PLANTS SYNTHESIZE THE PRIMING MOLECULE β-AMINOBUTYRIC ACID (BABA) IN RESPONSE TO STRESS. **I. Baccelli[1], D. Thevenet[2], A. Balmer[1], V. Pastor[1], A. Vallat[3], R. Neier[2], G. Glauser[3], B. Mauch-Mani[1].** *[1]Institute of Biology, University of Neuchâtel, Neuchâtel, Svizzera. [2]Institute of Chemistry, University of Neuchâtel, Neuchâtel, Svizzera. [3]Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Neuchâtel, Svizzera. E-mail: ivan.baccelli@unine.ch*

Plants can be sensitized to respond faster and/or stronger to stress situations by application of the non-protein amino acid β-aminobutyric acid (BABA), a well-known priming agent. BABA can increase resistance against a wide range of stresses, such as attacks by pathogens, nematodes, and arthropods, as well as abiotic stressors like heat, cold or salt. Plants treated with BABA can deploy more rapidly the signaling pathway most appropriate to counteract the given stress situation. For instance, against *Plectosphaerella cucumerina* infection, BABA priming leads to an ABA-dependent enhancement of the callose response at the sites of attempted penetration, and thus to increased resistance. With the present study we provide evidence that BABA, which has been considered a xenobiotic for more than 50 years, is actually produced by plants and regulated by stress. By using a sensitive and selective protocol developed in our laboratory and based on ultra-high pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), we were able to separate BABA from its two isomers alpha (AABA) and gamma (GABA) that are present in plant tissues, and to quantify it. Subsequent analyses revealed that BABA is present in various plant species, including Arabidopsis and crops like maize and wheat. Importantly, BABA levels were found to increase following infection with necrotrophic, biotrophic and hemibiotrophic pathogens, as well as after abiotic stress. The biosynthetic pathway and the regulation of BABA are currently under investigation. At the present, our results suggest that BABA may be a novel hormone helping plants to cope better with stress.

IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS EMITTED BY DIFFERENT GRAPEVINE GENOTYPES IN RESPONSE TO DOWNY MILDEW INFECTION. **V. Lazazzara[1,2], C. Bueschl[2], A. Parich[2], I. Pertot[1], R. Schuhmacher[2], M. Perazzolli[1].** *[1]Fondazione Edmund Mach, Research and Innovation Centre, Department of Sustainable Ecosystems and Bioresources, Via E. Mach, 1 - 38010 S. Michele all'Adige, Trento. [2]University of Natural Resources and Life Sciences, Vienna (BOKU), Department IFA-Tulln, Center for Analytical Chemistry, Konrad-Lorenz-Strasse 20, A-3430 Tulln, Austria. E-mail: valentina.lazazzara@fmach.it*

Vitis vinifera is susceptible to several pathogens including *Plasmopara viticola*, the causal agent of downy mildew. American grapevine species are resistant or tolerant to *P. viticola* and breeding programs have introduced resistance traits to susceptible cultivars. Although grapevine resistance to *P. viticola* has been widely characterized in resistant genotypes, the possible contribute of volatile organic compounds (VOCs) was not yet investigated. The aim of this work was the characterization of VOCs produced by resistant and susceptible genotypes in response to *P. viticola* inoculation, in order to identify VOCs associated to grapevine resistance. The susceptible *V. vinifera* cultivar Pinot noir, and the resistant genotypes Kober 5BB, SO4, BC4 and Solaris were grown under greenhouse conditions and they were subsequently inoculated with *P. viticola*. Leaves were harvested immediately before (0 dpi) and six days (6 dpi) after inoculation, and the lower disease severity in resistant genotypes as compared with Pinot noir was confirmed. A solid-phase microextraction-gas chromatography-mass spectrometry approach (SPME/GC-MS) was used to analyze VOCs emitted by the five genotypes studied. GC-MS chromatograms showed specific VOC emission profiles of the four resistant genotypes as compared with Pinot noir at 6 dpi. VOCs specifically emitted by resistant genotypes were then selected, and pure compounds were tested against *P. viticola* by leaf disc assays. Particularly, three sesquiterpenes, two aldehydes and one heterocyclic compound significantly reduced downy mildew severity on Pinot noir, demonstrating that VOCs could play an important role in the resistance against downy mildew by direct toxicity against *P. viticola*.

GENE RESPONSES OF GRAPEVINE AND *BOTRYTIS CINEREA* DURING THE LATENT INFECTION OF BERRIES ("NOBLE ROT"). **A. Lovato, T. Colombo, S. Negri, F. Guzzo, G.B. Tornielli, A. Polverari.** *University of Verona, Department of Biotechnology, Verona, Italy. E-mail: arianna.lovato@univr.it*

High throughput technologies allow deep investigations of molecular mechanisms involved in plant-pathogen interactions. In yet uncharacterized environmental conditions, *Botrytis cinerea*, the agent of grapevine grey mould, can develop as a latent infection, a phenomenon known as "noble rot", determining favourable berry modifications responsible for the typical aromas of "passito" wines.

In this work, we aimed at identifying *B. cinerea* genes deployed during "noble rot" process and concomitant grapevine responses. Healthy Garganega berries were artificially inoculated with *B. cinerea* B05.10 by injecting conidia under the berry skin and placed in controlled conditions to reproduce the *pourri plein* stage of "noble rot". Biological triplicates of berry samples inoculated with water and *in vitro*-grown *B. cinerea* mycelium were used as controls. Samples were analysed at transcriptomic and metabolomic levels by RNAseq and HPLC, respectively. Large-scale expression analyses revealed 2503 and 2871 differentially expressed genes in grapevine and *B. cinerea*, respectively. In response to "noble rot" grapevine induced 1738 genes belonging to functional categories related to plant-pathogen interaction such as response to stress, transcription regulation and the production of secondary metabolites, likely to contrast fungal infection. On the other hand, genes related to cell death, defence responses and transport were found repressed. *B. cinerea* gene expression profiles were also found substantially reprogrammed during "noble rot", with a trend of *B. cinerea* virulence gene expression differentially affected during the latent infection compared to published bunch rot data. A deep investigation of grapevine and *B. cinerea* pathogenicity-related gene reprogramming during noble rot will be presented.

INSIGHTS ON THE INTERACTIONS AMONG NON-NATIVE AND NATIVE *HETEROBASIDION* SPECIES AND THE ECTOMYCORRHIZAL SYMBIONT *TUBER BORCHII* ON *PINUS PINEA*. **L. Giordano[1,2], E. Zampieri[1], G. Lione[1], A. Vizzini[3], F. Sillo[1], R. Balestrini[4], P. Gonthier[1].** *[1]University of Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo Paolo Braccini 2 - 10095 Grugliasco, Italy. [2]University of Torino, Centre of Competence for the Innovation in the Agro-Environmental Field (AGROINNOVA), Largo Paolo Braccini 2 - 10095 Grugliasco, Italy. [3]University of Torino, Department of Life Sciences and Systems Biology (DBios), Viale P.A. Mattioli 25 - 10125 Torino, Italy. [4]Institute for Sustainable Plant Protection, CNR, Torino Unit, Viale P.A. Mattioli 25 - 10125 Torino, Italy. E-mail: paolo.gonthier@unito.it*

The protective role played by symbionts against plant pathogens has been extensively documented. However, little is known on the effects of pathogens on the ectomycorrhizal (ECM) symbiosis. In this work, through a six-months inoculation experiment, we studied the effects of fungal plant pathogens on both the ECM symbiosis and the expression of genes putatively involved in the symbiosis,

in a three-actors model system including the non-native and the native pathogens *Heterobasidion irregulare* and *H. annosum*, the ECM symbiont *Tuber borchii* and the common host species *Pinus pinea*. The two pathogens induced the same macroscopic reaction in the plant-symbiont complex with mycorrhizal density increasing with the pathogen colonization along the stem. The gene expression analyses showed that genes regulated in *T. borchii* were more than twice in plants inoculated with the native pathogen compared to that inoculated with the non-native one. Although the consequences of this differentiated gene expression is largely unknown, our results suggest that a recognition mechanism between the native symbiont and the native pathogen through a host plant-mediated signal transduction may be involved.

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GENOME-WIDE MICROARRAY ANALYSIS OF TOMATO PLANT IN THREE-WAY INTERACTION WITH *TRICHODERMA HARZIANUM* OR ITS METABOLITE HARZIANIC ACID AND *RHIZOCTONIA SOLANI*. **A. Sacco[1], G. Manganiello[1], F. Vinale[2], M.R. Ercolano[1], R. Marra[2], N. Lombardi[2], G. d'Errico[1], M. Lorito[1,2], S.L. Woo[1,2].** *[1]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100 - 80055, Portici, Italy. [2]Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), Via Università 133 - 80055 Portici (NA), Italy. E-mail: adriana.sacco@unina.it*

A microarray analysis was used to study tomato gene expression during the interaction with the fungal biocontrol agent *Trichoderma harzianum* M10 or its secondary metabolite harzianic acid (HA), in presence of the soil-borne root pathogen *Rhizoctonia solani*. The analysis allowed identifying differentially expressed genes (DEGs) in both treatments during the three-way interaction. As result, 1218 and 2507 DEGs were identified in the comparisons infected plant (IP) treated with M10 vs IP and IP treated with HA vs IP, respectively. A strong over-expression of genes involved in the hypersensitivity response was ascertained in infected plant treated with *T. harzianum* as well as with HA. The protective action of *T. harzianum* on the host is related to the over-expression of genes able to detoxify cells from ROS, that, if accumulated, can be highly toxic to the plant itself. Furthermore, the presence of genes coding for 'Multi bridging factor 1', 'ER-24' and 'EIN3' indicated the production of ethylene (ET) and the related activation of jasmonic acid (JA) pathway. Interestingly, in the IP treated with HA numerous genes coding for pathogenesis related proteins, including the PR-1, resulted differentially expressed with simultaneous over-expression of genes mapped in the biosynthetic pathway of the salicylic acid. Similarly, several genes involved in the JA-ET pathway have been found. These data supported the hypothesis of a simultaneous activation of ISR and SAR pathways.

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RNA-SEQ BASED TRANSCRIPTOME ANALYSIS OF *PSEUDOMONAS CORRUGATA* LuxR REGULATORS PcoR AND RfiA. **G. Licciardello[1], A. Caruso[2], P. Bella[3], R. Gheleri[4], C.P. Strano[5], E.A. Trantas[6], P.F. Sarris[7], N.F. Almeida[4], V. Catara[2].** *[1]Parco Scientifico e Tecnologico della Sicilia, Catania, Italy. [2]Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, Catania, Italy. [3]Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Palermo, Italy. [4]School of Computing, Federal University of Mato Grosso do Sul, Campo Grande MS, Brazil. [5]Dipartimento di Agraria - Università "Mediterranea" di Reggio Calabria, Reggio Calabria, Italy. [6]Department of Agriculture, School of Agriculture and Food Technology, Technological Educational Institute of Crete, Heraklion, Greece. [7]Department of Biosciences, University of Exeter, Exeter, UK. E-mail: vcatara@unict.it*

Cyclic lipopeptides production in the phytopatogenic bacterium *Pseudomonas corrugata* is regulated by two transcriptional regulators, belonging to the LuxR superfamily, PcoR and RfiA. Mutational analysis revealed they also have a role in *P. corrugata* interaction with plants and in antimicrobial activity. PcoR is the cognate receptor of the N-acyl-homoserine lactones, synthetised by PcoI in *P. corrugata* quorum sensing (QS) system. The QS system also directly activates the transcriptional regulator gene *rfiA***.** Our previous studies showed that RfiA, via-QS directly or indirectly, regulates genes for two transmembrane transporter systems *pcoABC* and *crpDE*, and a gene coding for a nonribosomal peptide synthetase *crpC*. In order to define the complete regulon of PcoR and RfiA, deep sequencing of cDNA library (RNA-seq) was used to analyse the whole transcriptomes of GL2 (*pcoR*-) and GLRFIA (rfiA-) mutants at early stationary phase of growth on a minimal medium. Differential expression analysis between the Wt and the mutants showed that 93 and 62 genes were identified as significantly different in the GL2 and GLRFIA mutants, respectively. Fifty-five genes appear to be co-regulated by both mutants. This is reasonable since the PcoR-AHL complex control the *pcoI-rfiA* transcription. By the fact, in the GL2 mutant there is no expression of *rfiA*. Overall, RfiA orchestrates a number of secondary metabolite biosynthesis clusters putatively involved in bioactive peptide and exopolysaccharide production. PcoR- and RfiA-dependent transcriptional regulation of a subset of differentially expressed genes was confirmed with relative transcript levels determined by qRT-PCR in different bacterial growth conditions.

RNA-SEQ BASED TRANSCRIPTOME ANALYSIS OF DIFFUSIBLE SIGNALING FACTOR REGULATED TRAITS OF *XYLELLA FASTIDIOSA* subsp. *FASTIDIOSA*. **C.P. Strano[1,2], S.E. Lindow[1].** *[1]Department of Plant and Microbial Biology, University of California, Berkeley - 94720. [2]Dipartimento di Agraria - Università "Mediterranea" of Reggio Calabria, Feo di Vito - 89122 Reggio Calabria (RC), Italy. E-mail: cinzia.strano@unirc.it*

The Gram-negative bacterium *Xylella fastidiosa* causes serious plant diseases of several important agricultural crops, including Pierce's disease of grapevines, a devastating and chronic problem in the grape industry in California. *X. fastidiosa* is obligatory transmitted from one plant to another by xylem-sap-feeding insects. In susceptible hosts, the pathogen multiplies and spreads from the site of infection to colonize the xylem. Bacterial cells attach to the vessel walls and multiply, forming biofilm-like colonies that can completely occlude xylem vessels, blocking water transport. *X. fastidiosa* produce several different diffusible signaling factors (XfDSF), to regulate its behavior in a cell density-dependent manner. Virulence, motility and biofilm formation are regulated by XfDSF production, which are synthesized by RpfF. *ΔrpfF* mutant of *X. fastidiosa*, is hypervirulent to grapevine but unable to colonize and be transmitted by insect vectors, suggesting that DSF signaling is used as a context-dependent lifestyle switch. To shed some light into the determinants involved in these processes, the global transcriptome profile of *X. fastidiosa* Temecula strain and its *ΔrpfF* mutant was investigated under *in vitro* and *in planta* conditions. Moreover, to evaluate if the bacterium differently responded to various XfDSF species altering its behavior, the effect of different XfDSF was tested *in vitro*. In total, 611 genes differentially expressed ($P < 0.05$, false discovery rate) were identified. Among them, 334 known genes were down-regulated, whereas 276 genes were over-expressed. Moreover, 256 genes were differentially expressed in the Temecula - *ΔrpfF* mutant-XfDSF treatment combination. A large number of differentially expressed genes with unknown functions were identified.

TRANSCRIPTOME ANALYSIS OF TWO OLIVE CULTIVARS IN RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION. **A. Giampetruzzi[1], M. Morelli[2], M. Saponari[2], G. Loconsole[1], M. Chiumenti[2], D. Boscia[2], V.N. Savino[1], G.P. Martelli[1], P. Saldarelli[2].** *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro, 70126 Bari. [2]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari, 70126 Bari, Italy. E-mail: pasquale.saldarelli@ipsp.cnr.it*

The CoDiRO strain of *Xylella fastidiosa* subsp. *pauca* (*Xfp*) is ravaging olive (*Olea europaea*) groves in southern Italy, causing a destructive disease denoted Olive Quick Decline Syndrome (OQDS). Field observations show that the *Xfp*-infected plants of the cv. Ogliarola salentina develop more severe symptoms than that of cv. Leccino. A global transcriptome profiling comparing the two olive cultivars, infected or not by *Xfp*, was performed to ascertain whether a tolerant condition of cv. Leccino exists, which could be exploited for lessening the economic impact of the disease on the local olive industry. The study revealed that 659 and 447 genes were differentially regulated upon *Xfp* infection, in cvs Leccino and Ogliarola salentina, respectively, whereas 512 genes resulted altered between the two infected cultivars. The analysis showed that plants of both cultivars perceive the presence of *Xfp*, mainly involving cell wall-associated proteins. The predominant response of cv. Leccino, which is missing in cv. Ogliarola salentina, consists on the up-regulation of genes encoding receptor-like kinases and receptor-like proteins. This different transcriptome response determines a lower pathogen concentration in the cv. Leccino, suggesting that it may harbor genetic constituents and/or regulatory elements which counteract *Xfp* infection. These findings suggest that cv. Leccino is endowed with an intrinsic tolerance to *Xfp*, which makes it eligible for further studies aimed at investigating molecular pathways underlying its different defense response.

***POTATO SPINDLE TUBER VIROID* UPREGULATES DNA METHYLATION-RELATED GENES AND ANTAGONIZES THE INFECTIVITY AND THE ACCUMULATION OF A GEMINIVIRUS.** **E.M. Torchetti[1], M. Pegoraro[2], B. Navarro[1], M. Catoni[3], E. Noris[2], F. Di Serio[1].** *[1]Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy. [2]Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Torino, Italy. [3]University of Cambridge, The Sainsbury Laboratory, Cambridge, United Kingdom. E-mail: francesco.diserio@ipsp.cnr.it*

Tomato is a natural host of *Potato spindle tuber viroid* (PSTVd) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV), which are representative members of pospiviroids (infectious non-coding circular RNAs) and geminiviruses (single-stranded DNA viruses), respectively. While molecular events during infection have been explored separately for each one of these two nuclear replicating pathogens, plant responses during mixed infections are unknown. In this context, dissection of DNA methylation pathway is particularly interesting because it is well known that plants may methylate viral DNA to impair geminivirus infection, while whether viroids interfere with host DNA methylation pathways is unknown. Exploiting an experimental system based on PSTVd and TYLCSV co-infecting the same tomato plant, and applying qRT-PCR, methylation-sensitive restriction and bisulfite conversion assays, we found that: i) when plants were co-infected, TYLCSV infectivity and accumulation were strongly impaired, indicating an antagonistic action of PSTVd; ii) PSTVd alone or in double infection with TYLCSV significantly upregulated the expression of key genes governing DNA methylation in plants; iii) PSTVd promoted a strong hypermethylation of TYLCSV DNA in tomato plants co-infected by both pathogens, thus supporting a mechanistic link with the antagonism of the viroid on the virus during co-infection. Besides providing the first solid evidence that a viroid may interfere with host regulatory networks involved in DNA methylation, these data open new perspectives on the possible involvement of viroid-induced epigenetic changes in plant responses against other biotic and abiotic stresses.

DISTINCT EFFECTS OF TOMBUSVIRAL p19 RNA SILENCING SUPPRESSOR ON SMALL RNA MEDIATED PATHWAYS IN PLANTS. **L. Kontra[1,2], T. Csorba[1], M. Tavazza[3], A. Lucioli[3], R. Tavazza[3], S. Moxon[4], V. Tisza[1], A. Medzihradszky[1], M. Turina[5], J. Burgyán[1].** *[1]National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute, Szent-Györgyi A. 4., Gödöllő H-2100, Hungary. [2]Szent Istvan University, Gödöllő, Hungary. [3]UTAGRI Centro Ricerche Casaccia, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile, Rome, Italy. [4]The Genome Analysis Centre, Norwich, UK. [5]National Research Council, Institute for Sustainable Plant Protection, Torino, Italy. E-mail: burgyan@abc.hu*

Viral suppressors of RNA silencing (VSRs) are specialized arms deployed by viruses to neutralize the antiviral RNA silencing plant defense mechanism. Since the endogenous and antiviral functions of RNA silencing pathway rely on common components, it was suggested that VSRs interfere with endogenous silencing pathway contributing to viral symptom development. In this work, we aimed to understand the effects of the tombusviral p19 VSR on endogenous and antiviral silencing during genuine virus infection. To this end, we generated a *Nicotiana benthamiana* plant (p19syn) capable of sustaining the ectopic expression of the *Cymbidium ringspot virus* (CymRSV) p19 upon infection with a suppressor-deficient CymRSV (Cym19stop). By using wt and p19syn plants in combination with CymRSV and Cym19stop, we were able to analyze the effects of p19 provided "*in trans*" and "*in cis*" during the viral invasion of the plant. We showed that ectopically expressed p19 sequesters endogenous small RNAs (sRNAs) in the absence, but not in the presence of virus infection. Our presented data question the generalized model in which the sequestration of endogenous sRNAs by the viral suppressor contributes to the viral symptom development. We further showed that p19 preferentially enriches the perfectly-paired ds-viral small interfering RNAs (vsiRNAs) but does not select based on their sequence or the type of the 5' nucleotide. Finally, using AGO1- and AGO2-immunoprecipitation experiments we observed that p19 specifically compromises vsiRNAs' loading into AGO1 but not AGO2. Since antiviral silencing is strongly inhibited by p19, this suggests that AGO1 is the main effector protein against tombusviruses.

IDENTIFICATION AND CHARACTERISATION OF THE GRAPEVINE ATL GENE FAMILY AND FUNCTIONAL ROLE OF AN *ATL* GENE FROM *VITIS RIPARIA* IN DOWNY MILDEW RESISTANCE. **P. Ariani[1], A. Regaiolo[1], A. Lovato[1], A. Giorgetti[1], A. Porceddu[2], S. Camiolo[2], D. Wong[3], S. Castellarin[3], C. Zadra[4], E. Vandelle[1], A. Polverari[1].** *[1]Università degli Studi di Verona, Dipartimento di Biotecnologie, Ca' Vignal 1, strada le Grazie 15 - 37134 Verona, Italy. [2]Università degli Studi di Sassari, Dipartimento di Agraria, SACEG, Sassari, Italy. [3]University of British Columbia, Wine Research Centre, Vancouver, Canada. [4]Università di Perugia, Dipartimento di Scienze Agroambientali e della Produzione Vegetale, Sez. Chimica Agraria, Perugia, Italy. E-mail: elodiegenevieve.vandelle@univr.it*

Plasmopara viticola is the causal agent of downy mildew, one of the most economically important grapevine diseases. In an attempt to decipher the resistance mechanisms evolved in naturally resistant

American grapes, we previously performed a comparative microarray analysis between resistant *V. riparia* and susceptible European *V. vinifera* following infection with *P. viticola*. An in-depth data analysis revealed the specific up-regulation, in infected resistant grape, of 10 genes encoding proteins sharing common features of ATLs, a subgroup of the RING-finger E3 ubiquitin ligase family. These enzymes, which mediate protein ubiquitination, may play an important role in plant defense signaling. To go deeper into grapevine ATL family, we carried out a complete survey of *V. vinifera* translated genome and found 96 members, further analyzed in terms of specific molecular characteristics, phylogenesis and gene expression profiles in different grapevine tissues and developmental stages. Finally, we performed a co-expression analysis in order to define ATL putative functions. We then produced transgenic *V. vinifera* plants expressing one of the 10 up-regulated ATLs in *V. riparia* to perform a functional analysis. The choice of the ATL was driven by its high expression level and its high homology with AtATL2, already described as responsive to elicitors and hormones in *A. thaliana*. The phenotypic analysis of transgenic *V. vinifera* plants revealed a higher resistance to infection with *P. viticola* at macroscopic and microscopic levels. Transgenic plants were further characterized at molecular levels, i.e. gene expression profile, hormone and ubiquitination levels.

MILD AND SEVERE '*CANDIDATUS* PHYTOPLASMA ASTERIS' STRAINS INDUCE DIFFERENT miRNA EXPRESSION PROFILES IN INFECTED PERIWINKLES. M. Morano, A. Carra, L. Galetto, S. Palmano. *CNR - Istituto di Protezione Sostenibile delle Piante, IPSP, Strada delle Cacce 73 - 10135 (TO), Italy. E-mail: martinamorano86@gmail.com*

Symptoms triggered by phytoplasmas in plants suggest that the infection mechanism of these pathogens can affect several host metabolic pathways. Recently, microRNAs (miRNAs) were identified as a family of regulatory molecules modulating gene expression in plant-microorganism interactions, mainly through post-transcriptional gene silencing. It was observed that two 16srI phytoplasma strains, CY and L163, showing more than 99% genome identity and originally isolated from infected daisy (CY) and lettuce (L163) plants, induce symptoms of different severity upon graft inoculation of periwinkle (*Catharanthus roseus*) plants. Expression profiles of selected miRNAs were analysed and compared between healthy, CY and L163 graft inoculated plants. Thirteen miRNAs, chosen because of their involvement in hormone signalling (miR159, miR160, miR167, miR390, miR393), plant development (miR156, miR164, miR166, miR172, miR319, miR394, miR396) and stress response (miR398) were selected for the study. miRNA expression profiles were analysed by qRT-PCR, in midribs of leaves collected at three times after grafting (4, 12, 18 weeks) in plants harbouring comparable phytoplasma populations. Preliminary data analysis indicated a general miRNA upregulation in phytoplasma infected plants. Moreover, the observed over-expression was more pronounced in the phytoplasma strain causing milder symptoms. To better understand the role of selected miRNAs potentially involved in the infection process, specific target gene expression analysis in the infected plants is currently undergoing. The results will cast some light on the mechanisms of phytoplasma exploitation of the host plant molecular machinery, providing potential targets to address the issues of plant susceptibility/resistance to these pathogens.

PHYTOPLASMA-HOST INTERACTIONS: A CLOSER LOOK THROUGH LASER MICRODISSECTION AND *IN SITU* HYBRIDIZATION. M. Pesando[1,2], R. Balestrini[2], D. Bosco[1,3], C. Marzachì[1], L. Galetto[1]. *[1]CNR Istituto per la Protezione Sostenibile delle Piante, Strada delle Cacce 73 - 10135 Torino, Italy. [2]CNR Istituto per la Protezione Sostenibile delle Piante, Viale Mattioli 25 - 10125 Torino, Italy. [3]Università degli Studi di Torino, Largo Braccini 2 - 10095 Grugliasco, (TO), Italy. E-mail: massimo.pesando@ipsp.cnr.it*

Phytoplasmas are phloem-limited plant pathogens transmitted by phloem-sucking Hemipteran insects. They are wall-less bacteria causing severe economic damage on many crops worldwide. Molecular mechanisms involved in the interaction between phytoplasmas and their hosts are still poorly understood, mainly because phytoplasma cultivation in purity is not yet feasible. This work aimed to set up a laser microdissection (LMD) protocol that could be useful for further studies to elucidate the signaling pathway in phytoplasma colonized host cells, both in plant- and insect-phytoplasma interactions. Two unrelated phytoplasmas (Flavescence dorée, FD, 16SrV-C and Chrysanthemum yellows, CY '*Candidatus* Phytoplasma asteris', 16SrI-B), the model plant *Arabidopsis thaliana* and the leafhopper *Euscelidius variegatus*, vector of both pathogens, were studied in this work. Protocols were successfully optimized to isolate vascular tissues of *A. thaliana* from leaves and stems through LMD, and also to extract RNA from collected cells. To verify the optimized procedure, the presence of three plant transcripts (one ubiquitarious and two tissue specific) and of four phytoplasma transcripts (one abundant and one rare for each pathogen) was evaluated by RT-PCR on RNA extracted from isolated phloem cells. *In situ* hybridization experiments were performed on *E. variegatus*, by using specific DIG-conjugated probes to localize insect transcripts known to be expressed either in gut or in salivary glands, as well as phytoplasma cells. These experiments were performed to identify specific tissues inside an insect section, which is an essential step for the application of an LMD protocol to dissect them.

HETEROLOGOUS RNA-RNA INTERACTIONS INVOLVED IN THE PRESERVATION OF *BEET NECROTIC YELLOW VEIN VIRUS* GENOME INTEGRITY. M. Dall'Ara[1,2], C. Ratti[1], E. Klein[2], S.E. Bouzoubaa[2], D. Gilmer[2]. *[1]Dipartimento di Scienze Agrarie, Area Patologia Vegetale, Università di Bologna, Bologna, Italy. [2]Institut de Biologie Moléculaire des Plantes, Integrative Virology, CNRS UPR2367, Université de Strasbourg, Strasbourg, France. E-mail: gilmer@unistra.fr, claudio.ratti@unibo.it*

Multipartite phytoviruses are characterized by having two or more genomic segments packaged in independent particles. To ensure a productive infection, such heterogeneous group of viruses has to preserve their genome as a functional unit ensuring the presence of at least one of each viral segment in the target cell. Such condition implies a mechanism that eludes the biological costs of having the genome split in physically separated components.

The high level of multiplicity of infection (MOI) together with the physical constrains that a multipartite virus faces during its journey in the host vascular tissues, make, in fact, the uncoordinated trafficking of viral particles an unrealistic model of viral movement across the plant.

We, therefore, hypothesized a model for multipartite phytoviruses ssRNA(+) whereby each genomic RNA is specifically recognized by a network of RNA/RNA interaction and packaged in a modular ribonucleoprotein (RNP) complex.

Beet necrotic yellow vein virus (BNYVV) possesses the higher number of genomic segments (RNA1 to 5) within ssRNA(+) viruses. Among them, RNA1 together with RNA2 is sufficient for viral replication and long distant movement in susceptible hosts. Consequently, we decided to investigate the existence of specific heterologous interactions between these RNAs evaluating their role in the formation of genome complexes.

By Electro Mobility Gel Shift Assays (EMSA) and reverse genetic approaches, we identified specific interaction domains in each RNAs possibly involved in the BNYVV infectivity.

ROLE OF *XylA* GENE ON THE VIRULENCE OF THE FUSARIUM HEAD BLIGHT PATHOGEN *FUSARIUM GRAMINEARUM*. **F. Tini[1,2], A.H. Benfield[2], L. Covarelli[1], D.M. Gardiner[2].** *[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74 - 06121, Perugia, Italy. [2]Queensland Bioscience Precinct, CSIRO Agriculture, Brisbane - 4067 QLD, Australia. E-mail: francesco.tini@studenti.unipg.it*

Fusarium graminearum is the most important causal agent of Fusarium head blight (FHB), a dangerous disease of small grain cereals worldwide. During colonisation of plant tissues, *F. graminearum* biosynthesizes hydrolytic enzymes such as endo-1,4-β-xylanase which facilitate the invasion of host cells. Transcriptomic analyses revealed that five genes from fungal pathogens encode xylanases on wheat. A study was conducted to investigate the role of *F. graminearum XylA* gene during infection (FGSG_10999 locus). Knockout mutants of *XylA* gene of CS3005 strain were realised by DNA homologous recombination: a construct with a resistance gene for the nourseothricin antibiotic was placed in the FGSG_10999 locus. Virulence assays on seedlings and heads were carried out to compare the virulence of five different *XylA* knockouts with respect to the wild type strain on wheat shoots, seedlings and heads of the cv. Kennedy. The growth rate of the knockout strains was compared to the wild type on synthetic media containing xyolose or xylan as unique carbon sources at five days post inoculation. Assays on shoot roots and seedling stem bases proved a virulence reduction of the transformed strains with respect to the wild type of 36% and 31%, respectively for the two tissues. A FHB test, performed by evaluating the percentage of necrotic spikelets, showed an average lower virulence of 70% of mutants with respect to the wild type. The growth rate of the knockout strains was lower with respect to the wild type of about 33% and 32% on the media containing xylose and xylan as unique carbon source, respectively.

STEM-TO-HEAD COLONIZATION BY THREE *FUSARIUM* SPECIES AND DEOXYNIVALENOL TRASLOCATION IN BARLEY (*Hordeum vulgare* L.). **F. Pecoraro[1], M. Giannini[1], G. Beccari[2], L. Covarelli[2], A. Pisi[1], P. Nipoti[1], A. Prodi[1].** *[1]Dipartimento di Scienze Agrarie (DipSA), Università degli Studi di Bologna, Viale G. Fanin 44 - 40127, Bologna, Italy. [2]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, Borgo XX Giugno 74 - 06121, Perugia, Italy. E-mail: antonio.prodi@unibo.it*

Fusarium crown rot (FCR) is an important disease of wheat and barley mainly caused by *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*, which are also responsible for the production of the mycotoxin deoxynivalenol (DON) during plant colonization. While most of studies on fungal colonization are focused on wheat, this is the first investigation on the colonization process of barley by these pathogens. The three above mentioned species were inoculated at the stem base level. At maturity, symptoms were visually evaluated and then plants were cut into four segments: Segment 1 (the area adjacent to the crown, including the first node) Segment 2 (the area including the second node) Segment 3 (the area adjacent to the flag leaf) and Segment 4 (head). Fungal DNA quantification of each inoculated strain was performed, on each segment, by Real Time qPCR. DON presence along the plant was determined by ELISA method. All inoculated strains caused browning, symptom particularly evident up to the second node, and the *F. culmorum* strain seemed to be the most virulent. Real Time qPCR assays showed that both *F. graminearum* and *F. culmorum* DNA was found up to the head, while *F. pseudograminearum* DNA was found up to the second node. For every pathogens, DON was detected up to the head. This study shows that barley, as already demonstrated in wheat, may be subject to head contamination by DON produced during FCR, thus representing a DON source in addition to *Fusarium* head blight contaminations.

CAREFUL WITH THAT *AXE* GENE. GENOME PERTURBATION AFTER A PEG-MEDIATED PROTOPLASTS TRANSFORMATION IN *FUSARIUM VERTICILLIOIDES*. **V. Scala[1], A. Grottoli[2], R. Aiese Cigliano[3], M. Beccaccioli[2], C. Fanelli[2], C. Dall'Asta[4], P. Battilani[5], M. Reverberi[2], W. Sanseverino[3].** *[1]CREA-PAV, Via C. G. Bertero 22 - 00156 Roma, Italy. [2]Università La Sapienza, Dept. Environmental Biology - 00185 Roma, Italy. [3]Sequentia-Biotech, Barcelona, Spain, [4]Università di Parma, Italy. [5]Università Cattolica, Piacenza, Italy. E-mail: alessandro.grottoli@uniroma1.it*

Fusarium verticillioides causes ear rot disease in maize and its contamination with fumonisins, mycotoxins toxic to humans and livestock. Lipids, and their oxidized forms, may drive the fate of this disease. In a previous study, we have explored the role of oxylipins in this interaction by deleting in *F. verticillioides* a linoleate diol synthase-coding gene, *lds1*, by standard transformation procedures. A profound phenotypic diversity in the generated mutants has prompted us to investigate deeper the whole genome of two *lds1*-deleted strains. Surprisingly, bioinformatic analyses pinpoint significant differences in the genome sequences emerged between the wild type and the *lds1*-mutants further than those trivially attributable to the deletion of the *lds1* locus such as single nucleotide polymorphisms, small deletion/insertion polymorphisms and structural variations. Difference among the three genomes were analyzed by using a high degree of filtering to decrease the bias due to sub- culturing practices and parasexual cycle operating in these fungi. The results suggest the possibility that the effect of a (theoretically) punctual transformation event might have caused an overall genomic instability, and that transformation practices, commonly used in the reverse genetic of fungi, may potentially be responsible of unexpected, stochastic and henceforth off-target rearrangements throughout the genome.

FATTY ACID METABOLISM IN THE FUNGAL MAIZE PATHOGEN *FUSARIUM VERTICILLIOIDES*. **M. Beccaccioli[1], A. Grottoli[1], D. Magali[1], C. Fanelli[1], M. Reverberi[1], M. Ludovici[1], V. Scala[2].** *[1]Department of Environmental Biology, University of Rome "Sapienza", Piazzale Aldo Moro 5 - 00185, Rome, Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale, Via C.G. Bertero - 00156 Rome, Italy. E-mail: marzia.beccaccioli@uniroma1.it*

The fungal maize pathogen *Fusarium verticillioides* produces fumonisins, secondary metabolites harmful to humans and animals (classified as IARC2B). As shown in previous studies, the production of some mycotoxin (e.g. fumonisins) is related to fatty acids metabolism and oxylipins signaling. Fungal lipids play a crucial role in regulating the fungal growth and the interaction with the host. We studied the role of the linoleate diol synthase coding gene, *lds1,* in *F. verticillioides*. This gene is involved in the synthesis of oxylipin and its inactivation strongly influences the interaction with the host, the secondary metabolism and, intriguingly, the polyunsaturated fatty acid (PUFA) amount in the fungal cell. This work aims at studying the expression of some genes related to the fatty acid metabolism and involved in the synthesis of oxylipin in *F. verticillioides* grown

under mycotoxins-inducing conditions. Genetic analysis in the model yeast *Saccharomyces cerevisiae* has provided valuable insights into the description of the fatty acids biosynthetic pathways. We focused our attention on the expression of different fatty acid desaturases and elongases. Fatty acid elongases coordinately with fatty acid desaturases generate several long chain mono- and polyunsaturated fatty acid, componing cellular lipids. Elongases and desaturases play critical roles in the regulation of the length and the degree of unsaturation of fatty acids and thereby their functions and metabolic fate. Gene expression profile was compared with the PUFA content in WT as well as in *lds1*-deleted strain of *F. verticillioides*. It emerges a close link between fatty acids desaturation and oxylipin synthesis.

NOVEL REGULATORS OF DEFENSE HORMONAL CROSSTALK UNRAVELED BY GENOME-WIDE ASSOCIATION STUDY. S. Proietti, L. Caarls, S. Coolen, S.C.M. Van Wees, C.M.J. Pieterse. *Plant-Microbe Interactions, Department of Biology, Padualaan 8 - 3584 CH, Utrecht University, The Netherlands. E-mail: s.proietti@uu.nl*

The plant hormones salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) play central roles in biotic and abiotic stress responses. To respond appropriately to specific attackers or to multiple attackers at the same time, their signaling pathways cross-communicate. Antagonistic and synergistic effect of ABA or SA on JA response have been extensively studied and some molecular players of ABA/JA and SA/JA crosstalk identified, however there are still many links that remain unknown. To identify new regulators of SA/JA and ABA/JA crosstalk, we performed a genome-wide association (GWA) study on natural genetic variation in *Arabidopsis* for the effect of SA and ABA on the JA pathway. Firstly, 349 wild *Arabidopsis* accessions were treated with MeJA, ABA+MeJA or SA+MeJA after which the level of expression of the JA marker gene *PDF1.2* was quantified. The results showed that *Arabidopsis* has a large genetic variation in the magnitude by which SA and ABA affect JA-responsive gene expression. GWA mapping of the quantitative gene expression data revealed several genomic regions that are potentially associated with SA/JA or ABA/JA crosstalk. Underlying candidate genes are involved in regulatory as well as metabolic pathways, signal transduction, transporter activity, oxidative stress and programmed cell death. T-DNA insertion lines confirmed the role of 6 candidate genes in SA/JA crosstalk and of 12 candidate genes in ABA/JA crosstalk. Moreover, we proved for some of them a role in the resistance against fungal pathogens and herbivorous insects. The results we obtained could be exploited to produce new tolerant crops.

PGE: A NATURAL ANTIFUNGAL PREPARATION FROM POMEGRANATE PEEL WITH A WIDE SPECTRUM OF ACTIVITY. S. Pangallo, M.G. Li Destri Nicosia, F.V. Romeo, G.E. Agosteo, S. Scibetta, P. Rapisarda, S.O. Cacciola, S. Droby, L. Schena. *Università Mediterranea di Reggio Calabria, Dipartimento di Agraria, località Feo di Vito - 89122 Reggio Calabria, Italia. E-mail: sonia.pangallo@unirc.it*

An alcoholic extract obtained from the peel of pomegranate (PGE) has proved to be an effective alternative control mean against a number of fungal pathogens including *Colletotrichum* spp. and *Fusarium* spp. on olive fruit, *Penicillium digitatum* and *P. italicum* on different citrus species, *P. expansum* on apples, *Monilinia laxa* and *Botrytis cinerea* on sweet cherries and *B. cinerea* on table grapes. Most experiments were conducted by simulating commercial conditions and PGE proved as effective or more effective than chemicals used as controls. PGE is characterized by curative and preventive activity, long persistence effect, wide spectrum of activity and high efficacy on different hosts under different conditions. Its mechanism of action is based on both a direct toxicity on fungal conidia and mycelia and on the induction of resistance in treated plant tissues. Furthermore, signs of possible phytotoxic effect were not observed in any of the investigated species. According to the above features and the wide availability of the pomegranate peel as a waste product of the processing factories, PGE may be easily implemented in commercial control strategies as a natural, safe and eco-friendly extract.

RESISTANCE COMPONENTS TO PLASMOPARA VITICOLA IN GRAPEVINE GENOTYPES. F. Bove, T. Caffi, E. Gonzalez-Dominguez, V. Rossi. *Università Cattolica del Sacro Cuore, DIPROVES, Via Emilia Parmense 84 - 29122 Piacenza (PC), Italy. E-mail: federica.bove@unicatt.it*

The use of resistant varieties against downy mildew (DM, *Plasmopara viticola*) is increasingly gaining ground in Europe to reduce fungicide applications. A research was conducted to characterize the resistance components to DM in 16 grapevine genotypes bringing one or more of the following resistance genes from *Muscadinia* and American *Vitis* spp.: *Rpv3*, *Rpv4*, *Rpv10*, *Rpv11*, and *Rpv12*. The following resistance components were assessed in monocyclic experiments with artificial inoculation on leaf discs: i) infection efficiency of sporangia (percentage of the inoculation sites showing DM symptoms); ii) AUDPC (area under the disease progress curve); iii) incubation length; iv) latency period (in degree-days); v) sporangia produced per unit of DM lesion (number of sporangia per mm^2 of lesion area); v) infectivity of the produced sporangia (by re-inoculation on susceptible variety); and vi) infectiousness (time a DM lesion produces sporangia, evaluated through repeated washings). Leaf discs were excised from fully developed young leaves collected in 2014 and 2015 at shoot growing, flowering and fruit set. Resistance components were expressed at different degrees in the different genotypes and at the different growth stages, with significant differences in comparison to the susceptible *Vitis vinifera* variety used as reference (Merlot). Data from monocyclic experiments were used for calibrating a DM epidemiological model able to predict disease progress in resistant genotypes.

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COMPARING CHESTNUT CULTIVARS AND THE WILD-TYPE FOR THEIR SUSCEPTIBILITY TO THE NUT ROT CAUSED BY *GNOMONIOPSIS CASTANEAE*. G. Lione[1], L. Giordano[1,2], G.L. Beccaro[1], P. Gonthier[1]. *[1]University of Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo Braccini 2 - 10095 Grugliasco, (To) Italy. [2]University of Torino, Centre of Competence for the Innovation in the Agro-Environmental Field (AGROINNOVA), Largo Paolo Braccini 2 - 10095 Grugliasco, Italy. E-mail: paolo.gonthier@unito.it*

The emerging nut rot caused by the fungal pathogen *Gnomoniopsis castaneae* stands among the most detrimental threats to chestnut (*Castanea* spp.). The goal of this study was comparing a large selection of chestnut cultivars and the wild-type of *C. sativa* for the levels of susceptibility to *G. castaneae*. In 2013, up to 40 nuts per tree were collected from 85 chestnut cultivars and from the wild-type growing in the Chestnut Regional Repository of Chiusa Pesio (Italy). The sampling was partially replicated in 2014 for validation purpose. Isolation trials and molecular analyses were performed to assess the incidence of *G. castaneae* at tree level. The incidence

of each cultivar was compared to the incidence of the wild-type, assumed as reference population, through an innovative approach based on the Pearson system of generalized frequency curves and on Monte Carlo simulations. In the wild-type, the incidence of *G. castaneae* increased from 2013 (4.8%) to 2014 (19.6%) and a similar trend was also observed, on average, in the chestnut cultivars (up to +29.7%). Cultivars significantly more susceptible ($P<0.05$) than the wild-type (22% of the total number of cultivars in 2013 and 55% in 2014) were detected by definite integration of curves associated with the Pearson system. The validation analysis revealed no significant association between the most susceptible cultivars detected in 2013 and 2014 (odds ratio 2.85; 0.18-176.61 95% CI), suggesting that the susceptibility to *G. castaneae* is substantially homogeneous between the chestnut cultivars and the wild-type.

COMPETITION ASSAYS REVEAL A NOVEL PUTATIVE BIOCONTROL AGENT: *PAENIBACILLUS PASADANENSIS* STRAIN R16. A. Passera, P. Casati, G. Venturini, F. Penaca, F. Quaglino, P.A. Bianco. *Università degli Studi di Milano, DISAA, Milano, Italia. E-mail: alessandro.passera@unimi.it*

Since European policies aim to reduce the environmental impact of agriculture and implement sustainable containment strategies for diseases, research of novel biocontrol agents is very important. In this study, the plant growth promoting potential and biocontrol effect against three important plant pathogenic fungi (*Botrytis cinerea*, *Fusarium verticillioides*, and *Phomopsis viticola*), exerted by the novel candidate biocontrol agent *Paenibacillus pasadenensis* strain R16, isolated during previous investigation carried out to characterize the microbiome of diseased and healthy plants, were assessed *in vitro*. Biochemical assays to determine plant growth promoting potential gave negative results in regard to siderophore production and phosphate solubilization, and positive results for ACC-deamination, IAA production, and activity of catalase and chitinase. Biocontrol assays showed that strain R16 is very effective against *B. cinerea* in several tests, reducing mycelial growth both in dual-culture and through volatile substances only, as well as reducing infection rate on berries and inhibiting conidia germination. Strain R16 also showed good biocontrol potential also against *P. viticola*, but was ineffective toward *F. verticillioides*.

Obtained results proved for the first time the efficient biocontrol activity against fungal pathogens and putative plant growth promotion traits of *P. pasadenensis* strain R16, opening an interesting scenario for further studies investigating the application of this endophytic bacterium as a biocontrol agent in open field.

SYNERGISTIC EFFECT OF TRICHODERMA AND CHITOSAN APPLICATION IN TOMATO FOR THE CONTROL OF CUCUMBER MOSAIC VIRUS INFECTION. N. Rendina, A. Sofo, A. Vitti, A. Scopa, M. Nuzzaci. *Università degli Studi della Basilicata, Scuola di Scienze Agrarie, forestali, alimentari ed ambientali, Potenza, Italy. E-mail: nunzia.rendina@unibas.it*

Soil and plant environments are characterized by a wide range of microorganisms able to interact with host plants and, in some cases, to differentially induce susceptibility or resistance to pathogens. Plant viruses cause chlorosis and necrosis, so decreasing plant growth and productivity. Differently, many fungi are able to trigger a beneficial relationship with plants. *Trichoderma* spp. are endophytic symbionts able to modify plants metabolism, increasing nutrient uptake by plants and photosynthetic efficiency, and protecting them from pathogens. A biopolymer able to elicit plant-immunity is chitosan, derived by the deacetylation of chitin, a component of some fungal cell walls. Chitosan improves the host hypersensitive response by the expression of pathogenesis-related proteins and the synthesis of secondary metabolites. *Trichoderma harzianum* T-22 (T22) induces defense responses against *Cucumber mosaic virus* Fny (CMV) in *Solanum lycopersicum*. On this basis, the aim of this work was to determine if the combination T22-chitosan has an antiviral activity against CMV in tomato plants. Plant physiological parameters (gas exchange, chlorophyll content and fluorescence) were followed throughout the experiment. Furthermore, ELISA test was employed to detect CMV. Results indicate that plants treated with T22 and chitosan had a strong attenuation of viral load, a higher chlorophyll content and a better photosynthetic performance compared to the untreated plants. Further investigations are in progress to determine plant antioxidant responses. In conclusion, combined treatment based on T22 and chitosan represents a highly effective strategy against CMV, embracing the criteria of sustainable agricultural practice and public health protection.

THE EU RESEARCH PROGRAMS IN RESPONSE TO THE *XYLELLA FASTIDIOSA* EMERGENCY. D. Boscia, M. Saponari. *CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari - 70126 Bari, Italy. E-mail: donato.boscia@ipsp.cnr.it*

The identification in 2013 of a large outbreak of *Xylella fastidiosa* (*Xf*) in olive groves in the Salento peninsula (southern Italy) has resulted in a plant health emergency of unprecedented proportions for the EU. Afterwards, in 2015 numerous *Xf* outbreaks were identified in Corsica and France. Because of the complexity of the *Xf*-associated diseases, the management and the control of the infections rely on deep knowledge of the hosts, of the biology and genetics of the isolate(s), and on their interactions with the autochthonous insect vector population(s), the climate conditions and the agriculture practices. As such, the EU Commission mobilized resources within the EU framework programme for research and innovation Horizon 2020. At the end of 2015, the project Pest Organisms Threatening Europe (POnTE) started covering among the other emerging pathogens the topic of *Xf*, whereas in 2016 (i) a dedicated H2020 action for *Xf* (Spotlight on critical outbreak of pests: the case of Xylella fastidiosa) has been launched, and (ii) a targeted *Xf*-project has been set within the EUPHRESCO network. These actions involve very large Consortiums with ambitious work-plans covering basic and applied researches on prevention, detection, surveillance and innovative control strategies for *Xf* and its vector(s). The multi-actor approach ensured by these large Consortiums will facilitate interactions among research groups, share previous experiences, establish new and strengthen current collaborations among European and non-European research organizations, and increase awareness about scientific work previously done. Best practices to manage the EU resources are put in place in order to maximize the efforts while avoiding research duplications.

POTENTIAL DISTRIBUTION OF *XYLELLA FASTIDIOSA* AND ITS INSECT VECTOR *PHILAENUS SPUMARIUS* IN THE MEDITERRANEAN BASIN. L. Bosso[1], M. Di Febbraro[2], G. Cristinzio[1], A. Zoina[3], D. Russo[1,4]. *[1]Dipartimento di Agraria, Università degli studi di Napoli Federico II, Via Università 100 - 80055 Portici, Napoli, Italy. [2]EnvixLab, Dipartimento Bioscienze e Territorio, Università del Molise, Pesche, Italy. [3]CNR, Istituto per la Protezione Sostenibile delle Piante, UOD Portici, Italy. [4]School of Biological Sciences, University of Bristol, Woodland Road BS8 1UG, Bristol, United Kingdom 4. E-mail: luciano.bosso@unina.it*

Xylella fastidiosa is a xylem-limited gram negative bacterium causing a high number of severe diseases to many agricultural and

forestry plants. *Philaenus spumarius* is the principal insect vector of this pathogen bacterium in Italy. We developed a Maxent model to detect the potential distribution of *X. fastidiosa* and *P. spumarius* in the Mediterranean basin. Maxent models achieved excellent levels of predictive performance as can be seen from AUC, TSS and AUC_{diff} values for both the organisms. Species distribution models showed a high probability of *X. fastidiosa* in Portugal, Spain, Italy, Southern France, Corsica, Albania, Montenegro, Greece and Turkey as well as all countries of Northern Africa and the Middle East. Maxent models also showed that *P. spumarius* was widespread in all countries of Europe, Northern Africa and the Middle East. *P. spumarius* could spread *X. fastidiosa* in all countries of the Mediterranean basin but, cold winter temperature seems to limit the spread of this pathogen bacterium in all countries located beyond the south of France. Our study highlights that *X. fastidiosa* may overcome the current boundaries outside Italy. Given the potentially high risk, the Phytosanitary Services of the listed nations are considering stringent phytosanitary measures to avoid the introduction of the bacterium in their own countries.

MONITORING OF *XYLELLA FASTIDIOSA* IN AN ITALIAN PEST-FREE AREA (LATIUM REGION). V. Modesti, N. Pucci, S. Lucchesi, L. Campus, S. Loreti. *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale (CREA-PAV), via C. G. Bertero, 22 - 00156 Roma, Italy. E-mail: nicoletta.pucci@crea.gov.it*

The occurrence of *Xylella fastidiosa* subsp. *pauca* on olive trees in the Salento area (Southern Italy) represent one of the most serious plant health emergencies of recent years in which the entire European Union have had to deal with. A monitoring plan for preventive purposes in the entire national territory was planned by the Italian Ministry of Agriculture by transposing the EU guidelines that foresee the measures to prevent introduction and spread of *X. fastidiosa.* As an example of 'pest-free' area is reported the experience of Latium region, in order to control the sanitary status of the territory and investigate on the presence/absence of *X. fastidiosa*. Taking into account that analyses were mainly focused on asymptomatic plant material, the diagnosis was based on the use of molecular methods characterized by high specificity and sensitivity: real-time PCR. Two assays based on the primers selected on the *rimM* gene and on the primers from the gene encoding the HL protein were used. These methods allowed to exclude the presence of *X. fastidiosa* in the processed samples in spite of the observation of some host plants with suspicious symptoms. An in wide comparison of the adopted approaches, by checking their analytical sensitivity and specificity, showed that the real-time PCR based on *rimM* selected primers was the most accurate for monitoring activity as it does not cause undetermined results when compared to the other real-time PCR (gene encoding HL protein). Other techniques, such as LAMP-PCR, are taken into account to improve the procedures by maintaining the performance of high sensitivity/specificity and in view of direct application in the field.

DETECTION OF *XYLELLA FASTIDIOSA*: VALIDATION AND IMPLEMENTATION OF ROUTINE TESTING METHODS. G. Loconsole[1], D. Boscia[2], O. Potere[1], S. Zicca[2], G. Altamura[2], F. Palmisano[3], V.N. Savino[1], M. Saponari[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, I-70126 Bari, Italy. [2]Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, SS Bari - 70126 Bari, Italy. [3]Centro di Ricerca, Sperimentazione e Formazione in Agricoltura, Via Cisternino 281 - 70100 Locorotondo (BA), Italy. E-mail: giuliana.loconsole@uniba.it*

Accurate and early detection of *Xylella fastidiosa* (*Xf*) is a major challenge due to the wide range of host plants (different matrices/tissues, rate of host colonization) and the occurrence of symptomless bacterial infections. The recent establishment of this exotic plant pathogenic bacterium in the EU territory and the large panel of EU susceptible host plants increased the need for rapid diagnostic tools suitable for processing large number of samples and from different sources. Although, several approaches are currently available for the detection of *Xf* in the host plants and vectors, there is a need for harmonized protocols and user-friendly diagnostic tests. In this study, we compared the sensitivity and the reliability of a selected panel of currently available protocols (ELISA, PCR, qPCR), in comparison with novel approaches based on automatized diagnostic platform and on DTBIA and LAMP-based assays. The overall results showed that: (i) although resulting in different diagnostic sensitivity all the approaches tested were able to detect the bacterium in samples from symptomless plants; (ii) Real-time LAMP assay based using crude plant sap can represent a rapid and reliable screening test; (iii) Real-time quantitative PCR assays had the higher diagnostic and analytical sensitivity; (iv) the use of automatized platform allowed to prepare PCR-templates with high and standardized quality for highly reliable diagnostic results; (v) DTBIA had the lowest diagnostic sensitivity, yet representing a useful approach when movement of *Xf* infected materials is limited due to the phytosanitary regulations.

TEST PERFORMANCE STUDY FOR VALIDATION OF DETECTION METHODS OF *XYLELLA FASTIDIOSA*. S. Loreti[1], N. Pucci[1], M. Saponari[2], G. Loconsole[3], F. Gaffuri[4], V. Modesti[1], S. Lucchesi[1], O. Potere[3]. *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale (CREA-PAV), via C. G. Bertero, 22 - 00156 Roma, Italy. [2]Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Sede Secondaria di Bari - 70126 Bari, Italy. [3]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy. [4]Laboratorio Fitopatologico SFR c/o Fondazione Minoprio, Viale Raimondi, 56 - Vertemate con Minoprio 22070 (CO), Italy. E-mail: stefania.loreti@crea.gov.it*

Xylella fastidiosa, a xylem-limited bacterium of the *Xanthomonadaceae* family, was recently associated with the "Olive Quick Decline Syndrome" (OQDS) widespread in the Salento area (Southern Italy). The rapid emergence of this regulated quarantine pathogen in new areas imposes the application of official diagnostic methods aimed to prevent its spread in other Italian regions and the movement of latently infected plant material. The present study summarizes the results of a test-performance study (TPS) to validate, at a national level, detection methods of *X. fastidiosa* by involving sixteen Italian laboratories that includes several Plant Protection Services (PPS), SELGE, UniMi, UniCT, CRSFA, CIHEAM. A working group was constituted, that organized: i) a PRE-TEST for the establishment of analytical-sensitivity of each method and repeatability, analytical-specificity, relative accuracy, ii) the final TPS to detect the reproducibility of the selected methods. The activity of the pre-test highlighted a higher analytical-sensitivity from samples of total-DNA with respect crude-extracts. In particular, LAMP-PCR was more sensitive than ELISA tests from crude-extracts. Using total-DNA, duplex (based on *rimM* and on *cox* primers) and single real-time PCR (*rimM* primers) resulted in the most sensitive methods followed by LAMP-PCR and, finally, conventional PCR (primers RST31/RST33). The high values of relative-accuracy and reproducibility (among 92-100% for both) confirmed a high reliability of duplex/single real-time PCRs and LAMP-PCR from total-DNA. LAMP-PCR from crude extracts gave values of accuracy and reproducibility respectively of 78% and 87% resulting a promising assay for its friendly and on-site-based use, thus implementing

phytosanitary inspections prior to import/export of plant material and controls in orchards or in nurseries.

A GENOME-WIDE APPROACH TO REDEFINE *XYLELLA FASTIDIOSA* TAXONOMY. S. Marcelletti[1], M. Scortichini[1,2]. *[1]CREA-Centro di ricerca per le Colture Arboree, Via di Fioranello, 52 - 00134 Roma, Italy. [2]CREA-Centro di ricerca per le Colture Arboree, Via Torrino, 3 - 81100 Caserta, Italy. E-mail: marco.scortichini@crea.gov.it*

Xylella fastidiosa is a xylem-limited, fastidious phytopathogenic bacterium of the *Xanthomonadaceae* family which colonizes a very large number of hosts. Recently, *X. fastidiosa* strains belonging to the subspp. *pauca* and *multiplex* have been isolated in Southern Europe from *Olea europea* (Salento area; Southern Italy) and several ornamental shrubs (Corsica, Maritime Alps; Southern France). The rapid emergence of this regulated phytopathogen in new areas imposes the application of rapid and reliable detection techniques to prevent the further introduction of latently infected plant material. The knowledge of the pathogen basic taxonomy and population structure is fundamental for the development of an efficient detection and prevention protocol. The incorporation of genomic data into bacterial taxonomies and systematic procedures has recently greatly contributed to the advancement of such disciplines. A total of 21 *Xylella fastidiosa* strains were assessed by comparing their genomes to infer their taxonomic relationships. The whole-genome-based average nucleotide identity (ANI) and tetranucleotide frequency correlation coefficient (TETRA) analyses were performed. In addition, a consensus tree based on comparisons of 956 core gene families, a genome-wide phylogenetic tree and a neighbor-network were constructed with 820.088 nucleotides (i.e., approximately 30-33% of the entire *X. fastidiosa* genome). All approaches revealed the occurrence of three well demarcated genetic clusters that represent *X. fastidiosa* subspecies, namely *fastidiosa*, *multiplex* and *pauca*. Moreover, the proposed but never formally described subspecies '*sandyi*' and '*morus*' are instead members of the subspecies *fastidiosa*. These analyses also revealed the existence of a new *Xylella* species that was isolated in Taiwan from *Pyrus pyrifolia.*

A METAGENOMIC INVESTIGATION OF THE MICROBIOME OF *XYLELLA FASTIDIOSA*-INFECTED OLIVES. A. Giampetruzzi[1], M. Saponari[2], G. D'Attoma[1,2], M. Morelli[2], M. Chiumenti[2], D. Boscia[2], P. Saldarelli[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro - 70126 Bari. [2]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari - 70126 Bari, Italy. E-mail: pasquale.saldarelli@ipsp.cnr.it*

Following the introduction and establishment of the plant pathogenic bacterium *Xylella fastidiosa* (*Xf*) in the Apulia Region (southern Italy), olive turned to be the main host of the Salentinian bacterial strain and the majorly devastated crop. The mechanism of pathogenicity of *Xf* is still not completely understood and no means to cure the bacterium in the infected plants are available yet. Nevertheless, the alteration of microbial communities and effects in the expression of symptoms of *Xf*-infected plants is poorly studied. We are investigating the microbiome of *Xf*-infected olives by a shotgun metagenomic DNA sequencing approach that avoids the limitations of amplicon sequencing. Data obtained (28,333,924 and 29,096,610 reads from *Xf*-infected and healthy plants) were analyzed by MetaPhlAn, a metagenomic abundance estimation tool which maps reads to a set of selected marker sequences. Libraries from xylem tissues revealed a complex community in which small symbiotic bacteria of insects, i.e. *Candidatus* Zinderia insecticola and *Candidatus* Carsonella ruddii represented the 31% and 22% of the total population. *Xf* reaches in infected plants the 12% of the total microbial community. Studies are ongoing to characterize the microbial communities in the xylem sap of tolerant and susceptible olive cultivars, to envisage a control strategy based on the manipulation of these resident communities and to identify endosymbiont(s) which may be used to reduce the severity of symptoms. To this end, the evaluation of an endosymbiont bacterium for its potential to colonize *Xf*-infected olive tissues is underway.

RAPID SCREENING TESTS FOR DIFFERENTIATING *XYLELLA FASTIDIOSA* ISOLATES. M. Saponari[1], M. Montes-Borrego[2], G. D'Attoma[1,3], L. De La Fuente[4], G. Loconsole[3], B.B. Landa[2]. *[1]CNR Istituto per la Protezione Sostenibile delle Piante UOS Bari (IPSP), SS Bari, 70126 Bari, Italy. [2]CSIC Instituto de Agricultura Sostenible, 14080 Córdoba, Spain. [3]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro, 70126 Bari. [4]Department of Entomology and Plant Pathology Auburn University, 36849 Auburn, AL, USA. E-mail: maria.saponari@ipsp.cnr.it*

The bacterial pathogen *Xylella fastidiosa* (*Xf*) is characterized by a wide plant host range and insect vectors, and on the basis of phylogenetic studies it was subdivided into different subspecies. Results from strain typing, phylogenetic analyses, and other data comparisons have shown that phylogenetic clusters exhibit host-based genetic relationships. Until now, different molecular tests can be used for the differentiation of *Xf* isolates, among which MLST/MLSA represents the most common method to determine classification and phylogenetic placement of novel isolates. *Xf* outbreaks in EU motivated the search for accurate and faster approaches for detection and identification of the bacterium in different plant matrices. Because MLST/MLSA requires several PCR reactions and sequencing analyses, we have developed two independent approaches for rapid taxonomic assignment of uncharacterized isolates: (1) single-nucleotide primer extension (SNuPE) method for the multiplex amplification of six *Xylella* DNA sequences (targeting all subspecies and three genotypes within *Xf* subsp. *pauca* including the type-isolate infecting olive in Italy); (2) high-resolution melting analysis of the amplicon recovered from the gene encoding the conserved HL protein. Both assays proved to clearly differentiate *Xf* isolates currently known to occur in the Italian and France outbreaks. Indeed, validation on a larger panel of isolates covering the different subspecies consistently allowed to rapidly differentiate the isolates in different clusters. In conclusion, these approaches represent a useful tool for pre-screening and selection of infected samples to be further analyzed by MLST or whole genome sequencing.

TRANSMISSION OF *XYLELLA FASTIDIOSA* TO DIFFERENT HOST PLANTS BY NATURALLY INFECTED *PHILAENUS SPUMARIUS*. V. Cavalieri[1], D. Cornara[2], C. Dongiovanni[3], G. Altamura[1], D. Boscia[1], F. Porcelli[2], D. Bosco[4], M. Saponari[1]. *[1]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari, 70126 Bari, Italy. [2]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro, 70126 Bari. [3]Centro di Ricerca, Sperimentazione e Formazione in Agricoltura (CRSFA) "Basile Caramia", 70010 Locorotondo (Bari), Italy. [4]Università degli Studi di Torino, DISAFA, Grugliasco, (TO) Italy. E-mail: vincenzo.cavalieri@ipsp.cnr.it*

The meadow spittlebug *Philaenus spumarius* (Hemiptera, Aphrophoridae) has been identified as a vector of *Xylella fastidiosa* (*Xf*) in southern Italy where the bacterium has established in the Salentinian Peninsula. This species is one of the most common potential

vectors in Europe, but limited information is available on spittlebugs as vectors of *Xf*. In this work, eleven transmission experiments were performed in 2015 from late spring to late autumn, when adult spittlebugs were present in the *Xf*-infected olive groves. Insects were collected by sweeping net on the olive canopies of two selected *Xf*-infected olive groves and transferred in groups of five on to the following recipient plants: olive, oleander, citrus, grapevine, GF677 (*Prunus persica*×*Prunus amygdalus*) and periwinkle. Following an inoculation access period (IAP) of 7-days, the insects were recovered from the cages in order to estimate i) the survival rates ii) the presence of *Xf* by real-time qPCR in single insects. Transmissions were determined by testing with qPCR the recipient plants. The results showed that the proportion of *P. spumarius* that tested positive for *Xf* ranged from 25 to 71%. *P. spumarius* transmitted *Xf* to all the recipient plants except grapevine; however, citrus and stone fruit plants were not systemically infected. More than 75% of the insects survived the 7-day IAP on olive, grapevine, GF677 and periwinkle. A lower survival rate was recorded on citrus and on oleander. These data show that field-collected *P. spumarius* in the Salentinian olive groves have high rates of *X. fastidiosa* and are able to transmit the bacterium to different hosts.

ESTABLISHMENT OF AN EXPERIMENTAL FIELD TO EXPLORE THE DIFFERENTIAL OLIVE CULTIVAR RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION. M. Saponari[1], F. Palmisano[2], C. Dongiovanni[2], V. Cavalieri[1], G. Altamura[1], G. D'Attoma[1,3], G. Loconsole[3], M. Morelli[1], A. Saponari[2], D. Tavano[1], S. Zicca[1], D. Boscia[1]. *[1]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari, 70126 Bari, Italy. [2]Centro di Ricerca, Sperimentazione e Formazione in Agricoltura (CRSFA) "Basile Caramia", 70010 Locorotondo (Bari), Italy. [3]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.) Università degli Studi di Bari Aldo Moro, 70126 Bari. E-mail: maria.saponari@ipsp.cnr.it*

While different sources of natural resistance to *Xylella fastidiosa* (*Xf*) have been described in grapevines and citrus, lack of consolidated information exists on the wide panel of cultivars characterizing the vast olive germplasm. Preliminary observations on few cultivars, support the evidence that differential cultivar responses to *Xf* infections may exist. To explore the response of a larger panel of cultivars, in April 2015, an experimental olive plot, located within the *Xf*-heavily affected olive groves, was established in the Apulia Region (Italy). Twenty-four trees for each of the ten different cultivars were planted in randomized blocks. Each tree was caged with 15-20 specimens of *Philaenus spumarius* collected from the neighboring infected olive groves. Upon removing the cages, the trees are then continuously exposed to the natural vector populations occurring in the area. Nine and 12-months after planting, the trees were sampled, tested for *Xf* and inspected for symptoms. The first data confirmed the infectivity of the vector populations occurring in the Apulian contaminated area and the *Xf* susceptibility of the olive cultivars tested. Almost 50% of the trees tested positive, with an infection incidence ranging from 25% (Leccino) to 78% (Koroneiki). Symptoms of shoot dieback started to appear 1-year after planting, limitedly on few replicates of Cellina di Nardò. In April 2016, the number of cultivars has been increased up to 30. Periodical surveys for symptoms and quantitative analyses to monitor the differential bacterial titer and expression of target genes involved in the host response, are underway.

REPORT OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' IN COMMERCIAL APIACEAE SEEDS IN ITALY. V. Ilardi[1], E. Di Nicola[1], V. Lumia[1], M. Tavazza[2]. *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale (CREA-PAV), via C.G. Bertero, 22 - 00156 Roma, Italy. [2]ENEA, SSPT-BIOAG, Via Anguillarese 301 - 00123 - Rome, Italy. E-mail: vincenza.ilardi@crea.gov.it*

'*Candidatus* Liberibacter solanacearum' (CaLsol) has been recently shown to be seed-borne in carrot (*Daucus carota*), family Apiaceae. Therefore, exclusion of infected seedlots is necessary to prevent its introduction in new areas, such as Italy. Here, we tested seedlots of five carrot varieties sold in Italy during 2015 for CaLsol by i) real-time PCR assay (rtPCR) of the 16S rRNA locus; ii) PCR assay of the intergenic region between the 16S and 23S rRNA genes (ISR16/23S); and iii) PCR assay of the 50S rpIJ/rpIL ribosomal protein genes (50SrpIJ/L). CaLsol DNA was detected in seedlots of four varieties regardless of the method used. Sequence analysis of PCR-derived 50SrpIJ/L DNA and ISR16/23S DNA amplicons identified two homogeneous groups of CaLsol isolates. The single-nucleotide polymorphism analysis revealed that the first group was closely related to the CaLsol haplotype-E (except for having A at nucleotides 1620 and 1632 of ISR16/23S), while the second to the haplotype-D (except for having A at nucleotide 1648 of ISR16/23S, and T at nucleotides 920 and 1068 of 50SrpIJ/L). The identification of CaLsol in carrot seeds prompted us to investigate its presence in seedlots of another Apiaceae, the parsley (*Petroselium crispum*). Of note, rtPCR and PCR analyses identified CaLsol in seedlots of all three parsley varieties analyzed. Our data indicate that CaLsol-infected seedlots of different carrot and parsley varieties are commercialized in Italy, a country where the presence of CaLsol has not been reported yet, thus highlighting the requirement of coordinated and harmonized measure to limit its spread.

COMPARATIVE STUDY AMONG *NEOFABRAEA* spp. ITALIAN ISOLATES. I. Cameldi, F. Neri, M. Meneghini, I.M. Nanni, M. Collina, M. Mari. *DIPSA-Dipartimento di Scienze Agrarie, Viale Fanin 46, 40127, Bologna, Italy. E-mail: irene.cameldi2@unibo.it*

Bull's eye rot is one of the main post harvest disease of pome fruits. It is caused by four species of *Neofabraea* spp., however in Italy the most common species is *N. vagabunda*. Recently, an isolate of *N. malicorticis* was isolated for the first time from Italian 'Cripps Pink' apple. In order to characterized this isolate, its biological traits and its pathogenicity were compared with two representative isolates of *N. vagabunda* derived from our collection. Both *Neofabraea* species produced more conidia at low temperatures (0-10°C, depending on isolate) than at 25-30°C, while their highest mycelial growth was observed at 20°C. *Neofabraea vagabunda* and *N. malicorticis* isolates, artificially inoculated in wounded apple, after 60 days of storage at 0°C, showed a high disease incidence. In addition, the two species of *Neofabraea* increased the ambient pH *in vitro* and *in vivo* trials. The main differences between *N. vagabunda* and *N. malicorticis* observed in colony morphology and symptoms on fruit were discussed. The increased knowledge on the biology of two *Neofabraea* species is crucial for an efficient control of bull's eye rot.

AMPLICON METAGENOMICS ANALYSIS OF THE FUNGAL MICROBIOME IN APPLE FRUIT. A. Abdelfattah[1], M. Wisniewski[2], S. Droby[3], S. Mosca[1], S.O. Cacciola[4], L. Schena[1]. *[1]Università degli Studi Mediterranea, Dipartimento di Agraria, Loc. Feo di Vito - 89122, Reggio Calabria, Italy. [2]USDA-ARS-AFRS, 2217 Wiltshire Road, Kearneysville, WV, USA. [3]Department of Postharvest Science ARO, The Volcani Center, Bet Dagan - 50250, Israel. [4]Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi, Via S. Sofia 100 - 95123 Catania, Italy. E-mail: lschena@unirc.it*

An amplicon metagenomics approach was utilized to evaluate the fungal diversity of organic and conventional apples in 4 different locations (stem end, calyx end, peel and wounded flesh) soon after fruit purchasing (T1) and after two weeks of storage (T5). A total of 5,760,162 high quality fungal sequences were recovered and assigned to 8,504 OTUs. Members of the phylum *Ascomycota* were dominant in all samples and accounted for 91.6% of the total number of detected sequences. This was followed by *Basidiomycota* (8%), *Chytridiomycota* (0.1%) and unidentified fungi (0.3%). The incidence of the Phyla varied significantly between the examined fruit parts. For example, *Ascomycota* and *Basidiomycota* had a relative abundance (RA) of 88.2 and 11.4 % in the stem end (SE) and a RA of 95.3 and 4.6% in the calyx end (CE), respectively. Beta diversity analyses revealed significantly different fungal populations in all investigated fruit parts. Among detected fungi, the genus *Penicillium* prevailed in the peel (PE) and in the wounded flesh (WF) while *Alternaria* spp. prevailed in the core of the apples (CE and SE). Putative human fungal pathogens were particularly abundant in PE and WF. Significantly different populations were revealed in organic and conventional apples and this result was consistent in all investigated fruit parts (CE, PE, SE and WF). On the contrary little differences were revealed in the two assessment times (T1 and T5). Results of the present study represent an advancement of the knowledge on the fungal microbiome in the carposphere.

CHICORY AND *NEOALITURUS FENESTRATUS* AS NATURAL HOSTS OF *SPIROPLASMA CITRI* IN FRIULI VENEZIA GIULIA (NORTH-EAST ITALY).

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During the process of drafting the genome of a chicory phyllody (ChiP) phytoplasma strain (16SrIX-C) transmitted to periwinkle by the vector *Neoliturus fenestratus*, the unexpected presence of co-infecting microorganism belonging to the genus *Spiroplasma* was revealed. Sequence analysis of 16S rDNA showed that the spiroplasma strain shared about 99% sequence similarity with *Spiroplasma citri*, the causal agent of citrus stubborn disease. We reconstructed the spiroplasma genome and further investigated its presence in periwinkle and its occurrence in field samples by molecular tools. *S. citri* infection was also confirmed by electron microscopy observations of ultrathin sections of periwinkle leaf midribs. Spiroplasmas were observed both in sieve elements and in companion cells, while phytoplasmas, less numerous in appearance, were found only in the sieve elements. The spiralin gene was chosen as target for molecular detection, thus a new reverse primer SpiralinR2 was designed for use with the previously devised SpiralinF for the amplification of a 690 bp long fragment. PCR with SpiralinF/R2 primer pair was performed on a collection of DNAs extracted from chicory, other herbaceous plants and *N. fenestratus*, collected throughout an extensive field survey during 2011-2013 to determine epidemiological characteristics of ChiP phytoplasma. According to preliminary results *S. citri* was detected in *Chicorium intybus, Lotus carniculatus* and the vector *N. fenestratus.* The majority, but not all, of spiroplasma infected chicory plants tested positive for ChiP phytoplasma. For the first time *S. citri* has been reported in North-east Italy and a mixed infection with ChiP phytoplasma (16SrIX-C) has been demonstrated.

GENETIC DIVERSITY AND POPULATION STRUCTURE OF *FUSARIUM FUJIKUROI* CAUSING THE BAKANAE OF RICE IN ITALY.

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Bakanae of rice, caused by the ascomycete *Fusarium fujikuroi* Nirenberg, (teleomorph: *Gibberella fujikuroi* Sawada) is a disease of economic importance and, in the last decades, has emerged as a problem in Italian rice production areas. Few studies have been published on the genetic diversity of *F. fujikuroi* populations and no data are available on the genetic structure of Italian pathogen populations. In this work, 19 polymorphic microsatellites have been developed to characterize the genetic variation of 334 isolates of *F. fujikuroi* coming from eight populations representative of eight Italian rice-cultivated areas. A high degree of haplotype diversity has emerged and 107 unique haplotypes were scored. High values of expected heterozygosity (UHe) and Shannon's Information index showed a high genetic diversity in each population. AMOVA showed that the vast majority of variance (98%) was within populations. According to these data, F_{st} values showed a weak genetic differentiation among populations. NJ and STRUCTURE analyses did not find any association between the genetic dissimilarity or structure of the whole collection and the geographic origin, host tissue, cultivar and mating type. The presence of 1:1 ratio of mating type alleles in six out of eight populations tested suggests the potential for sexual reproduction in the field. However, the high fraction of clonality (43%) and the high level of Linkage Disequilibrium observed indicate that clonal propagation is predominant. All data suggest that the observed genetic variability was probably mediated by human activity and transmission by rice seeds.

THE COMPLEX *FUSARIUM* COMMUNITY ASSOCIATED TO HEAD BLIGHT OF MALTING BARLEY CAUSES MULTI-MYCOTOXIN GRAIN CONTAMINATIONS.

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A study was conducted in 2014 by collecting 52 malting barley kernel samples from different areas of the Umbria region (Central Italy). Samples were subjected to fungal isolations and the species belonging to the *Fusarium* genus were molecularly identified. *Fusarium graminearum* and *F. culmorum* strains were also subjected to chemotype determination. Malting barley kernels were analyzed by a LC-MS multi-mycotoxin method. Furthermore, strains of each *Fusarium* species were characterized for their *in vitro* mycotoxigenic profiles. The fungal community present in the samples was composed by the genera *Alternaria* (77%), *Fusarium* (27%), *Epicoccum* (19%), *Aspergillus* and *Penicillium* (2%). The *Fusarium* Head Blight complex was represented by *Fusarium poae* (37%), *F. avenaceum* (23%), *F. graminearum* (22%), *F. tricinctum* (7%), *F. incarnatum/equiseti* species complex (FIESC) (4%), *F. sporotrichioides* (3%), *F. acuminatum* (2%), *F. culmorum* (1%), and *F. sambucinum* (1%). Species co-occurrence was also determined. The most frequent *F. graminearum* chemotype was 15-acetyl-deoxynivalenol and the only *F. culmorum* strain was 3-acetyl-deoxynivalenol chemotype. The most detected mycotoxins in the kernels were enniatin B, enniatin B1, nivalenol (NIV), enniatin A1, beauvericin (BEA), T-2 toxin, enniatin A and HT-2 toxin. Masked mycotoxins (HT-2 and DON glucoside)

were detected for the first time in malting barley cultivated in Umbria. *Fusarium avenaceum* and *F. tricinctum* strains showed the ability to biosynthesize enniatins. *Fusarium sporotrichioides* strains were BEA and NIV producers like *F. poae*, which also biosynthesized low amounts of T-2 toxin. The FIESC strain produced high amounts of equisetin.

BROWN APICAL NECROSIS (BAN) OF WALNUT FRUIT: A CASE OF STUDY OF A COMPLEX FUNGAL DISEASE. **M. Scotton[1], E. Bortolin[2], A. Fiorin[3], A. Belisario[4].** *[1]Dipartimento di Agronomia Animali Alimenti Risorse naturali e Ambiente, Università di Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy. [2]Nogalba, Consorzio Produttori di Noce, 45010 Pettorazza, Rovigo, Italy. [3]Il Noceto, Società Consortile s.r.l., 31040 Chiarano, Treviso, Italy. [4]Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale (CREA-PAV), Via C.G. Bertero, 22 - 00156, Rome, Italy. E-mail: alessandra.belisario@crea.gov.it*

Brown apical necrosis (BAN) is a recently described disease affecting English (Persian) walnut fruit. BAN was recorded only in intensively managed walnut orchards and was found to be a disease complex mainly caused by *Fusarium* species, but also *Alternaria alternata* complex is involved. All fungi, associated with this disease are polyphagous and ubiquitous, not specific to walnut. Consequently, BAN occurrence is more strictly dependent on the interaction between pathological features and environmental conditions. Environmental variables influent in modifying disease severity were identified with regression analysis. The highest influence on severity of BAN fruit drop was due to maximum temperature and only subordinately, factors are associated with relative humidity. A significant evidence on BAN was also found for the angle of main wind direction versus tree row orientation, which is associated with the inner orchard ventilation affecting the temperature/humidity conditions. BAN symptoms were fully reproduced with an *in planta* artificial inoculation method set up specifically for this type of complex disease.

MORPHOLOGICAL, MOLECULAR AND BIOLOGICAL APPROACHES TO CHARACTERIZE CITRUS-ASSOCIATED *ALTERNARIA* SPECIES. **F. Garganese[1], L. Schena[2], M.I. Prigigallo[2], D. Spadaro[3], A. De Grassi[4], A. Ippolito[1], S.M. Sanzani[1].** *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari, Italia. [2]Dipartimento di Agraria, Università Mediterranea, Reggio Calabria, Italia. [3]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Grugliasco (TO), Italia. [4]Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi Aldo Moro, Bari, Italia. E-mail: simonamarianna.sanzani@uniba.it*

Alternaria brown spot is one of the most important diseases of tangerines and their hybrids. Recently, a disease outbreak in Southern Italy was recorded. A collection of 180 *Alternaria* spp. isolates from citrus fruit and leaves was gathered. Twenty representatives were selected and characterized along with reference strains of *Alternaria.* Morphological characteristics separated *Alternaria* isolates into six morphotypes referable to *A. alternata* (5) and *A. arborescens* (1). Phylogenetic analyses based on endopolygalacturonase (*endopg*) and internal transcribed spacer (ITS), confirmed this finding. A five-gene phylogeny including two anonymous genomic regions (OPA 1-3 and OPA 2-1), and the beta-tubulin gene (*β-tub*), produced a further clustering of *A. alternata* into four clades, being OPA 1-3 region more suitable to highlight intra-species variability. The isolates showed different levels of virulence on leaves and fruit. In particular, pathogenicity on fruit seemed to be correlated with tissue of isolation and clade. Numerous isolates produced the mycotoxins alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), and tenuazonic acid (TeA). TeA resulted the most abundant. A significant correlation between expression of phytotoxins gene *ACTT1* and pathogenicity on leaves was recorded; whereas, the expression of *pksJ/pksH*, biosynthetic genes of AOH/AME, was related to pathogenicity on fruit. The occurrence of *Alternaria* spp. on citrus fruit and their ability to produce toxins might represent a concern for producers and consumers.

CHARACTERIZATION AND VARIABILITY OF *LASIODIPLODIA* spp. ASSOCIATED WITH CROWN ROT DISEASE UNDER ORGANIC FARMING OF BANANAS IN THE DOMINICAN REPUBLIC. **M.A.M. Kamel[1,2], P. Cortesi[1], M. Saracchi[1].** *[1]Università degli Studi di Milano, Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Via Celoria 2 - 20133 Milano, Italy. [2]Plant Pathology Research Institute, Agricultural Research Center, 9 Gamaa St. - 2619 Giza, Egypt. E-mail: mohamed.kamel@unimi.it*

The genus *Lasiodiplodia* has a wide host range including bananas, and *L. theobromae* is one of the main pathogens involved in crown rot disease. This work aims to assess the presence of this pathogen under organic farming conditions, and to study the phenotypic characters and variability of all isolated strains. Approximately 750 ha of organic bananas (Musa AAA, Cavendish) and their packing station, located in the Dominican Republic were investigated. Morphology and cultural characters of 43 strains isolated from crown tissues were examined on different media under different conditions, and then identified based on ITS and β-tubulin gene nucleotide sequences. Pathogenicity of representative strains was evaluated on both fresh and 16 days-after-harvest green bananas. Strain variability was assessed producing fingerprints based on PCR products obtained using three primers designed on nucleotide sequences of mini- and microsatellites. Thirty-seven strains shared ≥ 99% sequence homology with *L. theobromae*, and six shared ≥ 99% sequence homology with *L. pseudotheobromae.* The latter was isolated only from flowers and crown parts in field and showed high virulence activity. The results obtained suggest complexity of *Lasiodiplodia* populations in the Dominican Republic and an increase of fruit susceptibility during conservation. This is the first report of *L. pseudotheobromae* associated with crown rot symptoms. Some of these pathogenic fungi were also found on decaying leaves of banana plants used as mulching materials in organic farming.

THE FIRST ARBOVIRUS FROM A PHYTOPLASMA INSECT VECTOR. **S. Abbà, L. Galetto, M. Vallino, M. Turina, C. Marzachì.** *IPSP-CNR, Istituto per la Protezione Sostenibile delle Piante, Strada delle Cacce 73 - 10135 Torino, Italy. E-mail: luciana.galetto@ipsp.cnr.it*

The leafhopper *Euscelidius variegatus* is an efficient natural vector of chrysanthemum yellows phytoplasma (CY) and a laboratory vector of Flavescence dorée phytoplasma (FD). A 10,616 nucleotide-long contig, highly similar to picorna-like viruses, was identified through RNA-seq in *E. variegatus* and named Euscelidius variegatus virus 1 (EVV-1). The virus presence was confirmed by Northern blot and its prevalence determined by RT-PCR: EVV-1 was detected in all the tested insects from an Italian laboratory colony, but absent in two other *E. variegatus* lab colonies from France and California. The whole viral genome was cloned in 11 overlapping fragments and completely re-sequenced by Sanger method. The amino acid

sequence, the shape and size of viral particles observed at TEM as well as the phylogenetic analyses suggest that EVV-1 is a new species of the genus *Iflavirus*. The presence of virus does not seem to be associated with any evident symptom on *E. variegatus* laboratory colony. The virus was detected both in phytoplasma-exposed and in non exposed insects, but the viral load measured by RT-qPCR in FD-infected samples was significantly lower than the one of phytoplasma-free insects. This result suggests possible interesting interactions among insects, endogenous bacteria and viruses. The availability of EVV-1 free *E. variegatus* colonies will allow to characterize viral replication and transmission mechanisms, and will offer the opportunity to produce infectious viral clones and manipulate the expression of endogenous genes by promoting virus-induced gene silencing.

BIOCONTROL COMPOUNDS FROM *TRAMETES VERSICOLOR* ARE NEW TOOLS TO CHALLENGE FUNGAL PATHOGENS. **A. Parroni[1], P. Cescutti[2], R. Rizzo[2], M. Scarpari[1], C. Pietricola[1], M. Reverberi[1], C. Fanelli[1].** *[1]Department of Environmental Biology, University of Rome "Sapienza", Rome, Italy [2]Department of Life Sciences, University of Trieste, Italy. E-mail: alessia.parroni@uniroma1.it*

Natural contaminants, such as the dangerous mycotoxins, synthesized in foods and feedstuffs by fungal contamination are mainly controlled by the use of chemicals Because of the importance to eat safe food and to live in safe environment for the human and animal health, European Community has banned since 2014 about 50% of chemicals used in agriculture, in fact they are responsible of heavy pollution of soils and plants. Due to these problems, the EC pushed the research to investigate for preventive and/or detoxification strategies using "green" approaches and eco-compatible tools. *Trametes versicolor* an edible and nontoxic basidiomycete, now considered "healing mushroom" for its bioactivity towards some human diseases, is considered an eco-compatible tool. In fact, biocontrol compounds from *T. versicolor*, such as polysaccharide Trametano®, oligosaccharides from its partial hydrolysis and protein fractions, are proposed to counteract mycotoxigenic fungal growth (*Aspergillus flavus*, *A. carbonarius*, *A. ochraceus*) and the mycotoxins synthesis (aflatoxin B1, AF; ochratoxin A, OTA). These compounds were assayed in presence of different fungi and the inhibiting effect on germination, fungal growth and toxin synthesis was evaluated. Further, some aspects of their mechanism of action were presented. The goal is to propose an environmental friendly tool for a significant control of mycotoxin production, in order to obtain feedstuffs and foods with a high standard of quality and safety. Results indicated that biocontrol agents from *T. versicolor* can be considered new eco-compatible tools for mycotoxins control, in line with EU directives.

EFFECTIVENESS OF PLANT ESSENTIAL OILS AGAINST ZUCCHINI POWDERY MILDEW. **L. Donnarumma[1], E. Sturchio[2], F. Milano[1], P. Boccia[2], M. Zanellato[2], T. Annesi[1].** *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale (CREA-PAV), via C. G. Bertero, 22 - 00156 Roma, Italy. [2]INAIL, Dipartimento Innovazioni tecnologiche e sicurezza degli impianti, prodotti e insediamenti antropici (DIT), via Roberto Ferruzzi 38/40 - 00143 Roma, Italy. E-mail:lucia.donnarumma@crea.gov.it*

Excessive use of agrochemicals in conventional crop management has caused serious environmental and health problems including loss of biodiversity and human disorders. Plant essential oils of three aromatic herb species of the family Lamiaceae (*Origanum* sp. and *Rosmarinus* sp.) and Myrtacee (*Syzygium aromaticum* L.) were investigated for antifungal effect against zucchini powdery mildew. Furthermore, phytotoxicity effects and germination index on *Vicia faba* roots exposed to different concentrations of three essential oils were evaluated. The assays showed a dose dependent effect to essential oils concentrations tested. Based on the findings in this research and previous studies, it was concluded that use of essential oils can effectively control infections by powdery mildew on zucchini crop.

BOOSTING SUSTAINABLE BIOENERGY PRODUCTION BY MEANS OF *TRICHODERMA*. **G. Manganiello[1], A. Sacco[1], A. Pascale[1], F. Vinale[2], M.R. Ercolano[1], J.P. Vogel[3], I.V. Grigoriev[3], H. Shanakhat[1], M. Lorito[1,2], S.L. Woo[1,2].** *[1]Dipartimento di Agraria, Università degli Studi di Napoli 'Federico II', Via Università 100 - 80055, Portici, Italy. [2]Istituto per la Protezione Sostenibile delle Piante (IPSP-CNR), Via Università 133 - 80055 Portici (NA), Italy; [3]Joint Genome Institute (JGI)-United States Department of Energy (DOE), 2800 Mitchell Drive, Walnut Creek - 94598 CA, USA. Email: gelsomina.manganiello@hotmail.it*

Numerous *Trichoderma* strains can stimulate plant growth and increase yield in many crops. However, the mechanism(s) why the growth promotion varies between plant species and why only some fungal beneficial strains promote growth is unknown. *Brachypodium distachyon* is a model plant for genetic studies of monocots (i.e. cereals) and switch-grass (*Panicum virgatum*) used for biofuel production. Our goal is to obtain sequence information useful for scientific and industrial interests of renewable and sustainable energy resources from plant and microbe interactions. Ten *Trichoderma* isolates (*T. harzianum, T. virens, T. atroviride, T. viride*) were tested in a *B. distachyon-Trichoderma* experimental system for their ability to promote plant growth. Most *Trichoderma* strains were beneficial even at the earliest stage, increasing seed germination. The growth promotion extended to later phases of development with an increase in above- and below-ground biomass up to fourfold. RNA-sequencing of plants treated with different *Trichoderma* strains, and genome sequencing of two beneficial fungi are underway at the Joint Genome Institute. Fungal sequencing will also permit the identification of secondary metabolism gene families involved in disease suppression and plant growth promotion. We focused on a polyketide synthase (PKS) gene family, an important enzymatic machinery for polyketide-metabolite production in fungi. Bioinformatic analysis of nine *Trichoderma* genomes demonstrated that *T. harzianum* contains the greatest number of PKS clusters, making them an attractive target for gene synthesis and heterologous expression, in order to assess effects of secondary metabolites on plant. This is a project in the JGI Community Science Program 2016.

BIOSAFETY AND ENVIRONMENTAL CONCERNS RELATED TO THE USE OF BACTERIAL BIOCONTROL AGENTS IN CROP PROTECTION. **E. Stefani.** *Università di Modena e Reggio Emilia, Dipartimento di Scienze della Vita, via Amendola 2 - 42122 Reggio Emilia, Italy. E-mail: emilio.stefani@unimore.it*

The use of microorganisms in agriculture for the biological control of plant pathogens is increasing, due to a rising awareness among citizens for sustainability of agricultural production systems, coupled with a deeper knowledge of the relationships among the microbial communities in agricultural environments. In the EU, 54 microorganisms are currently approved for a possible use in plant protection and many more are under approval/authorization. Regulating the use of microbials in agriculture is a challenge: several

countries regulate them as chemical pesticides, without taking into consideration their biological properties. Challenges might refer to: i) identification, characterization and biological properties; ii) toxicology and environmental risks; iii) residues of microorganisms and their metabolites on food crops; iv) level of efficacy. Microorganisms may pose risks to the environment, as they are able to survive, proliferate and disseminate. Registration data requirements for biocontrol agents are currently concerning their toxicity, pathogenicity and/or infectivity. Environmental safety is assessed according a case-by-case evaluation. Mensik & Scheepmaker (2007) proposed a procedure, adopted by the OECD. The decision scheme starts with data on microbial characterization, followed by assessment on contamination/exposure in soil/surface water, together with fate and behavior of inoculum. Environmental toxicology is done on terrestrial and aquatic organisms, checking any adverse effects and, eventually, mitigation options. The environmental risk assessment terminates in "RISK ACCEPTABLE" or "RISK NOT ACCEPTABLE". The regulatory authority may, nonetheless, consider those biocontrol agents, whose risk is assessed as "not acceptable", if mitigation of adverse effects are possible or their use will replace a toxic pesticide.

DESIGN OF A PROTOTYPE FORMULATION FOR THE IMPROVEMENT OF LEAF COLONIZATION AND PLANT PROTECTION EFFICACY OF THE BIOCONTROL AGENT *LYSOBACTER CAPSICI* AZ78 UNDER FIELD CONDITIONS. G. Puopolo, G. Segarra, E. Porcel-Rodríguez, S. Tomada, O. Giovannini, I. Pertot. *Department of Sustainable Agro-Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via Edmund Mach, 1 - 38010 San Michele all'Adige (TN), Italy. E-mail: gerardo.puopolo@fmach.it*

When applied in the field, bacterial biocontrol agents must withstand environmental stressors such as desiccation, rain wash-off, and UV light. Recently, *Lysobacter capsici* AZ78 (AZ78) was selected for its efficacy in controlling *Plasmopara viticola* under greenhouse conditions. Since little is known about how *Lysobacter* spp. may respond to environmental stressors, the aim of this work was to design an effective formulation of AZ78, which can improve its plant protection efficacy and its persistence in the vineyard phyllosphere. Fermentation conditions were optimized to achieve a harvest of 10^{10} AZ78 cells/ml after 24 h and the shelf-life at 4°C indicated only a decrease of an order of magnitude after one year. Polyethyleneglycol, corn steep liquor and lignosulfonate were selected for the protection of AZ78 cells against desiccation, wash-off and UV light and were combined to implement a liquid formulation. The efficacy of AZ78 formulated cells against *P. viticola* on grapevine under field conditions was higher than the unformulated ones. To assess the fate in the environment, an AZ78 specific qPCR protocol was developed. Results showed that the negative effect played by solar radiation, rainfall, unfavourable temperature and relative humidity on the unformulated AZ78 cells was mitigated by the formulation. In conclusion, a first prototype formulation of AZ78 made this biocontrol agent more efficient in colonizing grapevine leaves and, consequently, controlling *P. viticola* under field conditions. The protocol adopted to design the AZ78 formulation can be replicated on other bacterial biocontrol agents fostering the development of new biopesticides.

ACTINOBACTERIA: ISOLATION, IDENTIFICATION, CHARACTERIZATION AND PRELIMINARY EXPERIMENTS FOR THEIR POSSIBLE USE AGAINST *CLAVIBACTER MICHIGANENSIS* subsp. *MICHIGANENSIS*. M. Ferrari[w], O. Kaewkla[2], C. Franco[2], D. Giovanardi[1], E. Stefani[1]. *[1]Università di Modena e Reggio Emilia, Via Università 4 - 41121 Modena, Italy. [2]Flinders University of South Australia, Sturt Road, Bedford Park 5042 - South Australia. E-mail: mferrari1984@gmail.com*

In recent years, new actinobacteria species have been isolated as endophytes from plants and are sought after for the role of bio-control inoculants for sustainable agriculture. In particular, our studies focus on the isolation of some endophytic actinobacteria from tomato healthy plants, with a potential antagonistic activity against the causal agent of bacterial canker of tomato: *Clavibacter michiganensis* subsp. *michiganensis* (Cmm). Cmm is a plant-pathogenic bacterium belonging to the order of Actinomycetales. It infects tomato plants, spreads through the xylem and causes bacterial wilt and canker which can be considered to be the most important bacterial disease of tomato causing substantial economic losses worldwide.

A total of 50 endophytic actinobacteria strains were isolated from tomato healthy plants collected from Adelaide Province.

INDUCED RESISTANCE AS A TOOL THAT CAN CONTRIBUTE TO SUSTAINABLE MANAGEMENT OF PRE- AND POSTHARVEST DISEASES OF PLANTS. G. Romanazzi, L. Landi, S. Murolo, E. Feliziani, V. Mancini, A. Servili, R. Foglia, S. Sabovic, M. Magini, S. Sanzani. *Marche Polytechnic University, Department of Agricultural, Food, and Environmental Sciences, Ancona, Italia. E-mail: g.romanazzi@univpm.it*

Plants are exposed to a list of pathogens, which can cause severe diseases both in the field and after harvest. Currently the control of plant diseases is based on the use of synthetic fungicides. However, the appearance of pathogen isolates resistant to one or more classes of fungicides, and the request by consumers and/or retailers of fresh fruits and vegetables with a reduced number and amount of residues led to the need to limit the application of synthetic fungicides. This trend is sided by the concurrent increase of organic produce, in which application of synthetic fungicides is not allowed. One of the possible responses to some of these issues is based on control strategies that do not target only the pathogen, but also increase plant defenses. The resistance inducers, also known as elicitors or biostimulants, are treatments of biotic or abiotic nature, which can trigger the natural defenses of the plant, activating mechanisms leading to the increase of compounds with antimicrobial activity. Such compounds can slow down and in some cases even control, with an effectiveness comparable to that of synthetic fungicides, plant diseases. Indeed, compared to synthetic fungicides, resistance inducers can have broader spectrum and long lasting effects, fitting in strategies aiming to optimize the plant protection as planned by Directive 128/2009 on sustainable use of fungicides, implemented through the following corresponding National Action Plans. Some application of resistance inducers in the control of preharvest and postharvest diseases will be reviewed.

ALTERNATIVE MEANS FOR CONTROLLING POSTHARVEST DISEASES OF STORED FRUITS. S.M. Sanzani[1], A. Ippolito[1]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: simonamarianna.sanzani@uniba.it*

Stored fruits suffer huge losses because of rots caused by fungi. Usually synthetic fungicides control these diseases, but technical and sociological issues related to their use are pushing the search for alternatives. Several natural compounds were tested against major postharvest diseases. For example, the phenolic compounds quercetin, scopoletin, and scoparone despite a little (≤13%) control of *Penicillium digitatum* growth, proved to be effective against green

mould of oranges. In fact, at 8 dpi, they significantly reduced disease incidence and severity by 40-69 and 70-85%, respectively, as compared to the control. Similarly, the protein hydrolysates SoyA and Cas did not affect *in vitro Botrytis cinerea* growth, but controlled postharvest grey mould on table grapes. A dose effect was observed, being particularly active at 0.8 g/l. When the hydrolysates were applied in the field during growth phase of wine grapes cv. Corvina, significantly reduced grey mould incidence by 65 and 92%, as compared to control, respectively; whereas, a combination of pre- and postharvest application reduced storage rots by 40-56%. Finally, calcium chloride and lemongrass oil proved to control *Rhizopus stolonifer* growth *in vitro*, reaching a complete inhibition at 20 g/l and 1.5 ml/l, respectively. Both substances induced ultra-structure modifications in *R. stolonifer*. In *in vivo* tests on peaches using lemongrass oil at 1.5 ml/l, a 70% reduction of both rot incidence and severity was achieved; whereas, in presence of 1.5 g/l $CaCl_2$, a reduction of 30 and 59% for incidence and severity, respectively, was observed. Combined treatments gave a synergistic effect.

SURVIVAL VARIABILITY OF *CALONECTRIA* spp. EXPOSED TO LABEL AND SUB-LABEL RATES OF FUMIGANTS APPLIED UNDER VIF AND TIF MULCH FILMS. **D. Aiello[1], R.F. Alfenas[2], V. Guarnaccia[3], A. Vitale[1], A.C. Alfenas[4], G. Polizzi[1].** *[1]Dipartimento di Agricoltura, Alimentazione e Ambiente, University of Catania, Via S. Sofia 100 - 95123 Catania, Italy. [2]Instituto de Ciências Agrárias e Ambientais, Universidade Federal do Mato Grosso, Sinop, Mato Grosso, Brazil. [3]CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8 - 3584 CT Utrecht, The Netherlands. [4]Departamento de Fitopatologia, Universidade Federal de Viçosa UFV, Viçosa, Minas Gerais, Brazil. E-mail: dalia.aiello@unict.it*

Calonectria species are pathogens distributed worldwide and cause severe diseases on important horticultural and forest crops. Twenty-eight isolates belonging to 19 *Calonectria* species (17 alien species of Europe and two species widespread in the Mediterranean basin) identified by multi-gene sequence analysis were used in this study. The effects of label and sub-label rates of dazomet (Basamid Granulat 99%) and metham-sodium (Divapan 42.8%) on survival of microsclerotia were evaluated. Two experiments in plastic containers for the alien *Calonectria* species and three trials in open field for the spread species were performed. Basamid was used at 100, 160, 200, 400, and 500 kg/ha while Divapan rates were 250, 350, 400, 700, and 1000 l/ha. The fumigants were applied under virtually impermeable film (VIF, Ecobrom®) and totally impermeable film (TIF, Kuraray-Eval™). The containers and the plots were artificially infested with microsclerotia of all *Calonectria* species produced on carnation leaf agar medium. All treatments were replicated three times, in a completely randomized design. The survival of each *Calonectria* isolate was evaluated after 21 days. Basamid applied at 200, 400 and 500 kg/ha and Divapan at 400, 700 and 1000 l/ha were totally effective for microsclerotia suppression of 16 *Calonectria* species while showed partial efficacy against *C. hongkongensis*, *C. naviculata* and *C. sulawesiensis*. At all lower rates, Basamid was more effective followed by Divapan. Most isolates were totally suppressed when Basamid was applied under TIF film. Moreover, variability in levels of inocula eradication was related to the soil temperature, film and isolate tested.

COMBINING CHEMICAL AND BIOLOGICAL MEANS FOR THE CONTROL OF CALONECTRIA DISEASES IN ORNAMENTAL PLANTS. **A. Vitale, A. Cinquerrui, D. Aiello, G. Cirvilleri, G. Polizzi.** *Università degli Studi di Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente, Via S. Sofia 100 - 95123 Catania, Italy. E-mail: alevital@unict.it*

Chemical control represents the main effective strategy to manage Calonectria diseases in ornamental plants. The occurrence of fungicide-resistant strains and the European Directive on "Sustainable Use of Pesticides" forced the technicians to setting-up effective IPM strategies to control Calonectria infections. Three-years period of nursery experimental trials was carried out to detect the best combination between fungicides and BCAs to control both leaf spot caused by six *Calonectria* species (*C. mexicana, C. morganii, C. pauciramosa, C. polizzii, C. pseudomexicana, C. tunisiana*) on bottlebrush and metrosideros, and stem rot caused by *C. morganii* on Florida hopbush. The cyprodinil + fludioxonil mixture combined with bioformulates containing *Bacillus* spp., *Trichoderma* spp. and *Streptomyces griseoviridis* provide the best performances in reducing leaf spot and stem rot caused by *Calonectria* species. Data also show as boscalid + pyraclostrobin and cyprodinil + fludioxonil mixtures could be suggested for large-scale application in reducing Calonectria infections, since they were able to effectively manage leaf and stem infections. Performances of BCAs alone were instead lesser than remaining treatments since they were depending on the host, pathogen species, symptomatology and nursery conditions involved. Thus, their application may be encouraged only in combination with fungicide mixtures. Comprehensively, this study allows to acquire useful information for Calonectria diseases management in nurseries.

EFFECTIVE DIFFERENCES OF CARBOXYLIC ACID AMIDES FUNGICIDES TOWARDS *PLASMOPARA VITICOLA* POPULATIONS: *IN VIVO* TESTS AND MOLECULAR STUDIES ON PVCesA3 GENE. **I.M. Nanni, M. Collina.** *Università degli Studi di Bologna, Dipartimento Scienze Agrarie (DipSA), Bologna, Italia. E-mail: irenemaja.nanni2@unibo.it*

Plasmopara viticola is controlled by fungicides with different modes of action, including carboxylic acid amides (CAAs). Dimethomorph was the first CAA introduced in 1988, followed by iprovalicarb, flumorph, benthiavalicarb, mandipropamid, valifenalate and latest pyrimorph in 2010. The mode of action of CAAs is linked to the inhibition of cellulose synthesis in the Oomycete plant pathogens. The mutations conferring CAA resistance in *Plasmopara viticola* located on the CesA3 gene are G1105S and G1105V. The aim of this work was to evaluate the activity of dimethomorph and mandipropamid on *P. viticola* populations. Bioassays on leaf discs, and on seedlings were carried out. In leaf discs assay, CAAs tested on Italian populations showed different level of activity and in particular dimethomorph showed lower levels of EC_{95} and this behavior was also confirmed from seedling tests.

All CAA resistant populations carried the G1105S/V mutations, which were detected by RFLP-PCR and qPCR. In order to gain a better understanding of the different behavior and to define the possible different resistant mechanism, molecular modelling and docking studies are ongoing.

INTEGRATED AND INNOVATIVE KEY ACTIONS FOR MYCOTOXIN MANAGEMENT IN THE FOOD AND FEED CHAIN (HORIZON2020 PROJECT MYCOKEY). **A. Moretti, A. Logrieco.** *Istituto di Scienze delle ProduzioniAlimentari (ISPA-CNR), Via Amendola 122/O - 70126 Bari, Italy. E-mail: antonio.moretti@ispa.cnr.it*

Contamination of cereals by toxigenic fungi is a global concern. MycoKey project aims to generate innovative and integrated concepts for management of mycotoxins along food and feed chain. Multi-disciplinary consortium composed of scientific, industrial and association partners (32) from Europe, China, Nigeria and

Argentina will conduct the 4-year programme in a framework of international networks. The project focuses on aflatoxin, ochratoxin A, deoxynivalenol, fumonisin and zearalenone challenges in important food crops such as mainly maize, wheat and barley, and, at a lesser extent grape. These crops represent nearly 60% of global cereal production. This project aims to: 1) improve global mycotoxin knowledge and communication 2) select and develop methodologies for monitoring of toxigenic fungi and mycotoxins 3) assess mitigation strategies in order to avoid fungal contamination in the field and to intervene toxin production in high risk years. This presentation will provide insights to new MycoKey approaches with special emphasis on the field control strategy. Mycotoxins represent an unavoidable risk. As they cannot be avoided, they need to be managed.