Esca disease of grapevine.** Z. KARÁCSO-NY and K.Z. VÁCZY. *Food and Wine Research Institute, Eszterházy Károly Catholic University, Leányka str. 6/G, Eger, H3300, Hungary. E-mail: karacsony.zoltan@uni-eszterhazy.hu*

*Phaeomoniella chlamydospora* is an important pathogen causing esca disease of *Vitis vinifera*. Esca belongs to the group of grapevine trunk diseases (GTDs) caused by fungal infections of vascular tissues, leading to symptoms both perennial (wood necroses) and annual tissues (stunted shoots, foliar chloroses and necroses) of host plants. The pathogenesis processes of GTDs have still to be fully defined. Uneven expression of external symptoms on infected plants and the lack of correlation between pathogen abundance in hosts and occurrence of symptoms suggest that external factors and unique events can affect disease development. Critical pathogen cell population density within hosts, which shift otherwise harmless fungi to pathogenic behaviour, can be important. Microorganisms perceive cell populations by the "quorum sensing" mechanism, which affects the virulence of several bacterial and fungal pathogens. Results in this study have indicated that cell population density is important for development and metabo-

lism *P. chlamydospora*. Experiments in solid medium cultures indicate large cell populations promote germination of conidia and later the formation of polysaccharide-rich mycelium patches, which are indicators of biofilm formation. High cell population densities promoted colony pigmentation and synthesis of antibacterial compound(s), indicating a shift towards secondary metabolism. These phenomena may also occur in infected plants, leading to disease development.

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**Aetiology and management of trunk and scaffold canker diseases of almond in California.** L.A. HOLLAND, F.P. TROUILLAS, M.T. NOURI, D.P. LAWRENCE and R. TRAVADON. *Department of Plant Pathology, University of California, Davis, 95616 Davis, CA, USA. E-mail: flotrouillas@ucanr.edu*

Trunk and scaffold canker diseases (TSCD) caused by fungal pathogens are destructive diseases of almond trees. Common TSCD symptoms include sunken bark lesions, wood discolouration and gummosis in trunks and branches. Recent surveys showed the occurrence of four major canker diseases in California, including *Botryosphaeriaceae* cankers, *Ceratocystis* canker, and *Cytospora* and *Eutypa* cankers. Up to 21 fungal pathogens were found to be associated with TSCD of almond. Pruning wounds made for scaffold selections and maintenance pruning were common entry sites for canker pathogens. Strategies were investigated for prevention of pruning wound infections. Fungicide trials were carried out to determine the best products for the protection of pruning wounds. These indicated superior efficacy of thiophanate-methyl against several canker pathogens. Additionally, the biocontrol agent *Trichoderma atroviride* SC1 provided excellent pruning wound protection, allowing sustainable control solutions against canker diseases. Additional studies were investigated seasonal susceptibility of pruning wounds and duration of pruning wound susceptibility, in relation to time (month) of pruning. These showed that duration of pruning wound susceptibility was least when pruning is carried out in January. Overall wound susceptibility declined substantially after 1 to 2 weeks following pruning. This research has indicated that one application of pruning wound protectant such as with the conventional fungicide thiophanate-methyl or the biocontrol agent *T. atroviride* SC1, following late prun-

ing in January, can reduce risks of infection of pruning wounds by canker pathogens.

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

*Botryosphaeriaceae* fungi have cosmopolitan distribution and a wide plant host range, including olive trees. Olive cultivation in southern Italy is important, with large areas and high yields. Different *Botryosphaeriaceae* have been associated with olive trunk diseases, causing cankers, die-back and olive decline. Several wood samples collected over 15 years from olive trees showing symptoms yielded a collection of fungal isolates, many of which were in this family. A phylogenetic study, carried out on representative strains and combining sequences from internal transcribed spacer region and translation elongation factor 1- $\alpha$  gene, allowed identification of four *Neofusicoccum* species such as *N. luteum*, *N. mediterraneum*, *N. parvum* and *N. vitisifusiforme*, and *Botryosphaeria dothidea*. Conidium size, colour and shape confirmed the molecular identifications. This study highlighted that the symptomatic olive wood analyzed was mainly infected by *Neofusicoccum* species. In particular, *Neofusicoccum* species above mentioned resulted all to be severe pathogens for olive by pathogenicity test carried out in vivo conditions.

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

Grapevine trunk diseases (GTDs) are important in viticulture. This disease complex may cause decay and death of grapevine arms and whole trunks with a wide range of symptoms. Losses of vines year by year accelerate the economic aging, so vineyards become prema-

turely unproductive. There are no effective preventative or curative treatments or agronomic practices against these diseases, because of the diversity of GTD pathogens, their protected niches within host woody tissues, which are difficult to reach with chemicals, and effects of abiotic factors on GTD incidence and symptom development. The present study aimed to determine the environmental parameters that may have significant influences on GTD development and spread. Determination of incidence patterns of different diseases within plantations, and the effects of climate and other environmental factors, require long time data collection. More than 3-year surveys were carried out in the Tarcál and in Pálag regions of Hungary, to detect incidence of GTDs and isolate fungi from cordón woody tissues. Results indicate that uneven rainfall, late spring frosts, high groundwater levels, and soil erosion have all increased GTD incidence. Species of *Botryosphaeriaceae* were the most common GTD pathogens in the examined plantations, based on isolations and isolate morphology and molecular identifications.

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

*Fomitiporia mediterranea* (Fmed) is a white rot agent that has been associated with esca in grapevines, but its biomolecular mechanisms of wood degradation by this fungus are not fully understood. Like all white rot agents, Fmed has an enzymatic pool (laccases, manganese peroxidases, endoglucanases and  $\beta$ -glucosidases) that can attack and depolymerizing cellulose, hemicellulose and lignin components of lignocellulosic biomass. Comparative genomics studies and experimental observations on the importance of iron in Fmed pathogenesis, retrieved from the literature, allowed formulation of the hypothesis that

Fmed could utilize non-enzymatic and enzymatic mechanisms for wood degradation in grapevine. This hypothesis is based on the Chelator Mediated Fenton (CMF) model, proposed in the late 1990s for brown rot fungi. Present study results, under appropriate experimental conditions and as close as possible to the physiological conditions of grapevine wood, have demonstrated the *in vitro* ability of Fmed to: i) produce low molecular weight chelating metabolites, ii) reduce ferric iron to ferrous iron, and iii) produce radical species such as the hydroxyl radical, thus satisfying all the conditions supporting non-enzymatic wood degradation mechanisms. The CMF model is also likely to be strain-dependent in Fmed. Further research is ongoing to study this model *in lignum* and *in planta*.

This research was financially supported by Université de Haute-Alsace.

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

Grapevine trunk diseases (GTDs) are increasing in vineyards. Many pathogens (e.g. *Phaeoemoniella chlamydospora* and *Neofusicoccum parvum*) are involved in these diseases, and there are no chemical treatments available for their control. Biocontrol of GTD pathogens using microorganisms has been developed. These include the oomycete *Pythium oligandrum*, strains of which naturally colonized grapevine roots in vineyards in several countries. The ability of *P. oligandrum* to induce grapevine resistance against *N. parvum* was evaluated. Two greenhouse assays showed that necrosis of Cabernet Sauvignon cuttings caused by *N. parvum* was reduced by 65% when *P. oligandrum* colonized the root systems of young vines. Expression levels of a set of 96 genes ("NeoViGen96" chip) involved in grapevine defence pathways were assessed by real-time PCR in grapevine trunks, to determine plant responses after inoculation

by *P. oligandrum* and/or *N. parvum*, at three different sampling time points. This showed that sampling time affected gene expression for all the treatments. At each sampling time, specific host responses to the different treatments (control, *P. oligandrum*, *N. parvum*, *P. oligandrum* + *N. parvum*) were also differentiated. When *P. oligandrum* colonizes grapevine root systems, infection with this pathogen is associated with intense up-regulation of PR protein and signalling pathway genes. A priming effect of the host defence system was induced in presence of *P. oligandrum*.

This research was supported by the Industrial Chair GTDfree, funded by ANR (French National Research Agency) and Jas Hennessy & Co.

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

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

## POSTER SESSIONS

**Pathogenesis-related proteins of *Arabidopsis thaliana* in response to combination of abiotic (salinity) and biotic (fungus gnats and dodder) stresses.** L. ZAGORCHEV, D. TEOFANOVA, K. GEORGIEVA and A. ATANASOVA. *Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski", 1164, 8 Dragan Tsankov blvd., Sofia, Bulgaria E-mail: lzagorchev@biofac.uni-sofia.bg*

This study was assessed the response of *Arabidopsis thaliana* L. to a combination of abiotic and biotic stresses, including: 1) salinity; 2) fungus gnat (Sciaridae) herbivores; and 3) parasitic plants – *Cuscuta campestris* Yunk. (dodder), as well as the indirect effect on the dodder. The major pathogenesis-related protein classes chitinases and proteases were profiled under single, double or triple stresses in directly and indirectly affected host organs. Enzymes were studied by zymography analyses after separation on polyacrylamide gel electrophoreses, and data were analyzed by ANOVA and principal component analysis. All stress factors affected the number of isoforms and the relative activity of both enzyme groups, with the effect of fungus gnats being the most pronounced. The combination of stresses leads to mostly antagonistic effects in roots and cumulative effects in leaves. Salt stress caused similar effects to parasitism, a differential effect in comparison to herbivores, and did not interact with biotic stresses. Herbivores and parasitism affected the PR-proteins differentially, but interacted strongly. Salinity and herbivores, applied to the host plants, also affected the parasitic plants.

This research was financially supported by grant KP-06-N31/10 of the National Science Fund, Ministry of Education and Science, Bulgaria.

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

The ascomycete *Monilinia fructigena* is among the most serious causal agents of brown rot in deciduous fruit trees. The pathogen genome is available, providing novel genomic resources for thorough characterization of particular gene families. WD40 proteins are scaffolding molecules in protein interactions, and although these proteins were extensively characterized in other organ-

isms, little is known in phytopathogenic fungi of their expansion and structural patterns, as well evolutionary selective pressures acting upon their WD40 repeats. Sixty-two WD40 proteins were identified in the *M. fructigena* genome (MfWD40s), and based on their phylogenetic classification and domain architectures they were categorized into five clusters and 17 classes, indicating their diverse expansion. Gene ontology analysis revealed that MfWD40s participate in protein binding and are involved in various biological processes. RNAseq data showed that the greatest number of MfWD40s genes had stage-specific expression profiles, with most being highly expressed during germination of conidia. The evolutionary signatures acting upon their WD40 repeats were accurately assessed. These results indicate existence of purifying selection acting across their phylogenies. However, a majority of amino acid residue sites were positively selected and were localized widely across the WD40 repeats, putatively affecting their ligand-binding specificities. These results will allow further deciphering of the diverse functions of the WD40 gene family in *M. fructigena*.

This research was supported by Hellenic Agricultural Organization HAO 'Demeter', Greece.

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

*Botrytis cinerea* is a widespread plant pathogen, which causes gray mould, and infects many economically important hosts, including kiwifruit. To decipher the induced defence mechanisms upon infection in kiwifruit, an RNA sequencing (RNA-Seq) approach was used to explore the transcriptome of mature affected fruits at 12, 24, and 48 h after infection (HAI). Eighteen cDNA libraries were produced generating 978,286,445 base pair-end reads. A total of 2,795 differentially expressed genes (DEGs) were identified, with most being up-regulated at late stages across a broad and time-dependent transcriptional reprogramming during infection. Suppression of photosynthesis-related pathways was detected at 12 h HAI, whereas DEGs involved in specific cell

wall modification processes may have facilitated early pathogen colonization. A strong shift towards defence responses and an induced transcriptional reprogramming was triggered but not earlier than 24 HAI. The majority of DEGs were up-regulated mainly at 48 HAI, and were predominately involved in the induction of biosynthesis of secondary metabolites, pattern-triggered immunity and signalling transduction cascades, activation of pathogenesis-related proteins, membrane-localized receptor-like kinases, transcription factors, and various transporters. A subset of *B. cinerea* genes that are linked to pathogen growth or manipulation of particular plant defence responses were also induced. These data provide novel insights into kiwifruit-*B. cinerea* compatible interactions, providing new knowledge for efficient management of this pathogen.

**Infection and colonization of grapevine propagation material by pathogens associated with young grapevine decline in Greece.** C. TSOUKAS, A.K. TZIMA, A. TRIANTAFYLLOPOULOU and E.J. PAPLOMATAS. *Laboratory of Plant Pathology, Agricultural University of Athens, Iera Odos 75, Athens, Greece.* E-mail: epaplom@aua.gr

Colonization of grapevine propagation material by young grapevine decline-associated pathogens, was investigated in eighty canes sampled (in July 2018) from four different varieties of mature mother plants ("Ksinomavro", "Agiorgitiko", "Asirtiko", or "Roditis"). In December 2018, forty 4-month-old vines ("Soul-tanina") grafted on rooted rootstocks (R110 or 41B) were also collected. Nested PCR reactions based on amplifications of the ITS region and species-specific primers showed that grafted young vines were heavily infected by the young grapevine decline-associated pathogens. Fourteen (35%) of 40 grafted cuttings were infected by *Phaeomoniella chlamydospora* and 35 (88%) were infected by Black Foot disease-associated pathogens. Multiplex PCR assays revealed that the dominant species was *Dactylonectria macrodidyma*. In July, pathogens could not be detected by PCR at the bases of growing season canes used for grafting. For the colonization studies, a *P. chlamydospora* isolate was transformed with the green fluorescent protein gene (*gfp*) using *Agrobacterium tumefaciens*-mediated transformation. Twenty four 4-month-old grafted vines were inoculated with either with the GFP *P. chlamydospora* strain or the wild type, and then kept in a greenhouse for 3 months. Fluorescence microscopy showed that, at 3 months post inoculation, the pathogen had colonized the cells around the

xylem vessels, parenchyma cells and primary xylem, but had not advanced more than 2 cm above or below the inoculation points.

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

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

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

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To investigate possible transmission of *Colletotrichum acutatum* by the olive fruit fly *Bactrocera oleae*, thus contributing to disease spread, pupae and healthy olive drupes were collected in October 2021 from olive orchards in Greece. The drupes were artificially inoculated by an aqueous suspension of  $10^6$  conidia mL<sup>-1</sup> of the pathogen, while control drupes were sprayed with ddH<sub>2</sub>O. Seven days later after incubation under appropriate conditions, the drupes were transferred for 2 d into separate entomological cages containing adult flies that had emerged from the collected pupae. All drupes were subsequently discarded and replaced by new sterilized drupes that remained for 2 days with the flies. Thereafter, molecular detection of *C. acutatum*, on flies and on drupes, was performed using Real-Time PCR assays based on TaqMan chemistry, with *C. acutatum*-specific primers targeting the HIS-3 gene. Pathogen DNA was detected in all artificially inoculated drupes and in some of the flies. Fungus DNA could not be detected in negative control drupes, while pathogen DNA was detected in drupes that remained for 2 d with adult flies previously caged with artificially inoculated fruits. To investigate the possibility that fungal spores were carried passively by the flies, adult insects were superficially sterilized using either hexane or water. Besides detection in non-sterilized flies, DNA of the pathogen was also detected in surface sterilized flies. These results indicate that *B. oleae* could be a vector of *C. acutatum* anthracnose on olive drupes.

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

Some microorganisms that promote plant growth can manipulate the level of ethylene in plants by decreasing ACC, an ethylene precursor, using ACC deaminase

(ACCd), Increasing host resistance to abiotic and biotic stresses. Despite the beneficial effect of ACCd-producing microorganisms, the role of ACCd in phytopathogens has been little studied. Whether ACCd of the soil-borne pathogen *Verticillium dahliae* was involved in pathogenesis and ethylene production was investigated. Overexpression and deletion mutants of the *V. dahliae* gene (ACCd) encoding this enzyme produced more ethylene than the wild type strain, with deletion mutants producing more ethylene than overexpression strains at most time points of observation. However, overexpression of the ACCd gene increased virulence of the pathogen in tomato, while disruption of ACCd resulted in reduced disease severity. ACC holds a key position in many plant physiological processes, with its main role as direct precursor of ethylene. Recent studies have shown that ACC may act as a potential signalling molecule independent from ethylene. To investigate whether the pathogenicity phenotype observed was due to ACC regulation, roots of WT and Never Ripe (Nr) tomato plants and Col-0 and *etr1-1 Arabidopsis* plants were treated with ACC prior to *V. dahliae* inoculation. Plants pre-treated with ACC displayed less severe symptoms than untreated controls. ACC application to roots of Col-0 and *etr1-1* plants *in vitro* triggered root hair formation and induced hormone-dependent defence responses. These results suggest a novel role of ACC as a regulator of plant defence and pathogen virulence.

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

The obligate biotrophic pathogen *Synchytrium endobioticum* causes potato wart, and is an important quarantine pest of cultivated potato (*Solanum tuberosum*). As chemical control is not effective in eradicating the pathogen, strict phytosanitary measures combined with the use of

resistant potato varieties are the only successful strategies to prevent the disease from spreading. The pathogen can overcome host resistance by developing new pathotypes. More than 40 pathotypes have been reported, of which pathotypes 2(G1), 6(O1) and 18(T1) are the most aggressive and widespread in Europe. Previous studies conducted under natural and controlled environment conditions have shown that only two of 50 tested European potato genotypes exhibited phenotypic resistance to pathotype 18(T1), which occurs in Greece. The potato varieties Kuba (resistant), Novano (tolerant) and Spunta (very susceptible) were selected to further study their molecular response to infection by pathotype 18(T1), using RNA-Seq transcriptomic analysis. Raw data genome-guided transcriptome assembly was carried out using STAR2.5.3.a and HTSeq. Differential Gene [removed]DGE) was used in R using DESeq2 1.32.0, followed by functional DEGs classifications using topGO in R and G-profiler. Many genes related to cell division, lipid catabolism, hormone transportation and peroxidase function were identified. GO-term analysis revealed enrichment regarding terms related to pathogen resistance, membrane bound organelle, cell cycle, defence response, structural molecule activity and response to external biotic stimulus, in intra- and inter-variety (infected or not) comparisons.

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

Fusarium wilt, caused by phytopathogenic strains of the soil-borne *Fusarium oxysporum* (Fo) species complex, is responsible for serious yield losses in >120 economically important crops. Because of the soil-borne nature

and the wide host range of the pathogen, *Fusarium* wilt management is difficult. Breeding for resistance has shown positive results in reducing the pathogen spread and increasing crop yields. The role of  $\beta$ -amylase (*BAM*) genes in host resistance to the *F. oxysporum* complex was investigated *in planta* using *Arabidopsis thaliana* ecotype Columbia-0 (wild-type, wt) and its mutants *bam1*, *bam2*, *bam3* and *bam4*, and their combinations, inoculated with *F. oxysporum* f. sp. *raphani*. The pathogenicity tests showed that *bam3* plants were less susceptible to infection than the wt plants. Disease intensity and fungal biomass in the host vascular system, as quantified by qPCR, were lower in *bam3* mutants compared to the wt plants. Transcriptomic and metabolomic analyses carried out using, respectively, DNA microarrays and gas chromatography-mass spectrometry (GC-MS), showed that the resistant phenotype of *bam3* plants is associated with differential gene expression and differential production of metabolites related to the cell wall integrity, carbohydrate metabolism, amino acids, and plant hormones.

This research was financially supported by the Benaki Phytopathological Institute.

#### **Evaluation of foliar resistance of Greek wine grape varieties to downy mildew by phenotyping methods and comparative transcriptomic and proteomic analyses.**

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The obligate biotrophic oomycete *Plasmopara viticola* (Berk & Curt.) Berl. & de Toni, the causal agent of grapevine downy mildew (DM), is a serious threat to viticulture. Sustainable and environmentally friendly DM management strategies rely mainly on host resistance. No information is available on the response of Greek winegrape varieties to *P. viticola* infections. The resistance level of 11 native wine grape varieties was assessed using leaf disc bioassays. The responses of the

grape varieties to DM was evaluated based on the host necrotic reactions and the intensity of pathogen sporulation during 15 d post-inoculation (dpi). The host varieties exhibited different responses to DM, while none were resistant. The least susceptible and the most severely affected varieties were selected for transcriptomic and proteomic analyses, to gain an insight into the molecular aspects of the host-pathogen interactions. Libraries prepared from total RNA and protein extracts from grapevine leaf disc samples (inoculated and non-inoculated) collected 5 dpi were analyzed, respectively, with the Illumina NextSeq500 and the Thermo Q-Exactive Orbitrap nanoHPLC-HRMS/MS platforms. Transcriptomic analysis was performed according to the “New Tuxedo” pipeline. Differential expression and functional classifications of the DEGs revealed genes involved in disease resistance, modulation of pathogen defence and leaf cell death, downy mildew-specific resistance, and plant hormone regulation. Proteomic analysis conducted using R programming revealed significant differences between the two varieties.

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#### **Exploring the selective signatures upon LRR-containing genes towards their functional diversification... the cases of cherries and mulberries.**

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Cherry (*Prunus avium*) and mulberry (*Morus notabilis*) crops are affected by several phytopathogenic fungi. Gene families that contain leucine-rich repeat (LRR) domains are key components of plant immune responses. The subfamily of LRR receptor-like serine threonine kinases plays a pivotal role in defence responses against phytopathogenic fungi. Resistance gene analogues (RGAs), which are closely related to the resistance (R) genes, are widely employed as functional molecular markers in breeding programmes towards host disease resistance against pathogens. The evolutionary profiles of these genes (173 RGAs of cherry and the 142 LRR receptor-like serine threonine kinases of mulberry) were

assessed to determine if successive episodes of diversifying selection contributed to acquisition of novel pathogen recognition repertoires. These genes were subjected to strong positive selection, while the majority of the positively selected amino acid residues were localized widely across gene sequences. These residues could have originally conferred specificity to a hypothetical ligand which was repeatedly altered to provide novel binding functions. The clustered distribution of these genes could give high birth and death rates, with diversifying episodes acting on their functional domains, putatively affecting their ligand-binding specificities. These evolutionary insights indicate how these genes are evolving, making them the foremost surveillance mechanism for durable resistance against rapidly evolving pathogens.

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

Sweet cherry is affected by many pathogens that are major threats to fruit production. As climate change affects the susceptibility of sweet cherry cultivars to rapidly evolved and emergent pathogens, it is important to thoroughly investigate the repertoire of high impact structural variations in disease resistance genes among cultivars, to facilitate selection of superior host genotypes. Whole-genome resequencing (WGRS) of 21 sweet cherry cultivars, representing the majority of wild and cultivated Greek germplasm, was employed to characterize genetic and structural variation among 119 defence-related genes (such as RPM1, RPP13, RGA2 homologues). A total of 2,468 structural variants were mapped on 107 disease resistance genes. The majority of the variants were heterozygous and were assigned as missense or synonymous variants. Forty-four NBS-LRR genes had single nucleotide polymorphism mutations in their coding sequences, with high impacts across the sweet cher-

ry genotypes. These data of this genome-wide analysis provide promising resources for expanding knowledge of diversity and evolution of variations in disease resistance genes across in different sweet cherry cultivars. As well, screening and genetic analyses of these genes would facilitate identification of functional variations contributing to variations in host resistance. This approach would facilitate evaluation of natural resistance resources, towards increasing durable disease resistance against sweet cherry pathogens.

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

Drought is a major abiotic stress with adverse effects on citrus. In grafted plants, drought tolerance is mainly determined by the rootstock. *Citrus macrophylla* Wester, a rootstock used in the Mediterranean region, is known to confer greater drought tolerance than other rootstocks. Transcriptional changes of *C. macrophylla* under water deficit was studied by Illumina RNA-seq technology, and responsive genes were identified. A total of 2745 differentially expressed transcripts (DETs, fold changes >2), were identified, of which 631 (23%) were up-regulated and 2114 (77%) were down-regulated. Gene ontology analysis identified “phenylpropanoid biosynthesis”, “amino sugar and nucleotide sugar metabolism” and “photosynthesis” as greatly down-regulated in response to drought stress. Up-regulated processes were soluble carbohydrates and amino acids, which contribute to osmotic balance and protect against oxidative damage. Glycolysis and photorespiration were also strongly up-regulated in *C. macrophylla* as well as transcripts for low molecular weight proteins such as late embryogenesis abundant protein. Citrus plants face abiotic and

biotic stresses. To assess the interplay of drought stress and Citrus tristeza virus (CTV) infection, twelve genes were profiled by quantitative PCR (qPCR) analysis, in control and CTV-infected *C. macrophylla* plants, with or without drought stress. The twelve analysed transcripts were correlated ( $r = 0.82$ ;  $P < 0.001$ ) with the RNA-Seq results, and gave insight into the response of *C. macrophylla* to drought and/or infection with CTV. Transcriptome results showed highly responsive genes to drought stress in stem tissues, indicating potential candidates for genetic selection of highly drought tolerant *C. macrophylla* plants.

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**Efficiency of different proactive measures in the management of Verticillium wilt of potato in Lebanon.** F. BAROUDY<sup>1</sup>, L. SAADÉ<sup>2</sup>, Z. MAHFOUZ<sup>3</sup>, C. SAAB<sup>1</sup>, E. GERGES<sup>1</sup> and W. HABIB<sup>1\*</sup>. <sup>1</sup>Laboratory of Mycology, Department of Plant Protection, Lebanese Agricultural Research Institute, P.O. Box 90-1965, Fanar, Lebanon. <sup>2</sup>Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, P.O. Box 55530, Dekwaneh, Lebanon. <sup>3</sup>Faculty of Agricultural and Food Sciences, Holy Spirit University of Kaslik, P.O. Box 446, Jounieh, Lebanon. \*W. HABIB current affiliation: Centro di Ricerca, Sperimentazione e Formazione in Agricoltura – Basile Caramia (CRSFA), Via Cisternino 281, Locorotondo, 70010 Bari, Italy. E-mail: whabib@lari.gov.lb

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is an economically important disease producing severe losses in Lebanese potato crops. Management of the disease is difficult, and requires integration of several methods because the pathogen can survive in soil as microsclerotia for up to 15 years. Two field experiments were carried in artificially inoculated soil over 2 years, to evaluate effects of different control methods on wilt incidence and severity, soil inoculum density, tuber yields and plant height. In the first trial, the fumigant allyl-isothiocyanate reduced microsclerotium population by 41% and wilt incidence by 18%. Application of fungicides (azoxystrobin + mefenoxam) at planting reduced wilt incidence by 20%. In the second trial, inoculum population density was reduced (58%) by soil solarization and integration of solarization and incorporation of broc-

coli residues (57%). Soil solarization decreased disease severity by 25%, and integration of fresh broccoli residues and soil solarization reduced disease incidence by 23%. Although incorporation of fresh broccoli residues before sowing did not significantly affect the disease parameters in both trials, this promoted average total tuber yield by 15.1 t ha<sup>-1</sup> compared to 7.9 t ha<sup>-1</sup> in the untreated controls. The results from both experiments indicate that integration of control methods, particularly soil solarization and incorporation of fresh broccoli residues are promising for management of Verticillium wilt of potato in Lebanon.

**Endophytic bacteria as potential biocontrol agents against *Phaeomoniella chlamydospora*, the dominant causal agent of Petri disease in grapevines.** C. TSOUKAS, A.K. TZIMA and E.J. PAPLOMATAS. Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece. E-mail: epaplom@aua.gr

Petri disease is most destructive disease of grapevines, and its occurrence in grapevine nurseries is widely reported. Due to the lack of agrochemicals to control the disease, utilization of effective biological control agents (BCAs) becomes important. The grapevine root bacteriome was assessed for biocontrol potential against the fungal pathogen *Phaeomoniella chlamydospora*, to further develop microbial communities capable of suppressing the disease. Bacteria were isolated from roots derived from vines of different ages (5 to >50 years) with no symptoms of trunk diseases. Twenty-three bacterial isolates were selected, based on colony morphology. The antagonistic abilities of the strains was assessed in *in vitro* experiments, using a modified dual culture technique. Inhibition was estimated as proportion (%) of mycelial growth compared with experimental controls. Four isolates significantly inhibited growth of *P. chlamydospora*, compared to the rest of the isolates. Sequencing of the 16S rRNA region showed that one strain was *Bacillus halotolerans* while the other three were *Bacillus* sp. To develop efficient microbial communities, interaction assays between the most effective bacterial strains based on antagonism is underway. These preliminary results have shown that, the endophytic microbiome has the potential to be utilized in control of grapevine trunk disease.

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

Optimization of agricultural practices is important. Copper (Cu) has been used as a fungicide in agriculture for more than a century, playing a key role in integrated disease management. Because of Cu accumulation in soil, use needs to be monitored and controlled, using modern technologies to minimize Cu use. We propose a novel approach based on the Active Thermography (AT) to monitor the persistence of Cu on leaf surfaces, and the use of a new polysaccharide-based adjuvant to prolong the persistence of Cu fungicides. Thermal responses were monitored after different treatments of grapevine and tobacco plants over a 3-week period. Spatial maps of leaf thermal recovery times were calculated and compared. These assessments showed that the adjuvant prolonged persistence of the fungicide on the leaves. The measurements were used to estimate the amount of the Cu on the leaves. Maximum difference in the amount of Cu between the plants treated with Cu alone or with Cu + adjuvant (after 2 weeks) was approx. 29% for grapevine and 32% for tobacco. These results demonstrate that an approach based on AT can effectively evaluate in real-time the presence of Cu on treated plants, allowing optimization of use of these fungicides in the agricultural practice.

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**Fungicide resistance of *Botrytis fabae* population isolated from faba bean in Morocco.** S. AOUZAL<sup>1,2</sup>, S. TOFFOLATTI<sup>3</sup>, H. ERRATI<sup>1,2</sup>, R. MENTAG<sup>4</sup>, H. HOU-MAIRI<sup>2</sup> and S. KRIMI BENCHEQROUN<sup>1</sup>. <sup>1</sup>*National Institute of Agricultural Research, (INRA, CRRA- Settati), P.O. Box 589, Settati, Morocco.* <sup>2</sup>*University of Hassan I<sup>st</sup>, Faculty of Science and Technology, P.O. Box 577, Settati, Morocco.* <sup>3</sup>*Department of Agricultural and Environmental Sciences, Milan, Italy.* <sup>4</sup>*Biotechnology Research Unit, National Institute of Agricultural Research, (INRA, CRRA-Rabat), Morocco. E-mail: sanae.krimibenchegroun@inra.ma*

Chocolate spot, caused by *Botrytis spp.*, is one of the most economically important diseases of faba bean. Management of this disease in Morocco largely depends on chemical treatments using several classes of fungicides, especially with pathogen site-specific fungicides. *Botrytis spp.* have high risks for the development of resistance to fungicides. Sensitivity was assessed of *Botrytis fabae* populations to fludioxonil, fenhexamid and boscalid, which are currently used for disease control in Morocco. Thirty-one isolates of *B. fabae* were collected from seven bioclimatic regions, where faba bean production is important. The sensitivity of the isolates to fludioxonil, fenhexamid and boscalid was evaluated with an automated quantitative test, using 96-well microtitre plates. Growth inhibition percentage (GIP) of pathogen was determined, using absorbance data (at 492 nm) after 72h of incubation, to determine sensitive isolates to each fungicide. The results showed that isolates with resistance to fludioxonil and boscalid were widespread. Seven resistant profiles were determined in the population (FluRFenRBosR, FluSFenSBosS, FluRFenRBosS, FluRFenSBosR, FluRFenSBosS, FluSFenSBosR and FluSFenRBosR). Among these, FluRFenRBosR was widely distributed and present in 58% of the isolates, in almost all of the assessed locations. The prevalence of resistant profiles found in this study will be useful in designing appropriate management of chocolate spot of faba bean in Morocco.

**Integrated management of *Aspergillus carbonarius* and ochratoxins in vineyards in Greece.** M.K. ILIADI, C.S. LAGOGIANNI, M.D. KAMINIARIS, E-F.N. VARVOUNI, M.N. VARVERI, E.G. POULAKI, C.K. KAVROUMATZI, A.X. VARYMPOPI, E.I. MARGARITIS, N.S. MASTRODIMOS, K.E. POLITIS and D.I. TSITSIGIANNIS. *Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Athens, Greece. E-mail: dimtsi@aua.gr*

*Aspergillus spp.* can cause severe pre- and post-harvest berry rots in grapevines, including sour rot of grapes. *Aspergillus carbonarius* is responsible for ochratoxin A production, a carcinogenic mycotoxin which affects humans and animals. Control of *Aspergillus spp.* is difficult, as application of many fungicides, including systemic compounds, is required in vineyards. The increasing use of pesticides in grapevine cultivation during the last decade, combined with low maximum residue levels, requires development of sustainable methods to control sour rots and ochratoxin A contamination in vineyards. This study aimed to develop an integrated

*Aspergillus* sour rot and ochratoxin A production. Biological and chemical plant protection products were evaluated for control of sour rot and OTA production. These were: 1) Grape yeasts (isolated from Greek vineyards) from the collection of the Laboratory of Plant Pathology of Agricultural University of Athens, 2) Botector®, 3) Trianium®, 4) Tusal®, 5) Serenade Max®, 6) Vacciplant®, 7) EM-Effective Microorganisms®, 8) Remedium®, 9) Mevalone®, 10) Switch®, 11) Quadris®, 12) Geoxe®, 13) Chorus®, 14) Cantus®, and 15) Scala®. Experiments were carried out in commercial vineyards for four years (2016–2019) on the two Greek grapevine varieties Malagousia and Savvatiano. A successful integrated ochratoxin management system combining biological and synthetic fungicides was developed.

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**IKOPROTECTA - Agricultural composted products as plant protection and growth regulators.** M. VARVERI<sup>1</sup>, A. BAKOS<sup>1</sup>, P. GIANNIOTIS<sup>2</sup> and D.I. TSITSIGIANNIS<sup>1</sup>. <sup>1</sup>Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece. <sup>2</sup>IKORGANIC, Greece. E-MAIL: [dimtsi@aau.gr](mailto:dimtsi@aau.gr)

Composts are natural products known for beneficial properties in plant nutrition by enriching soil with organic matter and nutrients. Composts contain high microbe populations, that are mainly non-pathogenic, and studies have highlighted their capacity to suppress or control plant diseases. This study assessed the bio-potential of two composted products, (IKORGANIC), using disease assessments in greenhouse and *in vitro* experiments. The bacterial/fungal strains that were isolated from the composted products were evaluated for their ability to inhibit growth of phytopathogenic fungi and bacteria, including *Verticillium dahliae*, *Colletotrichum acutatum* and *Pseudomonas syringae* pv. *tomato*. The *in vitro* antagonistic activity of the isolates was investigated using a dual culture technique. Subsequently, the plant growth promotion and disease severity reduction efficiency of the composted products against the three pathogens was evaluated in a greenhouse on tomato plants, and in planta on olive fruits, according to the host of each pathogen. Results of the *in vitro* experiments and the comparative study of the two composted products for ability to control foliar and soil-borne plant diseases was presented.

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**Investigation of DMI-fungicide sensitivity and resistance in grape powdery mildew (*Erysiphe necator*) populations in Hungary.** Z. SPITZMÜLLER<sup>1</sup>, X. KARÁCSONY-PÁLFI<sup>1</sup>, A. PINTYE<sup>2</sup>, O., MOLNÁR<sup>2</sup>, M. Z. NÉMETH<sup>2</sup>, Á.N. HORVÁTH<sup>2</sup>, L. KISS<sup>2,3</sup>, G.M. KOVÁCS<sup>2,4</sup> and K.Z. VÁCZY<sup>1</sup>. <sup>1</sup>Eszterházy Károly Catholic University, Food and Wine Research Institute, Leányka str. 6., Eger H3300, Hungary. <sup>2</sup>Centre for Agricultural Research, Plant Protection Institute, P.O. Box 102, Budapest H-1525, Hungary. <sup>3</sup>Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD 4350, Australia. <sup>4</sup>Eötvös Loránd University, Institute of Biology, Department of Plant Anatomy, Pázmány Péter sétány 1/C. 1117 Budapest, Hungary. E-mail: [spitzmuller.zsolt@uni-eszterhazy.hu](mailto:spitzmuller.zsolt@uni-eszterhazy.hu)

Powdery mildew caused by *Erysiphe necator* is a widespread disease of grapevine (*Vitis vinifera* L.). Continuous and intensive use of sterol demethylation inhibitor (DMI) fungicides has resulted in gradual development of insensitivity to these fungicides by alterations in several genes. Occurrence of resistant *E. necator* populations is generally low, and pathogens with reduced sensitivity to one DMI compound be sensitive to other DMIs. This study aimed to detect single nucleotide polymorphisms (SNPs) in genes causing DMIs resistance, and to determine fungicide sensitivity of Hungarian populations of *E. necator*. A commonly used method was adapted to determine sensitivity to propiconazole, tebuconazole, myclobutanil or penconazole of *E. necator* conidia collected from the vineyard of Eszterházy Károly Catholic University. The fungicides were tested at different concentrations as triplicates. The ratios of the germinating and non-germinating conidia and their germ tube lengths were determined. Reduced sensitivity was detected to propiconazole, tebuconazole and myclobutanil, but not to penconazole. With gene sequence analysis, conserved nucleotide variation was detected in the coding region of 14 alpha-demethylase (CYP51), the target of the commonly used DMI fungicides. SNP in CYP51 causing tyrosine to phenylalanine substitution at amino acid position 136 (Y136F) of the protein is associated with DMI resistance in *E. necator*. Further studies will test fungicides with other mechanism of action.

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

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

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

Tomato spotted wilt virus (TSWV) (Tospoviridae) infects a wide range of hosts. The TSWV genome has three linear negative-sense or ambisense RNA segments. Segment S RNA encodes the silencing suppressor NSs, and the nucleocapsid protein N. Segment mRNA encodes the cell-to-cell movement protein NSm and two glycoproteins (Gn and Gc). Antisense transcripts of the N, NSs and M viral genes were expressed in leaves of *Nicotiana benthamiana* infected with TSWV to assay for their ability to silence virus progression. Constructs in the binary vector pK7WG2 were transiently co-expressed with p7WG2-GFP in *N. benthamiana* leaves through agroinfiltration, followed by inoculation with TSWV after 48h. Inoculated leaves were harvested 5 d after agroinfiltration for RNA extraction. Antisense transcripts of partial sequences of the three genes were also expressed throughout the host tissues, using the Tobacco rattle virus viral vector (pTRV). New leaves were harvested 10 d after agroinfiltration of the pTRV viral vector. TSWV detection and absolute quantification was carried out using a TaqMan real-time RT-PCR assay. Leaves inoculated only with TSWV and new leaves showed had low virus titre, indicating host resistance to TSWV infection. In both assays, antiviral M mRNA transcripts limited TSWV genomic RNA accumulation and propagation compared to antiviral transcripts of N or NSs genes. These results indicate that the M gene transcripts in the antisense orientation is a suitable target for limiting virus progression in *N. benthamiana* plants.

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

*Verticillium dahliae* is a widely distributed and destructive soil fungus, infecting many economically important crops. The lack of efficient chemically based disease management strategies has focused research on the development of resistant cultivars or root stocks, biological control agents, or compounds to induce host plant resistance. Plant protective activity of starch and cellulose were investigated against *Verticillium* wilt of eggplants. Both polysaccharides reduced symptom development compared to controls. In a split root system experiments, starch and cellulose treated plants were less infected than controls. Transcriptomic analysis showed greater expression levels of PR1 and PR4 in the cellulose treated plants compared to the controls at 5 d post inoculation (dpi) with *V. dahliae*. Starch treated plants exhibited greater PR4 expression levels than controls at 10 dpi. Pathogenicity experiments on plants grown in sterilised soil demonstrated the efficacy of starch and cellulose to protect plants against *V. dahliae*, while the polysaccharides failed to protect plants in a split root system. These results indicate that starch and maltose may positively affect microbial population that trigger ISR in plants, and that starch and cellulose could direct effects against *V. dahliae*.

**Soil solarisation and biological control of soil borne pathogens in strawberry plantations.** A. TZIONIS<sup>1</sup>, I. KEFALOGIANNI<sup>2</sup>, I. CHATZIPAVLIDIS<sup>2</sup> and S.E. TJAMOS<sup>1</sup>. <sup>1</sup>Laboratory of Plant Pathology, Agricultural University of Athens, School of Crop Science, Iera Odos 75, 11855, Athens, Greece. <sup>2</sup>Laboratory of General & Agricultural Microbiology, Agricultural University of Athens, School of Crop Science, Iera Odos 75, 11855, Athens, Greece. E-mail: sotiris@aua.gr

Soil solarization is a suitable method for control of soil-borne pathogens in Mediterranean countries. Strawberry is a high value crop that is susceptible to fungus and oomycete pathogens. Soil fumigants have low efficacy against soilborne pathogens. Next generation polyethylene foils for soil solarization were assessed in strawberry plantations under greenhouse conditions. A totally impermeable 7-layer film (KRITIFIL® TIF, Plastika Kritis SA) was the most efficacious among the tested soil disinfection films. The main soil pathogens were *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *fragariae*, *Phytophthora* sp., *Rhizoctonia solani* and *Macrophomina phaseolina*. In the TIF covered soil there were 4–10 times fewer diseased plants less than in metam sodium treated soil. Analysis of the microbial community of the different treatments showed the presence of 18 prevailing and distinct bacteria. Dual culture tests showed that six of the isolated bacteria reduced growth of *V. dahliae* and *Fusarium oxysporum*. The six isolates were tested as single and mixed treatments against *Verticillium* wilt of eggplants. The consortium of the isolates was the most efficacious treatment. Further analysis of the six isolates showed that they had biocontrol and plant growth promoting traits, including cellulolytic activity, indole acetic acid and siderophore production, phosphorus dissolving activity and swarming motility.

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

Gray mold, caused by *Botrytis cinerea*, is an important postharvest disease of fresh-market tomatoes. Although fungicide treatments have been a major method for controlling gray mold, there is increasing international concern over the heavy use of fungicides on crops because of the possible harmful effects on human health and the emergence of pathogen resistance to fungicides. Therefore, there is requirement for alternative disease control methods, including the use of beneficial microorganisms. *Pseudomonas* includes species with biocontrol activity against various plant pathogens. Recently, we reported the isolation and identification of strain *Pseudomonas putida* Z13, a potent biocontrol agent against *Verticillium dahliae*. *Pseudomonas putida* is commonly used in environmental studies because it can degrade many aromatic compounds, cellulose and chitin, and also produces many antibiotics. The biocontrol activ-

ity of strain Z13 was assessed against *Botrytis cinerea* in tomato fruits. Application of Z13 to tomato fruits reduced disease severity and incidence by 50% compared to controls. This protective activity can be attributed to antibiotic production, since Z13 reduced *in vitro* growth of *B. cinerea* and also primed plant defence responses. qPCR analyses showed that expression of the defence associated genes PR1 and WRKY70 was upregulated in the Z13 treated fruit, and the upregulation was most prominent after *B. cinerea* infection. These results indicate that Z13 was a potential biocontrol agent targeting multiple pathogens in different host plants, as experiments have shown activity against *V. dahliae* and *B. cinerea* in, respectively, fruits of eggplant and tomato.

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

The Cyprus wine industry has increasingly focused on exploitation of indigenous grapevine varieties. Grapevine trunk diseases (GTDs) are the most destructive biotic complex, limiting vineyard productivity and longevity. Based on recent data, Cyprus vineyards are seriously affected by GTDs. The present study was the first attempt to describe rhizobacteria communities from the indigenous varieties, *Vitis vinifera* “Giannoudi”, “Maratheftiko”, “Promara”, and “Xinisteri”, to select effective biocontrol agents (BCAs) that could be used for GTD management. Antagonistic activities of 499 bacterial isolates were assessed *in vitro* against the important GTD pathogens *Phaeomoniella chlamydospora*, *Diplodia seriata*, *Neofusicoccum parvum*, *Eutypa lata*, *Ilyonectria liriodendri*, *Dactylonectria alcacerensis*, and *Dactylonectria torresensis*. Eighty-two isolates exhibiting  $\geq 40\%$  fungal inhibition were selected and further characterized. Based on 16S sequencing, isolates were assigned to *Acinetobacter* (1.2%), *Bacillus* (17.1%), *Bordetella* (2.4%), *Chryseobacterium* (3.7%), *Paenibacillus* (1.2%), *Pantoea* (6.1%), *Pseudomonas* (19.5%), *Olivibacter* (2.4%), *Rhizobium* (14.6%), *Serratia* (22%), *Streptomyces* (2.4%) and *Variovorax* (7.4%). Enzymatic traits of the selected isolates related to antifungal activities were also examined. Activities were detected for lipase (in 42% of isolates), esterase (63%), protease (78%), amylase (37%), xylanase (37%), pectinase (37%), cellulase (46%), chitinase (32%), HCN activity (11%), and biofilm formation (34%

of isolates). Assessments for antibiotic production, VOC activity and growth promotion traits are underway to complete characterization of a pool of promising novel, indigenous BCAs for GTD management.

This research was supported by the Cyprus University of Technology Start-up Grant EX200120 to Loukas Kanetis.

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

Bacteriophages have been extensively studied to explore new and environmentally friendly methods for managing phytopathogenic bacteria. *Pseudomonas syringae* pv. *tomato* (*Pst*) causes bacterial speck in tomato plants, with symptoms of black or brown spots with chlorotic margins on leaves and fruits. The most common disease management strategy is applications of copper-based pesticides. Biological control of *Pst* with bacteriophages could be an alternative environmentally-friendly approach to reduce damage from *Pst* in tomato crops. Potential preventive efficacy of the lysogenic bacteriophage Medea1 was investigated in Medea1 has been isolated from a tomato crop and has been characterized. This is a ds-DNA bacteriophage with a genome of 58,919 bp, having strong lytic activity against many strains of *Pst*, and ability to suppress bacterial populations *in vitro* for at least 10 h. The efficacy of Medea1 against *Pst* was further evaluated *in planta*, under greenhouse conditions. The *Pst* and Medea1 populations were monitored at 1 and 3 d post-infection, using RT qPCR. Host gene expression patterns involved in defence against *Pst* were also assessed. The bacterial population was reduced by up to 85%, and disease symptoms were delayed by up to 6-fold. These results indicate that the Medea1 phage could be an effective alternative control for *Pst* in greenhouse tomato crops. Further isolation of bacteriophages against *Pst*, and design of a phage-based cocktail, could provide an efficient bacterial speck management strategy.

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

*Alternaria dauci* causes *Alternaria* leaf blight, an important foliar disease in carrot production. The disease commonly occurs when carrots are cultivated during moderate temperatures, and the leaves are exposed to prolonged periods of wetness due to rainfall, dew, or sprinkler irrigation. Severe epidemics have been reported to reduce carrot yields by 40–60%. Under high disease pressure, no single control measure is sufficient to adequately manage the disease. Disease management relies on the combination of applications of synthetic plant protection products (PPPs), use of partially resistant varieties, and monitored disease prediction models. Efficacy of several commercial biopesticides, plant resistance inducers and synthetic PPPs against *Alternaria* leaf blight in carrots was evaluated under greenhouse conditions in two experiments. For the pathogenicity trials, an intermediately resistant and a susceptible variety were used, and these were inoculated with conidium suspensions of *A. dauci* 2 d after the application of PPPs. All the tested PPPs successfully controlled *Alternaria* leaf blight. Among the synthetic PPPs, *Luna Sensation*®, *Signum*® and *Dagonis*® consistently decreased disease severity on both cultivars. *LBG-01F34*®, *Trianium*® and *Sonata*® were the most efficient bio-PPPs on both hybrids. Results from the comparative study of the different PPPs were presented.

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

Development of biological products is important for health and environmental reasons. This study deter-

mined the chemical composition of some aromatic *Lamiaceae*, and their antifungal activity against the causal pathogens of *Ascochyta* blight and *Fusarium* wilt of chickpea. Three plant species that grow spontaneously in Morocco were selected: *Origanum compactum*, *Thymus vulgaris*, and *Mentha pulegium*. Oregano was the most effective against *Fusarium oxysporum* (MIC = 0.5 µL mL<sup>-1</sup>) for inhibiting mycelium growth and conidium germination, followed by thyme and pennyroyal (MIC = 1.5 µL mL<sup>-1</sup>). Complete inhibition of *Ascochyta rabiei* was also observed using oregano at 0.15 µL mL<sup>-1</sup>. No phytotoxic effects of the oregano and thyme oils at low doses were observed on chickpea seed germination, but the pennyroyal oil reduced seed germination. Thymol and carvacrol were the major compounds of oregano and thyme oil as indicated by GC/MS analyses, and these compounds are likely to be the main active ingredient for pathogens control. Greenhouse experiments were carried out to evaluate the efficacy of oregano and thyme oils against *A. rabiei* as a protective or curative treatments. In curative treatments, 0.15 µL mL<sup>-1</sup> of oregano oil decreased ( $P < 0.05$ ) *Ascochyta* blight severity by 66%, similar to the fungicide azoxystrobin at 250 g L<sup>-1</sup>. The thyme oil at 0.5 µL L<sup>-1</sup> also reduced disease severity by 53%. These essential oils or their major components could be investigated as natural antifungal products against chickpea pathogens.

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

Bryophytes were among the first land plants and they faced harsh terrestrial environments, and have interacted with different organisms during their long evolutionary history. They cope well with fungal cohabitants in mutualistic, parasitic or neutral relationships. Due to interesting chemical contents, they are assumed not readily infected by fungi. This feature was used to assess effects of selected bryophyte extracts on plant pathogenic fungi. Methanol extract of leafy liverwort (*Scapania nemorea* (L.) Grolle) was assessed for growth inhibition of *Botryosphaeria dothidea* isolated from rotted olive fruits and *Botrytis cinerea* isolated from diseased strawberries. Extract treatments containing 5, 10 or 15 µL

were assessed, while distilled water served as experimental controls. The bryophyte extracts reduced growth of *B. dothidea* and *B. cinerea*. The extract applied at 15  $\mu\text{L}$  inhibited mycelium growth ( $P < 0.01$ ) of *B. cinerea*, and at 10 or 15  $\mu\text{L}$  inhibited growth of *B. dothidea*. These inhibitory effects indicate potential for bryophytes as sources of natural active substances that could be used for development of environmentally-friendly alternatives as controls for important plant diseases.

This research was financially supported by the Serbia-Montenegro bilateral project “From Plantation to Table: Improving the Process of Berry and Stone Fruit Production by Biotreatment with Bryo-Extracts to Reduce Risk of Pesticide Use and Obtain a Healthy Product” 2019–2020 and by the project “Study on biological efficacy of newly synthesized compounds and plant extract to the most important diseases of grapevine in Montenegro (BIOEXTRA) 2018–2020.

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

Effects were assessed of sanitary status of the scions infected by grapevine trunk diseases (GTD) and five different disinfectants (one combined with thermotherapy (TT) on the phenolics in callus during grapevine propagation. The study included scions from healthy vines (HLT), GTD-infected asymptomatic vines (ASYM) and GTD-infected symptomatic vines (SYM), of Cabernet Sauvignon. Scions were treated separately with Beltanol, Serenade, sodium bicarbonate, Remedier, BioAction or with Beltanol plus TT. Calluses were assessed soon after callusing and later after classification in autumn. Different sanitary status of the scions affected the content of phenolic compounds in callus. After callusing the greatest contents of TAP (363–378  $\mu\text{g g}^{-1}$  FW), flavanols (FLA; 297–310  $\mu\text{g g}^{-1}$  FW) and stilbenes (STB; 25.7–27.0  $\mu\text{g g}^{-1}$  FW) in callus was measured from SYM and ZDR scions, while in autumn the greatest contents of TAP, FLA and STB were measured in callus from ASYM scions. However, the greatest content of TAP (599–783  $\mu\text{g g}^{-1}$  FW) was measured in callus from ZDR and SYM scions treated with BioAction. In autumn, the contents of TAP (6951–7110  $\mu\text{g g}^{-1}$  FW) were measured in callus from ASYM scions treated with Remedier or Beltanol, from ZDR scions (3231–3262  $\mu\text{g g}^{-1}$  FW) treated with Remedier and

sodium bicarbonate, and from SYM scions (5024  $\pm$  40  $\mu\text{g g}^{-1}$  FW) treated with sodium bicarbonate. This study contributes to the knowledge of the impacts of biocontrol agents on alteration of phenolics in the callus from scions with different GTD status.

This research was financially supported by the programme Horticulture P4-0013-0481, funded by the Slovenian Research Agency.

***Melia azedarach* induces the expression of marker genes involved in tomato defence responses against nematodes.** A. KROKIDA<sup>1</sup>, D. KIZIS<sup>2</sup>, M. SAMARA<sup>3</sup> and N. NTALLI<sup>3</sup>. <sup>1</sup>Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 8 Stefanou Delta Street, 14561, Kifissia, Athens, Greece. <sup>2</sup>Laboratory of Mycology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 8 Stefanou Delta Street, 14561, Kifissia, Athens, Greece. <sup>3</sup>Laboratory of Biological Control of Pesticides, Scientific Directorate of Pesticides' Control & Phytopharmacy, Benaki Phytopathological Institute, 8 Stefanou Delta Street, 14561, Kifissia, Athens, Greece. E-mail: n.ntalli@bpi.gr

Tomato is an important food crop, Root-Knot Nematodes (RKN) are important tomato pests. Environmental and health concerns influence the use of plant secondary metabolites as ecofriendly alternatives to the conventional nematicides. Previous research showed that ripe fruits of *Melia azedarach* yielded nematocidal extracts against *Meloidogyne* sp., with substantial activity under field conditions, and that these extracts triggered plant defence mechanisms in cucumber. However, the molecular processes by which these extracts activate plant defence against RKN remain largely unclear. qRT-PCR was used to monitor the expression of selected key defence genes and the  $\text{H}_2\text{O}_2$  degrading enzymes of catalase and ascorbate peroxidase in tomato roots at 5 and 30 d after RKN inoculation. *Melia azedarach* aqueous extract induced the expression of salicylic acid pathway genes (*PR-1*, *PR-5*), the *Mil* gene that confers resistance against RKNs, and the proteinase inhibitor *Pil*. Overexpression of *ACO* and *RAB18* genes, markers of, respectively, the ethylene and ABA signalling pathways, was also observed. Low induction of jasmonic acid pathway genes (*LOXD* and *SAM*) was detected, and expression of *CCD7*, involved in strigolactones biosynthesis, was reduced at 30 d after RKN inoculation. The expression profiles of catalase and ascorbate peroxidase genes were altered 30 d after RKN inoculation. These results provide evidence that *M. azedarach* extract can modulate

the expression of genes and metabolites associated with plant defence responses in tomato.

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**In vitro and in vivo antifungal properties of thymol against *Ascochyta rabiei*.** F.Z. IBN EL MOKHTAR<sup>1,2</sup>, H. ERRATI<sup>1</sup>, H. HOUMAIRI<sup>1</sup> and S. KRIMI BENCHEQROUN<sup>2</sup>. <sup>1</sup>University Hassan 1st, Faculty of Science and Techniques, P.O. Box 577, Settati, Morocco. <sup>2</sup>National Institute of Agriculture Research (INRA, CRRRA-Settat), P.O. Box 589, Settati, Morocco. E-mail: san-ae.krimibencheqroun@inra.ma

*Ascochyta* blight, caused by *Ascochyta rabiei* (Pass.) Labr., is an economically important disease of chickpea. With increasingly used organic farming practices, there is requirement for reduced chemical inputs in agricultural systems, and to develop new effective biological products. Thymol is a natural monoterpene phenol, found mainly in the essential oil from thyme (*Thymus vulgaris*) and other aromatic and medicinal plants. The antifungal properties of thymol against *A. rabiei* were evaluated *in vitro* and *in vivo*. The *in vitro* test was carried out on chickpea malt extract medium amended with six concentrations of thymol (0 to 0.3  $\mu\text{L mL}^{-1}$ ). Minimum inhibitory concentrations (MIC) were estimated by calculating the percentage of inhibition of mycelium growth (MGI). The fungistatic or fungicidal properties of thymol were determined using the Thompson method. Greenhouse experiments were also carried out to evaluate the efficacy of thymol as a curative or preventive treatment for *Ascochyta* blight control. Radial growth of the pathogen was completely inhibited by thymol at low concentration (MIC = 0.15  $\mu\text{L mL}^{-1}$ ). Thymol was fungicidal against *A. rabiei* at >0.3  $\mu\text{L mL}^{-1}$ . Application of thymol to plants was effective for control of the disease as preventive or curative treatments at 0.4  $\mu\text{L mL}^{-1}$  by reducing the mean disease index by 39% (preventive treatment) and 32% (curative). Thymol can be investigated as a natural antifungal product for management of *Ascochyta* blight.

**Potential of microbial agents for biocontrol of early blight of potato and tomato.** Ž. IVANOVIĆ, T.

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Biological control for management of plant diseases has been extensively investigated as an alternative to chemical pesticides. Use of antagonistic microorganisms could reduce environmental pollution avoid pesticide resistant plant pathogens. Early blight of *Solanaceae*, caused by *Alternaria* pathogens, is economically important, and is responsible for severe damage of production from these crops. Although the disease can be partially controlled by fungicides, *Alternaria* strains resistant to the compounds cause problems for early blight management. Thirty-nine bacterial isolates were obtained from rhizospheres and phyllospheres of field-grown potato and tomato plants. All the isolates displayed the antagonistic activity towards *Alternaria solani* and *A. tomatophila*, producing inhibition zones in dual culture assays of at least 5 mm. Based on 16S rDNA sequencing, the isolates with greatest antagonistic potential were identified as *Bacillus velezensis*, *Bacillus safensis*, *Bacillus subtilis* or *Bacillus amyloliquefaciens*. Although potential for these bacteria as biological control agents has been demonstrated, they need to be further studied using *in planta* experiments.

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

*Xylella fastidiosa* is increasingly causing diseases on olive trees and other crops in the Mediterranean region. This pathogen destroyed a number of olive groves in Italy and Spain in only a few years. The climate of the southern

European Union is ideal for *Xylella*. Due to rapid transmission across cultivation areas, *X. fastidiosa* is projected to cause yield losses of 35–70% in olive harvests and 13% in almond harvests. There are no products available against *X. fastidiosa*, which is spread by xylem-feeding insects, particularly the spittlebug *Philaenus spumarius* which occurs in many countries and in different climatic conditions. Only chemical insecticides are authorized to control xylem sap-feeding insects, but these are not permitted in organic crop production. In the BBI JU-H2020 project BIOVEXO, 11 partners from five countries, and belonging to industries, SMEs, RTOs and universities, aim to develop environmentally sustainable and economically viable plant protection solutions that can be used for integrated management of *X. fastidiosa* and its spittlebug vector. At least two best performing solutions will be progressed closer to market at the end of the project (Technical Readiness Level 7-8). Integrated pest management measures will be applied to existing and the newly established orchards during large-scale pilots in Apulia (Italy) and Mallorca (Spain), two most important *Xylella* outbreak regions of Europe. An overview of BIOVEXO was presented, including objectives and research plans. For more information about the BIOVEXO project: [www.biovexo.eu](http://www.biovexo.eu).

This project received funding from the Bio Based Industries Joint Undertaking (JU) under grant agreement No. 887281. The JU receives support from the European Union's Horizon 2020 research and innovation programme.

**An emerging threat to chestnut nuts: *Gnomoniopsis castanea* in the Northern Apennines.** E. SCALI<sup>1</sup>, C. AGLIETTI<sup>1</sup>, S. PINI<sup>1</sup>, A. GUIDOTTI<sup>3</sup>, P. CAPRETTI<sup>1</sup> and L. GHELARDINI<sup>1,2</sup>. <sup>1</sup>Department of Agriculture, Food, Environment and Forestry (DAGRI), Plant Pathology and Entomology section, University of Florence, Piazzale delle Cascine 28, I-50144, Firenze, Italy. <sup>2</sup>Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy. <sup>3</sup>Regione Toscana, Servizio Fitosanitario, Via Alessandro Manzoni 16, 50121 Firenze, Italy E-mail: [luisa.ghelardini@unifi.it](mailto:luisa.ghelardini@unifi.it)

*Gnomoniopsis castanea* (syn. *Gnomoniopsis smitholgyi*) is an emerging pathogen that damages nuts of sweet chestnut (*Castanea sativa*) nuts, and may cause twig and leaf necroses on sweet chestnut and hazelnut (*Corylus avellana*). Nut rot of sweet chestnut has long been observed in Northern Italy, but was only recently associated with presence of *G. castanea*, which is considered responsible for heavy pre- and post-harvest nut losses

that chestnut growers are experiencing in the Northern Apennines. Little is known of the epidemiology of this fungus, which is often reported as a natural endophyte in chestnut and was found associated with galls of the Asian gall wasp *Dryocosmus kuriphilus*. Presence of *G. castanea* was investigated in the Northern Apennines (Liguria and Toscana regions), where sweet chestnut has historically been grown for nut production and as coppices. Nuts and wasp galls were sampled, and *G. castanea* was isolated with high frequency from all sites including those where symptoms were moderate, revealing common and broad presence of the fungus. Temperature responses for mycelium growth and spore germination of the pathogen were assessed to assist modelling of disease spread and incidence at sites with different climatic conditions. Since application of management strategies relies on accurate and rapid identification of the pathogen before occurrence of symptoms, a specific diagnostic assay is also being developed based on Loop mediated isothermal AMPlification (LAMP) technology. This will be used as a field-deployable tool for helping risk assessment, damage prevention and disease control.

This research received funding from the Agreement for Scientific Cooperation between Regione Toscana and the University of Florence – DAGRI, on quarantine organisms harmful to agriculture and forestry.

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

Wheat stem rust had not been reported in Western Europe for several decades, but in the last 10 years an outbreak occurred in central Europe, first, mainly in Germany, and then sporadically, in Denmark, Sweden and the United Kingdom. The presence of *Puccinia graminis* f. sp. *tritici*, which causes wheat stem rust, was also recorded in Sicily with an epidemic outbreak in 2016. Stem rust symptoms were also reported in Tuscany during the last 2019, 2020 and 2021, and during the 2021 crop season all monitored wheat varieties showed symptoms. In Sicily, stem rust was found in 13 of 14 monitored sites. Monitoring and sampling wheat varieties is important to provide understanding of epidemics

and detect the rust races present in the different countries and regions. For this purpose, some varieties were sown in Tuscany and Sicily to: 1) follow the appearance of rust symptoms in different climatic and soil conditions; 2) evaluate an early warning system coordinated between Tuscany and Sicily, where symptom appearance occurs well ahead of the central-northern regions; and 3) assess the races present on the same varieties in different regions. First results from molecular analyses of collected samples from both regions showed that clades III-B and IV-F occurred in both regions, and that race TTRTF (clade III-B) and race TKKTF (clade IV-F) were detected in Sicily.

**Diversity of dangerous fungus and fungus-like pathogens of fruit and berry crops in Russia.** YU. TSVETKOVA<sup>1,2</sup>, D. SHUKHIN<sup>1</sup> and A. KUZNETKOVA<sup>1</sup>. <sup>1</sup>FGBU All-Russian Plant Quarantine Centre. Moscow region, Ramensky district, Bykovo, Pogrannichnaya st. 32, 140150 Russian Federation. <sup>2</sup>Lomonosov Moscow State University, 119234 Moscow, Russia. E-mail: yutska@mail.ru

Production of fruit and berry crops is important in Russian agriculture, but production of planting material in Russia is not sufficient to meet demands. Import of propagation material of horticultural crops in 2018 was estimated to be 19.8 million pieces (excluding grape). Fruit and berry crops are susceptible to fungal diseases, especially in humid conditions within the main zones of fruit production. Pathogen diversity in Russia is influenced by pathogens imported with the planting material. This study provided an overview of quarantine and other fungal pathogens found in imported berry fruit production material and fungal pathogens that are widespread in Russia. Anthracnose of strawberry, caused by *Colletotrichum acutatum*, was detected on strawberries samples in 2018–2019. The species *C. acutatum* species complex affecting strawberries, and species found on other fruit crops, were separated. Species of the *Colletotrichum gloesporioides* complex have also been studied. Several harmful prevailing pathogens have been identified on strawberries, including: *Pilidium concavum*, *Pestalotia longisetula*, *Diplocarpon earliana*, *Phytophthora cactorum*, and *Phytophthora nicotianae*. Bown rot, caused by *Monilinia* spp., was also important. *Monilinia fructigena* and *M. laxa* are present in the Russian Federation. *Monilinia laxa* causes damage to stone fruits, while *M. fructigena* damages pome fruits. Plant samples were collected in different regions of the European part of Russia and from the Far East. Fungi were isolated on PDA, and identifications were carried using cultural and

morphological characters, and analyses of nucleotide sequences of ITS regions.

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

Bacterial leaf blight, caused by *Pseudomonas syringae*, threaten wheat production in many regions. Approximately 500 wheat leaf samples showing symptoms suggestive of bacterial infections were collected from 107 fields in north, central and coastal areas of Syria during the 2021 growing season. Disease symptoms consisted of water-soaked dark green to brown lesions on expanding leaves which became necrotic and turned from grey-green to tan-white. A total of 56 isolates were selected using a semi-selective medium, and were characterized further using morphological, biochemical (LOPAT), and pathogenicity tests to identify the virulent properties of isolates, and molecular characterization with PCR using specific primers for the syringomycin production gene. Field observations showed that leaf blight severity was approx. 54%, whereas the coastal area isolates gave high disease severity (61.5%), followed by the central area isolates (56%). The pathogenicity tests showed that all the isolates were pathogenic to wheat under greenhouse conditions, with 63% of ranked as highly virulent. The PCR results confirmed that the isolates were *P. syringae* pv. *syrinae*. This study increased understanding of the ecology, distribution and severity of the microbial wheat communities in Syria.

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

Russian olive (*Elaeagnus angustifolia*, *Elaeagnaceae*) is a shrub or small deciduous tree that can grow in various provinces of Iran. During 2018–19, surveys for fungi associated with canker and dieback of Russian olive trees were conducted in Southern provinces

of Iran. Samples were collected from branches of trees showing disease symptoms, and fungal isolations were made from necrotic wood tissues onto potato dextrose agar (PDA) supplemented with streptomycin sulphate. Fifteen isolates of Diatrypaceous fungi were obtained from the symptomatic branches. Isolates were placed onto 2% water agar amended with autoclaved pine needles and incubated under a 12 h daily photoperiod for 3–5 weeks to induce sporulation. Based on morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS) nrDNA and a partial sequence of the  $\beta$ -tubulin gene, isolates were identified as *Diatrype stigma* (eight isolates) and *D. whitmanensis* (seven isolates). Pathogenicity of these species was assessed by inoculation of detached shoots of Russian olive trees under controlled conditions. Both species were pathogenic and caused significant wood discolourations on inoculated shoots 40 d post-inoculation. *Diatrype stigma* was more virulent than *D. whitmanensis*, based on the mean lengths of wood discolourations. This is the first report on the occurrence and pathogenicity of *D. stigma* and *D. whitmanensis* on Russian olive trees.

**Identification of *Venturia asperata* on scab-resistant apple cultivars in South Tyrol, Italy.** S. OETTL. Research Centre Laimburg I-39040 Auer/Ora (BZ), Italy. E-mail: sabine.oettl@laimburg.it

Reports of atypical fruit spots on Vf-resistant cultivars from South Tyrolean apple orchards increased in August 2020. Symptoms were similar to apple scab, caused by *Venturia inaequalis*, but the spots were less pronounced and light grey. Microscope examination of mycelia scraped off the spots from the fruit peels revealed rounded-tip conidia of 10–14  $\mu$ m length, and were morphologically assigned to *Venturia asperata*. Molecular analyses of the ITS regions using species-specific primers resulted in amplicons only for the atypical scab lesions, but not for typical leaf or fruit spots caused by *V. inaequalis*. Sequence analysis of the amplicons confirmed the presence of *V. asperata*. To date, the atypical scab symptoms were observed only on Vf-resistant cultivars, such as Bonita, Topaz, CIVM49 and Ipador, where low-impact plant protection strategies were applied. Further monitoring of the occurrence of *V. asperata* is necessary to develop targeted fungicide management, and identification of scab-resistant cultivars, to prevent emergence of new fungal diseases.

**Occurrence of *Monilinia* species in South Tyrolean (Italy) sweet cherry orchards.** U. SPITALER, A. PFEIFER, S. HAUPTKORN, E. DELTEDESCO and S. OETTL. Research Centre Laimburg, Laimburg 6, 39040 Auer/Ora (BZ), Italy. E-mail: urban.spitaler@laimburg.it

Sweet cherry could be a niche crop for small farmers in the mountainous province of South Tyrol (Italy), and commercial orchards have increased during the decade to 2020. The most important fungal pathogens of stone fruit are *Monilinia fructigena*, *M. laxa* and *M. fructicola*, causing brown rot. However, these species have not been found in South Tyrol. Thus, in 2020 a collection of fruit mummies from 17 orchards in the main cherry growing areas was analyzed using modified real-time PCR. On 30% of the mummified fruit samples at least one *Monilinia* sp. was detected. *Monilinia laxa* was the most common species, detected on 22 % of the samples, while *M. fructigena* as the single species was identified only on 4 % of the samples. Mixed infections with these species were identified on 6% of the samples. *Monilinia fructicola* was found on four samples from two bordering cherry orchards. This is the first detection of the former quarantine organism *M. fructicola* in South Tyrolean sweet cherry orchards.

**Chemical management of *Colletotrichum acutatum* causing olive anthracnose.** M. VARVERI, M.K. ILIADI, C.K. KAVROUMATZI and D.I. TSITSIGIANNIS. Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Greece. E-mail: dimtsi@aua.gr

Infections of olive fruit (*Olea europaea*) by *Colletotrichum* are very frequent and destructive. *Colletotrichum acutatum* is considered to be the causal agent of olive anthracnose (OA) in Greece. This fruit rot leads to fruit drop or mummification, resulting in significant yield losses and degradation of oil quality. Control of olive anthracnose is difficult, and is usually achieved through a combination of methods including chemical control. Several chemical plant protection products (PPPs) were evaluated for the control of *C. acutatum* in the two Greek olive varieties, “Koroneiki” and “Kalamon”, using 12 commercial fungicides. Fruits were surface sterilized and then immersed in a solution of each PPP according to the maximum certified dose for each fungicide. Two days later, treated fruits were sprayed with a conidium suspensions of a Greek *C. acutatum* isolate. Several of the PPPs inhibited colonization and sporulation of *C. acutatum* on olive fruits. Nine *C. acutatum* strains from

different regions of Greece were characterized, *in vitro*, for sensitivity/resistance to the active ingredients of the most effective fungicides. All isolates were sensitive to the selected sterol-inhibiting fungicides (SBIs), while most of the isolates were resistant to copper compounds.

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**Fungicide sensitivity and genetic diversity of *Botrytis cinerea* populations from conventional and organic tomato and strawberry fields in Cyprus and Greece.** G. MAKRIS<sup>1</sup>, A. SAMARAS<sup>2</sup>, N. NIKOLOUDAKIS<sup>2</sup>, G.S. KARAOGLANIDIS<sup>2</sup> and L.I. KANETIS<sup>1</sup>. <sup>1</sup>Department of Agricultural Sciences, Biotechnology, and Food Science, Cyprus University of Technology, Limassol, 3036, Cyprus. <sup>2</sup>Plant Pathology Laboratory, Faculty of Agriculture, Forestry and Natural Environment, Aristotelian University of Thessaloniki, POB 269, 54124, Thessaloniki, Greece. E-mail: loukas.kanetis@cut.ac.cy

*Botrytis cinerea* is a complex species prone to fungicide resistance and characterized by broad genetic diversity. Unravelling pathogen population diversity facilitates development of efficient disease management schemes. During 2016–2017, 360 *B. cinerea* isolates were collected from conventional and organic tomato or strawberry farms in Greece and Cyprus, to analyze the genetic diversity and population structure of *B. cinerea* from different hosts and farming systems, in terms of agrochemical-inputs. Results highlighted widespread fungicide resistance, with 61.9% of the isolates found to be resistant to at least one botryticide, while 15.5% of isolates from conventional farms, and 85.1% from organic farms, were sensitive to all the tested active ingredients. Resistance frequencies of the isolates collected from conventional and organic fields were, respectively, 67.7 and 8.2% to boscalid, 65.9 and 29.1% to cyprodinil, 7.3 and 0% to fludioxonil, 18.6 and 3% to fenhexamid, 18.6 and 0% to iprodione, 67.3 and 9.7% to pyraclostrobin, and 61.4 and 15.7% to thiophanate-methyl. Fludioxonil and fenhexamid resistance were not detected in any of the tomato farms or countries, while resistance to iprodione was not recorded from organic fields. Multiple fungicide resistance was common, with fungicide sensitivity classes ranging from >3 for conventional farms and <1 for organic farms. Lambda measure of association showed a symmetric value ( $0.563 \pm 0.065$ ;  $P < 0.001$ ), indicating that farming systems correlated

with fungicide resistance, across crops and countries. The *B. cinerea* populations were also genotyped using a set of seven microsatellite markers. *Botrytis pseudocinerea* was not detected, while the populations exhibited high genetic diversity and mixed modes of reproduction. Genetic variance among strawberry and tomato populations was evident, ranking host specificity higher than other selection forces. AMOVA suggested significant associations of population structure and resistance to thiophanate-methyl, pyraclostrobin, boscalid, and cyprodinil.

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

Infection of grapevines by grapevine trunk disease (GTD) fungal pathogens primarily occurs through annual pruning wounds made during the host dormant season. Efficacy was evaluated (in two field trials over one growing season) of liquid formulation fungicide (pyraclostrobin + boscalid) and paste treatments, as well as biological control agents (*Trichoderma atroviride* SC1, *T. atroviride* I-1237, or *T. asperellum* ICC012 + *T. gamsii* ICC080), for their potential to prevent natural infections of grapevine pruning wounds by trunk disease fungi. The vineyards were in Samaniego, Northern Spain (19-years-old; “Tempranillo”) and Madiran, Southern France (24-years-old; “Cabernet Franc”). Wound treatments were applied immediately after pruning in February 2020. Untreated controls were treated with sterile distilled water. In February 2021, canes were harvested from vines and brought to the laboratory for *Trichoderma* spp. and fungal trunk pathogen assessments. A total of 1,848 isolates were collected from the Samaniego vineyards, and 1,179 were from Madiran vineyards, associated with five GTDs. In some cases, it was not possible to establish statistically significant differences between treatments due to the low incidence of some GTDs in the untreated control treatments. The efficacy of each product varied according to the GTD fungi and the grape production region.

*Trichoderma* recovery percentages ranged from 16.7 to 67.5% from Samaniego, and from 32.5 to 89.6% from Madiran. The experiment will be repeated during the 2021–2022 season.

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**Fungal trunk pathogens associated with *Juglans regia* in the Czech Republic.** J. PECENKA<sup>1</sup>, M. SPETIK<sup>1</sup>, T. NECAS<sup>2</sup>, I. ONDRASEK<sup>2</sup>, J. ARMENGOL<sup>3</sup>, M. LEÓN<sup>3</sup>, C. BERLANAS<sup>4</sup>, D. GRAMAJE<sup>?</sup> and A. EICHMEIER<sup>1</sup>.

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The spectra of fungal trunk pathogens associated with wood necrosis of the English walnut trees in the Czech Republic was assessed in field surveys during 2016, in Moravia region. Branches of the walnut trees showing the typical symptoms of dieback were selected for the sampling. In total, 138 of the fungal isolates were obtained from six orchards. Based on the morphology and sequencing data, the isolates were identified in 10 species of trunk pathogens including: *Cadophora novi-eboraci*, *Cadophora spadicis*, *Cryptovalsa ampelina*, *Diaporthe eres*, *Diplodia seriata*, *Dothiorella omnivora*, *Eutypa lata*, *Eutypella* sp., *Peroneutypa scoparia*, and *Phaeoacremonium sicilianum*. The most predominant fungal taxon was *Cadophora* followed species of *Botryosphaeriaceae* and *Diatrypaceae*. Pathogenicity tests showed that isolates of *Cadophora* spp. and *Eutypa lata* were virulent to the walnut branches.

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**Preliminary observations on the interaction among *Neocosmospora solani* (syn. *Fusarium solani*), *Neofusicoccum batangarum* and *Opuntia ficus-indica*.** S. BURRUANO<sup>1</sup>, S. GIAMBRA<sup>1</sup>, G. GUSELLA<sup>2</sup>, G. CONIGLIARO<sup>1</sup> and G. SURICO<sup>3</sup>. <sup>1</sup>Dipartimento di Scienze Agrarie, Alimentari Ambientali (SAAF), Università degli Studi di Palermo, Viale delle Scienze 2, 90128 Palermo, Italy. <sup>2</sup>Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. <sup>3</sup>Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology Section, Università degli Studi di Firenze, Piazzale delle Cascine, 28, 50144 Firenze, Italy. E-mail: santella.burruano@unipa.it

*Opuntia ficus-indica* (L.) Mill. (Cactaceae) is a xerophilous plant native to Mexico and naturalized throughout the Mediterranean Basin, where it is a common landscape species. This plant *O. ficus-indica* is also cultivated on many continents. In Italy, production is mostly concentrated in some hilly Sicilian areas. A severe disease of cactus pear was reported on the minor Sicilian islands of Lampedusa and Linosa (Pelagic archipelago), Favignana (Aegadian archipelago), and Ustica. The disease was named “scabby canker”, and its causal agent was identified as *Neofusicoccum batangarum*, (*Botryosphaeriaceae*, *Ascomycetes*). Scabby cankers appear as radially expanding, concentric, necrotic cankers on cladodes. They first appear in April each year, following first rains, and continue to develop during summer. Occasionally, *Neocosmospora solani* isolates have been associated with symptomatic cladodes. Interactions between *N. solani* and *N. batangarum*, and between the two fungi and *O. ficus-indica*, were studied *in vitro* and *in planta*. Dual growth *in vitro* assays showed *N. solani* overgrowing *N. batangarum*, and dark reaction zones were observed at points of contact of the two colonies, suggesting antagonism. *In planta* assays carried out by inoculating cladodes with *N. solani*, individually or in association with *N. batangarum* (which was applied after 30 d) never showed lesions. Lesions were clearly visible on the cladodes after inoculations *N. batangarum* only, or simultaneously with *N. solani*. *In vitro* and *in planta* observations indicated that *N. solani* has a neutral interaction with *O. ficus-indica*, and that it has antagonistic activity towards *N. solani*. Furthermore *N. batangarum* is confirmed as the only causal agent of scabby cankers.

This research is dedicated to the memory of Dr Gaetano Conigliaro.

**An innovative protocol for the monitoring and onsite detection of *Erwinia amylovora* in Sicily.** F. VALENTINI, F. SANTORO, M. GALLO, L. OUALGUIRAH and A. M. D'ONGHIA. *Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy. E-mail: valentini@iamb.it*

*Erwinia amylovora* (Ea), which causes fire blight of pomefruits, remains a quarantine agent (list A2), despite widespread occurrence in the European Union. Effective control of the bacterium is based on the use of resistant varieties, the application of reliable tests for pathogen detection, knowledge of climatic conditions that can promote infections, and adoption of specific agronomic practices (e.g. pruning) and/or chemical treatments to reduce the inoculum of the pathogen. This study aimed to develop an effective protocol for monitoring Ea, to detect infections early before onset of symptoms, without moving infected plant material from infection outbreak areas. Infections were monitored in pomefruit groves in the Ea outbreak of Sicily. Visual inspections for fire blight symptoms and detection of the pathogen using real-time LAMP with an onsite device were carried out during two vegetative seasons (March–October, 2018 and 2019). The results were correlated to parameters measured at an agroclimatic station located in the study area, thus implementing the Maryblight model. The combination of the forecasting model and onsite pathogen detection by real time LAMP is an innovative approach for official monitoring of this pathogen.

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**Development of reagent kits for the identification of *Candidatus Phytoplasma mali* and *Candidatus Phytoplasma pyri* using Real-time PCR.** I.G. BASHKIROVA<sup>1,5</sup>, G.N. BONDARENKO<sup>1,5</sup>, A.A. SHVARTSEV<sup>2</sup>, Ya.I. ALEXEEV<sup>2,3,4</sup> and S.A. BLINOVA<sup>2</sup>. <sup>1</sup>All-Russian Plant Quarantine Center, 140150 Bykovo, Ramenskoe, Moscow Region, Russia. <sup>2</sup>LLC SYNTOL, 127434 Moscow, Russia. <sup>3</sup>Federal State Institution of Science Russian National Research Institute Viticulture and Winemaking “Magarach”, 298600 Yalta, the Republic of Crimea, Russia. <sup>4</sup>Institute for Analytical Instrumentation of the Russian Academy of Sciences, 198095 St. Petersburg, Russia. <sup>5</sup>Peoples' Friendship University of Russia, 117198, Moscow, Russia. <sup>6</sup>Russian Timiryazev State Agrarian Univer-

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The apple proliferation group phytoplasmas (16SrX) were included in the List of Quarantine Objects A2 of the European and Mediterranean Plant Protection Organization (EPPO), as well as in The Uniform list of quarantine objects of the Eurasian Economic Union. Kits of reagents were required to enable fast and accurate identification of these pathogens in plant material. The main and most susceptible hosts of *Candidatus Phytoplasma mali* and *Candidatus Phytoplasma pyri* are Rosaceae plants, species of *Pyrus*, *Malus*, *Cydonia* and *Prunus*. Phytoplasma DNA extraction was carried out using methods recommended by the EPPO, with modifications. Real-time PCR was carried out using four instruments: CFX-96 (BIO-RAD, USA), Rotor-Gene (QiaGen, USA), ANK (IAI RAS, Russia), and DTprime (DNA-Technology, Russia). The amplification programme was: hot start at 95°C for 5 min, then 50 cycles each of 95°C for 15 sec and 60°C for 40 sec. Primers and probes were designed for the *dihydrolipoamide acetyltransferase* (*aceF*) gene. Specificity of the reagent kits was tested on 37 samples of related objects, and DNA cultures were obtained from the DSMZ-GmbH, CIRM-CFBP, All-Russian Plant Quarantine Center and other collections. There were no false-negative results of analyses of plant samples containing phytoplasma DNA, nor false-positive results of analyses of plant DNA and bacterial DNA. Analytical sensitivity of the developed reagent kit for *Candidatus Phytoplasma mali* was  $\leq 14.0 \times 10^3$  copies of DNA per milliliter of sample, and for the *Candidatus Phytoplasma pyri* kit was  $\leq 6.0 \times 10^3$  copies of DNA mL<sup>-1</sup>.

**Development of an algorithm of recognition for automatic reading of nitrocellulose membranes processed by Direct Tissue Blot Immunoassay.** S. GUALANO, E. PANTALEO, F. SANTORO, D. FRASHERI and A. M. D'ONGHIA. *Centre International des Hautes Etudes Agronomiques Méditerranéennes – Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy. E-mail: gualano@iamb.it*

Serological tests are simple and rapid methods of detecting plant pathogens. Enzyme-Linked Immunosorbent Assay (ELISA) and Direct Tissue Blot ImmunoAssay (DTBIA) are commonly used for screening large numbers of plant samples with efficiency for most pathogens. Compared to ELISA, DTBIA is easy to use, shorter and

cheaper, and can also be used as on-site tests. However, the reading phase of the DTBIA under stereomicroscope is longer and manual, while an automatic optical reader is available for the ELISA plates. In this study, the first approach was, therefore, to develop an automatic reading procedure for the DTBIA. The main components of variability of the processed membranes (e.g. the membrane commercial brand, the storage period of the processed membrane) were analysed for print colour and membrane background using plant material infected by Citrus tristeza virus or *Xylella fastidiosa*. Significant reading variability correlated to some components was observed, confirmed by the optical characteristics. On the basis of these results, an algorithm was developed for recognition of positive prints using normalized high-resolution images.

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

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

*Xylella fastidiosa* (Xf) subsp. *pauca* (sequence type ST53) has severely affected olive groves in the Apulia region of Southern Italy, infecting approx. 11 million olive trees in more than 50,000 ha. Disease caused by Xf (olive rapid decline syndrome (OQDS)) causes phenotypic and metabolomic changes in host plants. The control strategy currently uses destruction of infected plants, that are detected on the basis of symptoms and positive diagnostic tests. Detection of infections before symptoms develop can provide an advantage against spread of the disease, allowing preventive action. The combination of hyperspectral reflectance (HSR) and nuclear magnetic resonance (NMR) can offer a non-destructive method to detect the host phenotypic and metabolic fluctuations caused by Xf. Young plants of the susceptible olive variety Cellina di Nardò, artificially infected with Xf, were

grown in a temperature-controlled environment, and were co-inoculated with additional xylem-inhabiting fungi. Asymptomatic leaves were exposed to HSR acquisition, and their extracts were subjected to an untargeted metabolomic study, to select diagnostic signals and wavelengths associated with Xf-infected plants. Covariance matrices between these methods were then used to assess linkage between HSR spectral features and NMR and HRMS diagnostic signals. These matrices showed that different wavelength ranges were associated with different levels of association with particular metabolites, with a few regions within the visual range of 420–520 nm, 570–720 nm, and more within the near-infrared range of 1000–1830 nm.

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

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

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**Characterization of *Alternaria* species associated with black point of wheat kernels in Lebanon.** M. MASIELLO<sup>1</sup>, W. HABIB<sup>2,\*</sup>, R. EL GHORAYEB<sup>3</sup>, E. GERGES<sup>2</sup>, C. SAAB<sup>2</sup>, A. SUSCA<sup>1</sup>, G. MECA<sup>4</sup>, J.M. QUILES<sup>4</sup>, A.F. LOGRIECO<sup>2</sup> and A. MORETTI<sup>2</sup>. <sup>1</sup>*Institute of Science of Food Production - ISPA, Research National Council – CNR, Via Amendola, 122/O, 70126 Bari, Italy.* <sup>2</sup>*Laboratory of Mycology, Department of Plant Protection, Lebanese Agricultural Research Institute, P.O. Box 90-1965, Fanar, Lebanon.* <sup>3</sup>*Faculty of Agricultural and Food Sciences, Holy Spirit University of Kaslik, P.O. Box 446, Jounieh, Lebanon.* <sup>4</sup>*Department of Preventive Medicine, Nutrition and Food Science Area, University of Valencia, Avenida Vicent Andres Estelles s/n, 46100 Burjassot, Valencia, Spain.* \*W. HABIB current affiliation: Centro di Ricerca, Sperimentazione e Formazione in Agricoltura – Basile Caramia (CRSFA), Via Cisternino 281, Locorotondo, 70010 Bari, Italy. E-mail: mario.masiello@ispa.cnr.it

*Alternaria* commonly infects wheat crops, pre- and post-harvest decay of wheat grains and symptoms on heads, such as dark brown discolouration (black point disease) of grain embryos. Several *Alternaria* species can produce a range of mycotoxins, associated with different toxicities to humans and animals. The aims of the present study were to: i) assess the incidence of black point disease of wheat in Lebanon and isolate the *Alternaria* species causing the disease; ii) evaluate the levels of contamination by *Alternaria* mycotoxins in 33 grain samples; and iii) to identify the main occurring *Alternaria* species, using a molecular approach, and study their phylogenetic relatedness. The disease occurred at the majority (97%) of sampled sites, with the greatest average incidence in Akkar district (55%) and least (2%) in Baalbeck. Chemical analyses, using HPLC-DAD, showed that altenuene, alternariol, alternariol monomethyl ether, and tenuazonic acid were not detected in any sample. The phylogenetic analyses, based on DNA sequences of  $\beta$ -tubulin, glyceraldehyde-3-phosphate dehydrogenase, allergen *alt-a1*, and calmodulin genes, showed that isolates were in two main clades: 37 strains belonged to section *Infectoriae* and 42 strains to section *Alternaria*. This study showed that although black point disease of wheat kernels is widespread in Lebanon, the risks of contamination by *Alternaria* mycotoxins remains low.

**Cultivar-dependent differences in the phyllosphere-associated mycobiome of grapevine (*Vitis vinifera*).** A. MOLNÁR<sup>1</sup>, J. GEML<sup>1,4</sup>, A. GEIGER<sup>1,3</sup>, C. M. LEAL<sup>3,4</sup>, G.M. KGOBE<sup>3,4</sup>, A.M. TÓTH<sup>2</sup>, S.Z. VILLANGÓ<sup>2</sup>, L. MÉZES<sup>1</sup>, A.M. CZEGLÉDI<sup>1</sup> and Z.S. ZSÓFI<sup>2</sup>. <sup>1</sup>*Food*

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Grapevines naturally host reservoirs of fungi affecting grape production and quality, and plant health. Above-ground host tissues, the phyllosphere, is a dynamic and harsh habitat for microbial colonizers, due to exposure to environmental factors. An important question is, what drivers shape the phyllosphere fungal composition, thereby contributing to plant health and possibly to crop quality and quantity? This study investigated potential differences of phyllosphere-associated mycobiomes inhabiting healthy leaves and berries of the three grapevine cultivars *V. vinifera* “Furmint”, “Kadarka” and “Syrah”, grown under very similar environmental conditions in the Eger wine region. The study assessed whether the grapevine cultivars affected richness, relative abundance and composition of the fungal phyllosphere communities, using high-throughput DNA sequencing. To better clarify the background community differences, host physiological parameters, macro- and micro-element composition of leaf samples, and sugar content of berries were also assessed. While richness and relative abundance of fungal functional groups did not statistically differ among the cultivars, strong compositional differences were detected among the cultivars. In contrast, The cultivar berry samples differed in community richness and relative abundance, but not in community composition, suggesting the existence of a core grape microbiome at least at terroir level.

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**Selecting strawberry and melon germplasm tolerant to *Macrophomina phaseolina*.** S.A. YOUSSEF<sup>1</sup>, N. DAI<sup>3</sup>, R. COHEN<sup>4</sup>, S. FREEMAN<sup>2</sup> and A.A. SHALABY<sup>1</sup>. <sup>1</sup>*Plant Pathology Research Institute, Agricultural Research Center, 9 El-Gamma St., Giza, Egypt.* <sup>2</sup>*Department of Plant Pathology and Weed Research, ARO, Volcani Cent-*

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*Macrophomina phaseolina* is a soilborne pathogen causing crown and root rot of strawberry and vine decline of melons, is a destructive pathogen of strawberry and melon in the Mediterranean region. This fungus was isolated from different cultivars of strawberry showing crown rot, foliage wilting, charcoal rot and plant death, as well as vine wilting and death of melon. Similar disease symptoms were recorded in melon and strawberry cultivation areas in Egypt and Israel. Effective management of these diseases on resistant host germplasm selected using reliable techniques. Pathogenicity tests of representative *M. phaseolina* isolates were carried out to determine virulence and viability using the toothpick method, whereby microsclerotia are produced aseptically on toothpicks that are subsequently used to inoculate stem and crown tissues of tested plants. In addition, artificial soil inoculation was also assessed by inserting plants in pots containing a soil mixed with  $2.5 \times 10^3$  sclerotia mL<sup>-1</sup>. All inoculated plants were grown at 30°C in greenhouse conditions. In strawberry, complete plant mortality was observed approx. 3 weeks after inoculation. However, microsclerotium inoculation was gave more rapid development of disease symptoms at 2 weeks after inoculation. Using the microsclerotia method, disease symptoms progressed more rapidly and faster in the susceptible cultivar 'Festival' compared to the more resistant 'Fortuna', which had the least disease progress. In melon, disease symptoms following toothpick inoculations in the greenhouse and field produced similar symptoms to those for plants growing in naturally infested soils. The toothpick method was reliable for differentiating between susceptible and resistant melon germplasm in but not for strawberry. Disease screening of host germplasm resistant *M. phaseolina* should rely on an accurate and reliable inoculation technique.

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**Identification and characterization of *Fusarium solani* and *Dematophora necatrix* on hemp (*Cannabis sativa*) in southern Italy.** R. SORRENTINO<sup>1</sup>, V. BATTAGLIA<sup>1</sup>, F. RAIMO<sup>1</sup>, D. CERRATO<sup>1</sup>, G. PICCIRILLO<sup>2</sup>, A.

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Since 2016, cultivation of industrial hemp is allowed in Italy according to Law no. 242/2016. Industrial hemp can be grown for production of fibre, seed, oil, or cannabinoids. In a survey commencing in 2018, two new diseases were highlighted as affecting hemp crops. The soil-borne pathogens *Fusarium solani* in indoor crops, and *Dematophora necatrix* in outdoor crops were identified. *Fusarium solani* caused development, at soil line, of brownish to dark brown areas along the main host stems, followed by wilting and shedding of leaves. In correspondence with the lesions, the stems swelled to create cankers. Removal of the bark of affected plants revealed that the inner cortices were also affected. *Dematophora necatrix* caused root rot followed by host death that generally occurred within 2 to 3 weeks after symptoms appeared, with approx. 10% of plants showing yellowing, canopy wilt, and roots covered with white fan-like mycelium under the bark. These were the first records for both pathogens in Europe. Since interest in hemp production is increasing, appropriate management tools are required for these two pathogens.

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**Behaviour characterization of durum wheat varieties to distinct strains of *Pyrenophora tritici-repentis* (tan spot) in field and controlled conditions.** S. TISSAOUI<sup>1</sup>, A. MOUGOU-HAMDANE<sup>1</sup>, N. OMRI-BENYOUSSEF<sup>2</sup> and B. NASRAOUI<sup>1</sup>. <sup>1</sup>LR14AGR02 Laboratory of Bio-aggressors and Integrated Pest Management in Agriculture, National Agronomic Institute of Tunisia, University of Carthage, Tunis, Tunisia. <sup>2</sup>Field Crop Laboratory, National Agronomic Research Institute of Tunisia, Tunisia. E-mail: salmatissaoui2@gmail.com

Tan spot of wheat, caused by *Pyrenophora tritici-repentis*, is of economic importance, and an emerging disease in Tunisia. Necrosis with chlorosis, and chlorosis on wheat leaves were distinct symptoms induced. The necrotrophic pathogen produces host-specific toxins responsible for symptom variation on susceptible genotypes. The reaction of 14 durum wheat varieties to *P. tritici-repentis* was evaluated in field and controlled con-

ditions, by inoculating with three characterized pathotypes. Susceptibility of commonly grown varieties was determined by percentage of leaf area infections. Tan spot lesions developed on all the inoculated wheat genotypes, which showed response variations in the two trial conditions. Differences ( $P < 0.05$ ) in strain virulence were recorded. The varieties also differed disease reaction to different pathogen strains. The most virulent was strain I1 induced chlorosis on varieties which showed reaction variation. The varieties Khia, Sculture and Monastir were moderately resistant in controlled conditions, but susceptible in the field. A statistically significant isolate  $\times$  variety interaction was detected, which demonstrated specificity between strains and varieties. The overall reaction of varieties indicated that the most resistant was Monastir, while the most susceptible varieties were Maali and Razzak. The use of distinct pathotypes could assist evaluation wheat varieties in order to select resistant lines for develop tan spot management.

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

Beside agronomic performance, attention is given to yield and composition of essential oils (EO) from citrus peels by some industries. Citrus bioactive components are reported to be influenced by environmental factors. Effects of host rootstocks were assessed in presence or absence of viroid infections for yield or peel EO composition differences in the the Tunisian sweet orange Maltaise. *Citrus exocortis viroid* (CEVd) and *Hop stunt viroid* (HSVd) or Cachexia viroid were inoculated to eight host rootstocks. Preliminary results showed that yields varied between 0.4 and 0.8%. However, infections with CEVd and Cachexia reduced by 50% for *Citrumelo swingle* but did not affect other rootstocks. Regarding the composition of EO, healthy *Citrus macrophylla* gave the 17% more oxygenated monoterpenes than infected plants. CEVd infections enhanced this class of monoterpenes in *Citrus macrophylla* by 32%, in *C. volkameriana* by 11%, and in *Citrange carrizo* by 22%, while infections by Cachexia infection in *C. macrophylla* caused monoterpene reductions of 17 to 3%.

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

Since 2008, Horta has implemented Decision Support Systems (DSSs) for sustainable crop management, following the “super consultant” approach for achieving acceptance by agricultural stakeholders. Horta’s DSSs are web-based platforms that collect real-time site-specific weather and crop data, through remote and proximal sensors, organize these data in cloud systems and analyse them via advanced modelling and big data techniques, provide automated agronomic interpretation of model outputs, and produce alerts and decision supports. Process-based mathematical models are the core of the DSSs. These provide advice to decision makers for overall crop management including: pest/disease management, weed control, fertilization, and irrigation. The DSSs support (but not replace) farmers in practical implementation of Integrated Crop Management. Benefits are related to all sustainability pillars including: economic (e.g., reduction of direct costs); environ-

mental (e.g., reduction of GHG emissions); and social (e.g., reduction of exposure to pesticides, workers' satisfaction). To date, DSSs are available for wheat, barley, wine and table grapes, olives, legumes, tomatoes, corn, potatoes, while others are in-progress. Users are farmers, technicians, private/public advisors, producer organizations, and agri-food companies. The DSSs are used in Italy and, increasingly, in other countries. For instance, vite.net® (grapevine) is widespread in Spain, grano.net® (wheat) and orzo.net® (barley) in Greece and Bulgaria, and mais.net® (corn) in Hungary. To quantify, the DSS for wheat and barley in Europe was used by more than 6,300 farms, yielding more than 575,100 tons of grains in 2019. Internationalization of DSSs is a resource-demanding process, which includes customization for each country and demonstration of the multifold benefits to local stakeholders from DSSs in relevant agricultural contexts.

**Metagenomics analyses of fungal communities associated with postharvest diseases in pear fruits.**

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An amplicon metagenomic approach was employed to identify the composition of fungal communities associated with postharvest diseases of pear fruits. Fruits were harvested at an orchard using routine management practices involving different fungicides, and were transferred to a storage packinghouse. Effective tag sequences clustered into OTUs and Ascomycota were the dominant phyla (83.4%), followed by Basidiomycota (15.8%). Pear fruits supported a high diversity of microbes, but *Penicillium*, *Rhodotorula*, *Alternaria* and *Cladosporium* were the most abundant, representing 59–95% of the relative abundance of all effective sequences. Pear fruits displayed different fungal communities, according to diversity analyses among untreated and treated samples. Interruption of chemical treatments for 1 month before harvest gave significant modifications in the structure of the fruit fungal communities. It is assumed that various antagonistic episodes occurred among the detected fungal genera in the fruit carpospheres, and relative abundances were affected by fungicide treatments.

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**DNA-based comparisons of plant pathogenic fungi between grapevine and wild woody *Rosaceae* with, a focus on trunk diseases.**

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Grapevine (*Vitis vinifera*) is a major cultivated plant, which is naturally colonized by commensal, beneficial or pathogenic microorganisms. Many of these can influence plant health and disease incidence and severity. Grapevine microbiome studies have primarily focused on plant parts and rhizosphere, while the influence of semi-natural ecosystems on the composition of the grapevine microbiome is practically unknown. This study focused on trunk diseases that can be caused by a variety of fungi, e.g., species of *Phaeoacremonium*, *Phaeomoniella*, *Eutypa*, or *Diplodia*. Many fungi in these genera can also infect wild and cultivated fruit-producing hosts, particularly *Rosaceae*, and can cause symptoms that are similar to those of grapevine trunk diseases. Possible connections were explored between the grapevine microbiome and that of wild woody *Rosaceae* present in surrounding landscapes. The compositional overlap was assessed for pathogenic fungi associated with grapevine and nearby wild *Rosaceae* shrubs and trees. Plant samples were taken from living trunks of the studied plants, with or without symptoms of trunk diseases. Several pathogens were present in all studied plants, including species of *Botryosphaeria*, *Phaeoacremonium*, and *Phaeomoniella* that are known to cause trunk diseases in vineyards. This overlap suggests that wild *Rosaceae* fruit species living near vineyards may be inoculum sources of plant pathogenic fungi capable of infecting grapevines.

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

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Plant growth and health are dependent on plant-associated microbes. Despite recent advances, there is still no systematic overview of the diversity of grapevine-associated fungi, and how the grapevine microbiome is influenced by crop cultivation methods. This study described the diversity and composition of plant pathogenic fungal communities occurring in grapevines under an organic or conventional management. The type of plant protection used in conventional and organic vineyards was hypothesized to greatly influence the microbial, especially fungal, community structure associated with grapevine plants. Leaves of the cultivar Bianca at the Eszterházy vineyard, in Eger, Hungary, were sampled in 2020. After DNA extractions and Illumina NextSeq sequencing, the quality-filtered and rarefied dataset reads were assigned to functional groups, of which 911 ASVs were considered plant pathogens. *Phaeomoniella* showed the greatest number of ASVs, followed by *Alternaria*, *Epicoccum* and *Diplodia*, from the 88 genera found (ASV richness). Differences in fungal richness were apparent among months, but not between organic and conventional crop management types. Similarly, community structure was primarily influenced by sampling month, as shown by NMDS and PERMANOVA analyses. Beside the strong temporal turnover of leaf-associated fungi, the organic vs. conventional management comparison did not significantly affect leaf fungal communities. It is unclear how much of this community turnover was driven by periodic fungicide applications or seasonality. The lack of significant differences among cultivation types suggests that leaf-associated fungi undergo a seasonal succession.

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