1. IDENTIFICATION OF PUTATIVE EFFECTOR GENES OF '*CANDIDATUS* PHYTOPLASMA AURANTIFOLIA' IN INFECTED LIME AND PRELIMINARY SUBCELLULAR LOCALIZATION OF ONE OF THEM IN *NICOTIANA BENTHAMIANA*. **A. Ananbestani[1], M. Morano[1], S. Palmano[1], A. Carra[1], M. Vallino[1], C. Marzachi[1].** *[1]CNR, Istituto per la Protezione Sostenibile delle Piante, Torino, Italy. E-mail: ameneh.anabestani@ipsp.cnr.it*

Phytoplasmas are phytopathogenic bacteria that induce several specific symptoms in the infected plants through secretion of effector proteins that induce changes in the architecture and defense response. Witches's-broom disease (WBDL) is an important disease of lime in Southern Iran. The disease is caused by '*Candidatus* Phytoplasma aurantifolia', for which full genome sequence is not available. To identify putative WDBL effector genes, the fully sequenced genome of the close relative peanut witches'-broom phytoplasma (PnWB) was mined using an appropriate pipeline. Primers were designed according to the retrieved genes, and total DNA of WBDL-infected lime was amplified with the PnWB-specific primer pairs. Eight putative effector genes were identified, and their similarity to PnWB homologs ranged from 50% to 100%. *In vivo* transcription of these putative effectors in infected limes was monitored. Bands of expected sizes were detected for five putative effector genes but not for the others, suggesting that only some of these putative effector genes may be active, at least under the experimental conditions of this study. Two of these putative effector genes (WBDLEff64 and WBDLEff99), were expressed at high levels during '*Ca*. P. aurantifolia' infection of lime, and both were selected for determination of their subcellular localization through a standard *Agrobacterium*-mediated transient transformation. The infiltrated leaves, harvested 72 h after infiltration, were examined by confocal laser-scanning microscopy (CLSM). Preliminary CLSM showed that GFP-WBDLEff64 was excluded from the cell nucleus and predominantly localized within the cytoplasm, suggesting a role for this protein at this cellular compartment.

2. PHYTOPATHOLOGICAL PROBLEMS IN POPLAR SHORT ROTATIONS FOR BIOENERGY PRODUCTION. **N. Anselmi[1], A. Giorcelli[2].** *[1]Dipartimento per la Innovazione nei sistemi biologici, agroalimentari e forestali (DIBAF),University of Tuscia, Via San Camillo de Lellis s.n.c. - 01100 Viterbo, Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Unità di Ricerca per le produzioni legnose fuori foresta, Strada Frassineto 35 - 15033 Casale Monferrato (AL), Italy. E-mail: anselmi@unitus.it*

Poplar short rotation, with high density of plants, are a very good opportunity for production of bioenergy. However, they present several characteristics that may induce disease attacks: high moisture and prolonged periods of wetness of the leaves in connection with limited gas exchanges and lack of light; constant sprouting of green tissue during the most part of the growing season; high competition for water and nutrient supply; presence of large wounds and cut surfaces due to repeated coppicing. Limited air exchange and weak light may increase the attacks by sooty mould fungi, associated with leaf sucker insects' attacks. The high moisture level under the close canopy is conducive to attacks by *Marssonina* spp. and *Melampsora* spp., which cause heavy losses of dry matter and, at times, limiting the survival of the stumps; they represent a constraint in genetic renewal. The weakening of plants due to water stress is a triggering factor for bark necrosis by *Discosporium populeum, Citospora* spp. and by *Phomopsis* spp. Fluctuations of the water table are putative factors for root rots by *Rosellinia necatrix* and *Armillaria mellea* which, especially after several coppicings, may extend throughout all stumps and remarkably increase their mortality. Presence of large wounds and cut surfaces due to repeated coppicing predispose the attacks of various root rots agents inside stumps, as *Auricularia spp*., *Chondrostereum purpureum*, *Stereum* spp., *Pholiota* spp., *Collibia velutipes*, which progressively induce declining and early mortality.

3. DIFFERENT REGULATION BETWEEN *VITIS VINIFERA* AND *VITIS RIPARIA* OF AN *ATL* GENE INVOLVED IN RESISTANCE TO DOWNY MILDEW: PROMOTER CHARACTERIZATION IN *ARABIDOPSIS THALIANA*. **P. Ariani, A. Regaiolo, D. Danzi, A. Lovato, E. Vandelle, A. Polverari.** *Università degli Studi di Verona, Dipartimento di Biotecnologie, Ca' Vignal 1, Strada Le Grazie 15 - 37134 Verona, Italy. E-mail: annalisa.polverari@univr.it*

In a previous microarray analysis, 10 E3 ubiquitin ligase ATL-encoding genes resulted strongly upregulated in the resistant *Vitis riparia* upon infection with *Plasmopara viticola*, while not modulated in the susceptible *Vitis vinifera*. One, in particular, showing high homology with *ATL2* from *A. thaliana*, known to be responsive to elicitors and hormones, displayed the highest expression 12 hpi specifically in resistant plants. To elucidate whether the different regulation of this *ATL* gene after infection could be due to specific features of the promoters, the regulative regions from both grapevine species were cloned and sequenced. The resulting sequences were then analysed to recognize any possible difference in terms of promoter structure or *cis*-acting elements composition. Despite the high level of similarity between the two sequences, some transcription factor-binding sites, likely related to disease resistance, were specifically predicted in the *V. riparia* promoter sequence, which also carries two putative TATA-boxes compared to the single one predicted in the sequence of *V. vinifera*. In order to functionally characterize the responsiveness of both promoters, they were then cloned into the pKGWFS7 binary vector to control the expression of *GUS* and *GFP* reporter genes. These two vectors were used to transform the model plant *Arabidopsis thaliana* and the GUS activity of the transgenic plants is currently analysed at different developmental stages and in response to different abiotic and biotic stresses.

4. UNFOLDOME VARIATION UPON PLANT-PATHOGEN INTERACTIONS: STRAWBERRY INFECTION BY *COLLETOTRICHUM ACUTATUM*. **E. Baraldi[1], E. Coller[2], L. Zoli[1], A. Cestaro[2], S.C.E. Tosatto[3], B. Zambelli[4].** *[1]Department of Agricultural Sciences (DIPSA), University of Bologna, Viale Fanin 46 - 40127, Italy. [2]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), via E. Mach 1 - 38010 San Michele all'Adige, Italy. [3]Department of Biomedical Sciences, University of Padova, Padua, Italy. [4]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy. E-mail: elena.baraldi@unibo.it*

Intrinsically disordered proteins IDPs lack secondary and/or tertiary structure under physiological conditions. In eukaryotes, these proteins play crucial roles in all molecular mechanisms regulating response to environmental stresses. In plants, different IDPs involved in stress response have been identified and characterized. Nevertheless, a comprehensive evaluation of the involvement of IDPs in abiotic or biotic stresses responses is not available so far. In the present work the transcriptome dataset of strawberry (*Fragaria×ananassa*) fruits interacting with the fungal pathogen *Colletotrichum acutatum* was actualized onto the woodland strawberry (*Fragaria vesca*) genome. The obtained cDNA sequences were translated into protein sequences and subjected to disorder analysis. The results provide a first estimation of the abundance of IDPs

associated to plant infection and show that the proteome activated in the strawberry red fruit colonized by the fungal pathogen is remarkably depleted in protein disorder. On the other hand, in the resistant white fruit, no significant disorder reduction is observed in the proteins expressed in response to fungal infection. Four representative proteins involved in defence response, predicted as mainly disordered and never experimentally characterized before, *Fv*SMP, *Fv*PRKRIP, *Fv*PCD-4 and *Fv*FAM32A-like, were isolated, and the absence of structure was validated using circular dichroism and differential scanning fluorimetry. Their quaternary structure was also established using light scattering. The results are discussed considering the role of protein disorder in plant defence.

5. IN SEARCH OF THE UNDISCOVERED MECHANISMS OF *COLLETOTRICHUM LUPINI* HOST SPECIALIZATION: ATM-MEDIATED GFP TRANSFORMATION OF THE PATHOGEN. R. Baroncelli[1], S. Sarrocco[2], N. Bonadies[2], G. Vannacci[2]. *[1]University of Western Brittany LUBEM Technopôle 29280 Plouzané-Brest, France. [2]Università degli Studi di Pisa, Dipartimento di Scienze Agrarie, Alimentari e Agro-Ambientali, Plant Pathology & Mycology lab, Via del Borghetto 80 - 56124 Pisa, Italy. E-mail: sabrina.sarrocco@unipi.it*

Since its first diagnose on lupin in 1939, anthracnose, caused by *Colletotrichum lupini*, has become a severe disease of lupins worldwide, causing meaningful yield losses as high as 100% and becoming a main limiting factor for production. Several morphological, cultural and molecular data confirm *C. lupini* as part of the *Colletotrichum acutatum* species complex. Although the *C. acutatum* species are considered polyphagous, *C. lupini* has been shown to have a strict host specialization that makes it a fascinating model for evolutionary and biomolecular studies. In the present work a *C. lupini* isolate (RB221) was submitted, for the first time, to AMT-mediated transformation in order to obtain a GFP marked isolate to be used as a tool to better understand the interaction at molecular level and the disease cycle of this pathogen on lupin. Five stable transformants have been characterized by morphological and physiological approaches and a phenotypic microarray test (Biolog) was performed in order to establish metabolic differences between the GFP marked strains and the wt isolate. In addition, the number and the localization of the gfp gene insertion is actually under evaluation by sequencing the entire genomes of the 5 transformed strains and comparing them with that of the wt. The availably of the *C. lupini* RB221 gfp-marked strain will allow to microscopically follow, step by step, the infection and colonization of the host by this emibiotrophic pathogen. This represents the first step for further analysis such as a transcriptomic evaluation of the *C. lupini*/lupin interaction.

6. AN ITALIAN POPULATION OF *ZYMOSEPTORIA TRITICI*: PATHOGENICITY AND STROBILURINS SENSITIVITY. G. Battistini, A. Ciriani, F. Cavina, P. Nipoti, A. Prodi, M. Collina. *Università di Bologna (Dipsa), Viale Fanin 44 - 40127 Bologna, Italy. E-mail: antonio.prodi@unibo.it*

Mycosphaerella graminicola (anamorph: *Zymoseptoria tritici*) is the causal agent of leaf blotch, the most important foliar disease of wheat in northern and Central Europe. In Italy only during the last few years, the incidence of the disease has been increased. During 2015 a *Z. tritici* population was collected from different cultivar of bread and durum wheat cultivated in experimental plots and commercial fields of Emilia-Romagna region (North Italy). The aim of the study was to test the pathogenicity of each *Z. tritici* strains on both (bread and durum) wheat species in greenhouse. In fact, some researchers observed that the majority of strains isolated from durum wheat are lowly virulent on bread wheat cultivars, whereas the majority of strains isolated from bread wheat are lowly virulent on durum wheat. Another aim was to test the sensitivity of *Z. tritici* population to fungicides as strobilurins by *in vitro* assays. The preliminary data about pathogenicity confirm the cultivar specificity in the infection process. Further data are required in order to confirm the hypothesis in an Italian *Z. tritici* population too. The results about sensitivity test showed an EC_{50} value of the wild types ranged from 0.1 to 2.88 mg/l of azoxystrobin, while the isolates collected from experimental plots and commercial fields showed EC_{50} value ranged from 0.01 to 5.1 mg/l. These first results show a slight decrease of sensitivity of *Z. tritici* isolates collected from Emilia-Romagna region.

7. INHIBITION OF GROWTH OF *FUSARIUM LANGSETHIAE* AND PRODUCTION OF T2 H-T2 IN DURUM WHEAT THROUGH THE USE OF BIOACTIVE COMPOUNDS EXTRACTED FROM THE BASIDIOMYCETE *TRAMETES VERSICOLOR*. A. Bellabarba[1], A. Parroni[1], M. Scarpari[1], C. Pietricola[1], A. Infantino[2], M. Aragona[2], M. Reverberi[1], C. Fanelli[1]. *[1]Environmental Biology Department, Università Sapienza,P.le Aldo Moro 5, I- 00185, Roma Italy. [2]Consiglio per la Ricerca e l'analisi dell'economia agraria (CREA-PAV), via C.G. Bertero, 22 - 00156 Rome, Italy. E-mail: agnesebellabarba@gmail.com*

Mycotoxins are a diverse group of bioactive compounds comprised of hundreds of secondary metabolic products from various fungal species, which are toxic and carcinogenic for humans and animals. Reducing the level of infection of cereal caused by *Fusarium* and thus associated mycotoxin accumulation in grains is of high priority in order to secure agronomic performance, and food and feed safety. *Fusarium langsethiae*, formally described as a new species over a decade ago, has been identified as the main producer of HT-2 and T-2 toxins in Europe in small cereal grains. The aim of the study is to investigate the effect of some bioactive compounds produced into the culture filtrates (CF) of the basidiomycete *Trametes versicolor* on *F. langsethiae* growth and type-A trichothecenes synthesis. *In vitro* experiments showed a strong inhibition of *F. langsethiae* growth, probably due to glycoprotein component of the culture filtrate. In particular, we focused on glycoprotein component of the culture filtrate to identify, *via* protein fractionation by HPLC, which protein fraction was more involved in the inhibition of fungal growth. *In planta* and in *semi-vivo* experiments were conducted by treating both wheat plants and autoclaved wheat kernels with different concentrations of CF and subsequently inoculated with *F. langsethiae*. Liquid chromatography-tandem mass spectrometry (LC-MS / MS) was used for the quantification of mycotoxins. As a result, a significant reduction in the production of H-T2 and T-2 mycotoxins in both experiments was observed. In conclusion, the compounds used could represent an innovative tool to reduce the amount of H-T2 and T-2 mycotoxins in foodstuffs and food.

8. WEB BLIGHT CAUSED BY *RHIZOCTONIA SOLANI* AG-1 RECENTLY OBSERVED IN ITALY ON NEW HOSTS BELONGING TO *LABIATAE*. D. Bertetti, P. Pensa, M.L. Gullino, A. Garibaldi. *AGROINNOVA, Centre of Competence for Agro-Environmental Innovation, University of Torino, Largo Braccini 2 - 10095, Grugliasco, (TO), Italy. E-mail: marialodovica.gullino@unito.it*

New web blights were recently observed in farms located near Albenga (Savona province, northern Italy) on new hosts belonging to the Labiatae family used as ornamentals and/or aromatics: lavender (*Lavandula officinalis*), butterfly lavender (*L. stoechas*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and winter savory (*Satureja montana* "Repandens"). Diseases appeared

on young rooted-cuttings grown in trays or on potted plants. Main symptoms consisted in water-soaked lesions of leaves and stems that started from the base of affected plants. As the diseases progressed, blighted tissues turned brown and withered. Finally, affected plants died. *Rhizoctonia solani* was easily isolated from affected tissues of all the hosts. The pathogen was identified by morphological characteristics of the isolates and by the ITS (Internal Transcribed Spacer) analysis. In pathogenicity tests, Koch's postulates were fulfilled for each new host. All the isolates of *R. solani* anastomosed with *R. solani* isolate AG-1 (ATCC 58946). Mycelium and sclerotia were typical of subgroup IA in the case of *R. solani* from rosemary and winter savory, whereas were typical of subgroup IB for *R. solani* isolated from lavender, butterfly lavender and oregano. The pathogen could cause important economic losses, particularly on *R. officinalis* and *L. stoechas* that are increasing crops in Italy. The possibility that each strain of *R. solani* from *Labiatae* can infect the other new hosts should be investigated.

9. VARIABILITY OF THE *WATERMELON MOSAIC VIRUS* GENES INVOLVED IN APHID TRANSMISSION: PRELIMINARY DATA IN ITALY. S. Bertin, A. Manglli, L. Tomassoli. *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: sabrina.bertin@crea.gov.it*

Watermelon mosaic virus (WMV) is a member of the genus *Potyvirus* and represents a serious threat for cucurbits in the Mediterranean basin. Three WMV molecular groups (G1-3) have been characterized so far, based on the coat protein (CP) gene sequence. The most recent G3 isolate was introduced in Europe since early 2000 and was responsible for severe symptoms on zucchini squash and melon plants. WMV-positive samples of zucchini and melon collected from different areas in Italy between 2002 and 2015 were analyzed at two different genomic regions: a fragment placed between the nuclear inclusion b and the CP genes, and the whole gene coding for the helper component-protease (HC-Pro). These regions are known to be variable within WMV populations and include recombination breakpoints. Both classic G1 and emerging G3 isolates were found in the Italian samples, and the two strains co-existed in few plants. The distribution of G1 and G3 isolates was not correlated with the geographical origin and/or host plant, but only with a temporal replacement of the classic strain by the emerging one. High levels of genetic variability were observed at both CP and HC-Pro N-terminal regions, which contain the DAG and KLSC motifs involved in aphid transmission. Even if these functional domains were conserved between G1 and G3, variations at adjacent amino acid positions may affect the strength of aphid-virus interaction and the transmission efficiency. These preliminary data provide a picture of WMV genetic evolution in Italy and open new questions about the transmissibility of the different isolates.

10. EVALUATION OF SSRs AND SNPs AS APPROPRIATE MARKERS TO CHARACTERIZE *PHYTOPHTHORA NICOTIANAE* POPULATIONS. A. Biasi[1], F.N. Martin[2], A. Abdelfattah[1], S.O. Cacciola[3], L. Schena[1]. *[1]Dipartimento di Agraria, Università degli Studi Mediterranea, Località Feo di Vito, 89124 Reggio Calabria, Italy. [2]United States Department of Agriculture - Agricultural Research Service, 1636 East Alisal Street, Salinas - 93905 CA. USA. [3]Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi, Via S. Sofia 100 - 95123 Catania, Italy. E-mail: antonio.biasi@unirc.it*

Mitochondrial Single Nucleotide Polymorphisms (mtSNPs) and genomic Simple Sequence Repeats (SSRs) were utilized to evaluate the diversity and the genetic structure of a collection of isolates from the cosmopolitan plant pathogen *Phytophthora nicotianae*. One hundred and eight isolates of worldwide origin from citrus, tobacco and several potted ornamental species, were analysed using 9 hypervariable genomic SSR regions and 2 mitochondrial SNPs. Microsatellites yielded 71 different multilocus genotypes (MLGs) and 112 different alleles with different rates of variability varying from 4 (locus P2039) to 23 (locus P1509). SNPs enabled the identification of 26 and 30 polymorphisms for each marker and a total of 42 multilocus mitochondrial haplotypes (MMH). Phylogenetic analyses were conducted for the two datasets, and the isolates grouped into five major consistent clusters for both markers. Particularly, the majority of Citrus isolates grouped together in spite of their geographical location. However, isolates collected from tobacco were rather similar according to their origin, while isolates from ornamental plants were scattered within the tree (Citrus' group excluded). Overall, SSRs provided a higher level of discrimination between closely related isolates. On the other hand, a major advantage of the mtSNP method was that results were unbiased (a list of nucleotides) and highly reproducible, thus enabling the comparison of data from different laboratories and time periods. The implementation of these two tools is therefore useful to achieve high levels of discrimination, reproducibility and reliability and to enable the analysis of both the maternal inheritance and the parental genotyping.

11. FIRST DETECTION OF *TOMATO RINGSPOT VIRUS* IN POMEGRANATE PLANTS. M. Calassanzio[1], A.R. Babini[2], R. Biccheri[1], A. D'Anniballe[2], C. Lanzoni[1], A. Mirotti[2], C. Poggi Pollini[1], C. Ratti[1]. *[1]Dipartimento di Scienze Agrarie, Area Patologia Vegetale, Università di Bologna, Viale Fanin 46 - 40127, Bologna, Italy. [2]Servizio Fitosanitario Regionale, via di Saliceto 81, Bologna, Italy. E-mail: claudio.ratti@unibo.it*

Cuttings of *Punica granatum* for fruit production and ornamental usage have been introduced in accordance with EC directives in the Emilia-Romagna region from the USDA/ARS (Agricultural Research Service) clonal germplasm repository (California) due to the increasing of pomegranate production all over the Italian territory. This planting material, before its delivering throughout the territory, was subjected to specific analysis in order to ensure a proper sanitary selection. Samples from symptomatic (yellowing, deformation or discoloration of leaves) and symptomless pomegranate trees were tested by ELISA assay using antibodies specific for several viruses affecting fruit crops. In many samples *Tomato ringspot virus* (ToRSV, EPPO A2 list) was detected and its infection confirmed by RT-PCR analysis. Biological characterization of the viral isolates was carried out. Buds were grafted into indicator peach rootstocks and sap from symptomatic tissues was mechanically inoculated onto herbaceous test plants on which typical symptoms of chlorotic ringspots, necrotic lesions, wilting and leaf curling were observed. Phylogenetic analysis revealed high sequence identity of RNA1 and RNA2 3' UTR regions of four pomegranate isolates. Official phytosanitary measures have been taken to eradicate the disease. All pomegranate plants of US origin have been uprooted and destroyed. These measures were aimed to avoid the ToRSV diffusion, with the intent to prevent the virus establishment in our territory where it would be harmful not only to pomegranates but also to other economically important fruit crops, as it is happening in U.S.A.

12. MYCOVIRUSES INFECTING *FUSARIUM CULMORUM* (W.G. SMITH) SACC. M. Calassanzio, A. Prodi, P. Nipoti, A. Pisi, G. Filippini, C. Ratti. *Dipartimento di Scienze Agrarie, Area*

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Fusarium culmorum is a plant pathogenic fungus that causes foot and root rot and 'Fusarium head blight' diseases in cereals and particular on wheat and barley. The fungus is also responsible for mycotoxins accumulation on grain. In the last years several studies identified mycoviruses in many fungal species that may cause hypovirulence and hence can be of value in the biocontrol of crop diseases. We analyzed the virome of 40 *F. culmorum* isolates from Italy, Iran and Syria and the resulting different dsRNA profiles have been associated with several species of mycoviruses. Each dsRNA fragment of the chosen strains was isolated and amplified through a random RT-PCR. PCR amplicons were cloned into plasmid vectors and sequenced. The overall rate of viral infection appears to be 60% and in particular we observed 18% of infection among Italian isolates (3 out of 16), 70% among Syrian isolates (11/16) and 87% among Iranian isolates (7/8). Sequencing results indicate the presence of single and mixed mycoviral infections by members of families *Endornaviridae*, *Narnaviridae* and *Partitiviridae*. Moreover a bipartitic viral species from an Italian fungal isolate has been characterized by virions isolation and full genome sequencing. Observing the low similarity of the genome with other previously described mycoviruses, we have proposed the name of Fusarium culmorum virus 1 (FcV1) for this new viral species. Further studies will focus on the effect of FcV1 on *F. culmorum* in order to determine its role in the biology and in particular in the fungal pathogenicity.

13. AN INTERNATIONAL MULTIDISCIPLINARY PROJECT TO ASSESS THE RISK OF INTRODUCTION OF *XANTHOMONAS CITRI* subsp. *CITRI*. **V. Catara[1], J. Cubero[2], O. Pruvost[3], G. Licciardello[1], Y. Aysan[4], R. Cetinkaya-Yildiz[5], G. Timpanaro[1], P. Caruso[6].** *[1]Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, Catania, Italy. [2]Instituto Nacional de Investigación y tecnología Agraria y Alimentaria, Madrid, Spain. [3]French Agricultural Research Centre for International Development CIRAD. La Reunion, France. [4]Plant Protection Department, Cukurova University, Adana, Turkey. [5]Plant Protection and Plant Quarantine Department, Biological Control Research Institute. Adana, Turkey. [6]Consiglio per la Ricerca e l'analisi dell'economia agraria - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Acireale, Italy. E.mail vcatara@unict.it*

Citrus crops in the Mediterranean region are threatened by several plant pathogens. The most important risk factor for the introduction of new pathogens is the importation of infected yet symptomless plant material and plant pathogen vectors. *Xanthomonas citri* subsp. *citri* (*Xcc*) is a quarantine pathogen not known to occur in the European community and entire Mediterranean region, responsible for Citrus Bacterial Canker (CBC). A way of entry of *Xcc*, as highlighted by EFSA, is the introduction of rutaceous ornamental plants, through both the commercial trade and passenger pathways since these species are extensively grown in Mediterranean countries, in nurseries, orchards and also in private and public gardens. Therefore, citrus relatives (*Rutaceae*), particularly those not covered by the 2000/29EC directive, represent a threat of the introduction of the bacterium. The project ORPRAMed - "Risk assessment of introduction of *Xanthomonas citri* subsp. *citri* through commercial trade of ornamental rutaceous plants in the Mediterranean basin" (ARIMNet2 2015 Transnational Call) aims to assess the risk of the introduction of *Xcc* in the Mediterranean region, by means of ornamental citrus relatives, generating and improving our understanding of the interactions between *Xcc* and ornamentals citrus. The project, which started the 1st of March 2016, involves Italy, France, Spain and Turkey and has work packages dealing with: Economics and trade of ornamental rutaceous plants in the Mediterranean region; Host status of ornamental Rutaceae species and mechanisms of *Xcc* survival and colonization in ornamental plants; Genomic and transcriptomic analysis of the *Xcc* resistant/susceptible genotypes.

14. *FUSARIUM VERTICILLIOIDES* AND *F. PROLIFERATUM* ASSOCIATED TO SEEDLING BLIGHT IN MAIZE. **F. Cavina[1], G. Urso[2], S. Rossi[2], P. Nipoti[1], E. Noli[2], A. Prodi[1].** *[1]Laboratorio di Micologia Fitopatologica. [2]Laboratorio di Ricerca e Analisi Sementi (LaRAS). Alma Mater Studiorum Università di Bologna, Dipartimento di Scienze Agrarie (DipSA). Viale G. Fanin 40 - 40127 Bologna, Italy. E-mail: federico.cavina6@unibo.it*

Fusarium species are responsible of severe ear rot on maize (*Zea mays* L.). Pink ear rot is caused mainly by *F. verticillioides* (Saccardo) Niremberg, *F. proliferatum* (Matsushima) Nirenberg and *F. subglutinans* (Wollenweber & Reinking), all mycotoxin producers. The most important mycotoxins are fumonisins, largely found in naturally contaminated foods and feeds. The presence of these fungi as endophytes was reported in leaves, stems, roots, grains, but not associated to particular symptoms. Only *F. verticillioides* is associated to a root and stalk rot in Italy. During routine testing of seed germination of maize samples, using standard methods, the presence of abnormal seedlings, characterized by mesocotyl rot, was observed with an incidence ranging from 4 to 42%. Mycological analysis, conducted on agar medium (PDA), revealed the presence of fast growing colonies with a white-dark violet aerial mycelium on 100% of the samples. Through morphological and molecular techniques, *F. verticillioides* and *F. proliferatum* were identified with a frequency of 87% and 13%, respectively. For both fungi Kock's postulates were verified by artificial inoculation either dipping seeds or seedling roots in a conidial suspension (1×10^6 conidia/ml). Both fungi reproduced the same symptoms. To our knowledge this is the first report confirming seedling blight caused by *F. proliferatum* on maize seed for the Italian market. In a situation of changing climatic conditions, this may become a relevant pathogen, and neglecting seed transmission could be potentially dangerous, due to severe post-emergence damping off of seedlings.

15. FACTORS INDUCING SOIL SICKNESS IN BABY LEAF CULTIVATION. **G. Cesarano, F. Ippolito, F. De Filippis, A. La Storia, D. Ercolini, G. Bonanomi, F. Scala.** *Università degli Studi di Napoli "Federico II", Dipartimento di Agraria, via Università 100 - 80054 Portici (NA), Italy. E-mail: gaspare.cesarano@unina.it*

Soil sickness (SS), a condition in which monocultures cause a decline in crop yield, is an emerging problem in baby leaf cultivation under plastic tunnels. Several mechanisms have been proposed to explain this condition including depletion of mineral nutrients, accumulation of toxic substances and build-up of soilborne pathogens. The objective of this study was to investigate the role of some biotic and abiotic factors in SS occurrence. Soils from 12 farms in which baby leaf lettuces have been cultivated for over 5 years were used for pot experiments. Soils from adjacent open fields were collected and used as control. Soil sterilization, supply of mineral nutrients and addition of active carbon were used alone or in combination and their effects on plant growth evaluated in comparison to untreated soils. The structure of microbial community including bacteria and eukarya was assessed by 454 Pyrosequencing. Compared to untreated soil, the addition of active carbon did not enhance the yield, whereas an increase of 50% and 90% was observed with the application of mineral nutrient and soil sterilization, respectively. Monocropping cultivation affected the structure of microbial community. The dominant bacteria phyla

across all samples were *Acidobacteria, Actinobacteria, Bacteroidetes Chloroflexi, Cyanobacteria, Firmicutes* and *Proteobacteria*. Significant differences in abundance of *Actinobacteria, Bacteroidetes* and *Cyanobacteria,* between open and under plastic tunnel soil were observed. The fungal genera *Chaetomium, Myrothecium, Rhizopus* and *Verticillium* were dominant and exhibited significant variations in most of the sampled soils compared with open fields.

16. ISOLATION, IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF *ALTERNARIA* SPECIES AFFECTING THE TUNISIAN HALOPHYTE *CAKILE MARITIMA*. A. Chalbi[1,2], M. Masiello[2], A. Susca[2], A.F. Logrieco[2], C. Abdelly[1], A. Moretti[2], B.S. Hammami[1]. *[1]Laboratory of Extremophile Plants, Center for Biotechnology of BorjCedria, CBBC, Technopole BorjCedria, University of Tunis El Manar, BP 901 - 2050 HammamLif, Tunisia. [2]Institute of Sciences of Food Production, Research National Council (ISPA-CNR), Via G. Amendola 122/O - 70126, Bari, Italy. E-mail: antonio.moretti@ispa.cnr.it*

Cakile maritima (*Brassicaceae*) is a halophyte plant of the Mediterranean coasts, tolerating abiotic stress conditions such as salinity and water stress, recently considered an excellent model to elucidate biochemical and physiological mechanisms of salinity tolerance. In addition to its medicinal (chemotherapeutic drugs), industrial (oilseed) and ecological properties, *C. maritima* plays an antiscorbutic, diuretic and purgative role in folk remedies. The plant is attacked by different fungal species, mainly belonging to *Alternaria* genus. Eighty *Alternaria* strains were randomly collected from the fresh stems of spontaneous plants showing symptoms of *Alternaria* infection and grown in four bioclimatic Tunisian areas (arid, semi-arid, semi-humid and humid). Phylogenetic analyses were carried out by sequencing the allergen alt1a, glyceraldeyde-3-phosphate dehydrogenase and translation elongation factor 1α genes, according with a multi-locus gene sequence approach. The analysis of the combined gene sequences resulted in a Maximum Parsimony tree showing two well defined clades, supported by high bootstrap values: Clade A, including reference strains of *A. alternata*, *A. tenuissima*, *A. arborescens* and 61 investigated strains; and Clade B, including 19 stains. A high level of genetic variability among and within the clades was observed. However, the geographical and climatic origin of the strains was not associated to their genetic traits. Therefore, further analyses by using a wider set of housekeeping genes are needed for acquiring more information on the phylogeny of *Alternaria* species affecting *C. maritima*. This is the first report on genetic diversity of a Tunisian population of *Alternaria* species isolated from *C. maritima*.

17. A NEW TOOL FOR INVESTIGATING FLAGELLIN-MEDIATED PLANT-PATHOGEN INTERACTIONS. S. Ciarroni[1,3], C. Clarke[2], H. Liu[2], A. Mazzaglia[1,3], B.A. Vinatzer[2], G.M. Balestra[1,3]. *[1]Università degli Studi della Tuscia, DAFNE, Via San Camillo de Lellis snc - 01100 Viterbo, Italy. [2]Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Latham Hall, Ag Quad Lane, Blacksburg, VA, United States. [3]Phy.Dia srl, Spin off Università degli Studi della Tuscia, DAFNE, Via San Camillo de Lellis snc - 01100 Viterbo, Italy. E-mail: seryc86@gmail.com*

Many plants can react to flg22 epitope of *Pseudomonas syringae* flagellin, initializing a cascade signal to trigger a plethora of defense mechanisms. Recently, a new epitope, flgII-28, within the same protein has proven to be a strong elicitor only for some tomato cultivars and also it seems to be recognized by a different and specific receptor. Deepening the defense mechanisms of plants which don't have receptors for already known epitopes could be very difficult because of the labour- and time-consuming process of *de novo* identification and purification of a new PAMP. We tried to setup a new protocol possibly applied for screening even a large set of different plant species for their ability to recognize part of the flagellin protein. The first 143 aminoacids of several *Pseudomonas syringae* flagellin alleles, including both flg22 and flgII-28 epitopes, were synthesized via a plasmid-*Escherichia coli* recombinant system and used as elicitors in downstream ROS assays on three different plants: Arabidopsis, tomato and kiwifruit. Arabidopsis generates oxidative burst after treatment with both flg22 and recombinant fragment, but not with flgII-28 epitope. Regarding tomato, every tested flagellin allele causes a response, but unfortunately amplitude of ROS synthesis curve wasn't precisely reproducible. As concerns kiwifruit, it doesn't recognize neither flg22 nor flgII-28, even when they are used simultaneously; however it seems that at least one receptor for flagellin should be present, since kiwifruit responds to all the recombinant fragments, regardless of the allele.

18. BASELINE SENSITIVITY OF *STEMPHYLIUM VESICARIUM* OF PEAR TO SDHIs AND FLUAZINAM. A. Ciriani, K. Gazzetti, M. Collina. *Università di Bologna, Centro di Fitofarmacia, DipSA, Bologna, Italy. E-mail: katia.gazzetti@unibo.it*

Brown spot of pear (BSP), a fungal disease caused by *Stemphylium vesicarium* (Wallr.) Simmons, is the most important pear fungal pathogen in Italy since late seventies. Many fungicide applications are required from petal fall to fruit ripening to protect orchards from BSP. The fungus showed field resistance against key products as dicarboximides and strobilurins. The introduction in field of new fungicides with different mode of action is thus fundamental. Boscalid was authorized in Italy as first SDHI (inhibitor of the succinate dehydrogenase in complex II) against BSP in 2007.

The aim of this study was to evaluate the baseline sensitivity of 43 isolates of *S. vesicarium*, collected before 2007 from pear orchards located in Po Valley area, to recent and broad-spectrum fungicide SDHIs (fluxapyroxad, fluopyram, penthiopyrad, bixafen, isopyrazam) and fluazinam, an uncoupler of oxidative phosphorylation. Sensitivity assays were carried out on spore suspension (YBA liquid medium; final density 2×10^4/ml) in microtiter plates using the low time consuming and validated spectrophotometric method. For each compound, concentrations of 0-0.02-0.05-0.5-1-2.5 mg/l were tested on each isolate, in four replicates. After two days of growth, absorbance at 450 nm was evaluated and EC50 values were calculated by probit analysis. Overall baseline sensitivity data, carried out on 43 isolates, showed for tested fungicides EC50 values ranging from 0.01 to 0.52 mg/l. These data describe the initial sensitivity level prior to the introduction of the fungicides and are essential information in resistance monitoring program to detect potential shifts in pathogen sensitivity.

19. INVESTIGATIONS ON '*CANDIDATUS* PHYTOPLASMA SOLANI' IN WESTERN SICILY, SOUTHERN ITALY. G. Conigliaro[1], G. Tuttolomondo[1], G. Lo Verde[1], P. Bella[1], G. Romanazzi[2], V. D'Urso[3], H. Tsolakis[1], S. Burruano[1]. *[1]Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze Ed. 4 - 90128 Palermo, Italy. [2]Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, Via Brecce Bianche - 60131 Ancona, Italy. [3]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Sezione di Biologia Animale, Università degli Studi di Catania, Via Androne 81 - 95124 Catania, Italy. E-mail: santella.burruano@unipa.it*

'*Candidatus* Phytoplasma solani', belonging to the 16SrXII-A subgroup, is associated with grapevine Bois noir (BN). It is usually

transmitted by *Hyalesthes obsoletus* from a wide range of weeds to grapevine, which is considered a dead-end host. Moreover, other alternative vectors can play a role in the epidemiology of the disease. In Sicily, the presence of BN was reported in 1996, both on typical and on newly introduced cultivars. Furthermore, high BN incidence was observed on native grape cultivars grown close to 'Chardonnay' vineyards. In summer 2014 and 2015, the spread and the severity of BN were recorded in a 10 year-old vineyard located in San Giuseppe Jato (PA, Western Sicily), grown with cultivars Chardonnay, Nero d'Avola, and Pinot Noir. Preliminary surveys showed the presence of a rich leafhopper fauna and the absence of *H. obsoletus*. The presence of '*Ca.* P. solani' in randomly sampled vines from all cultivars was confirmed by amplifying DNA through nested-PCR and RFLP, and no other phytoplasma were found. The percentage of symptomatic grapevines was the lowest in cv. Pinot Noir and the highest in cv. Chardonnay. *Ca.* P. solani' was also detected in some herbaceous plants and in leafhoppers collected inside the rows and at the border of the vineyard. Considering the importance of the disease and the recent finding of *Euscelidius variegatus*, *Neoaliturus fenestratus*, and *Exitianus taeniaticeps* as alternative vectors in vineyards of Eastern Sicily, there is the need to clarify the epidemiology of BN in Western Sicily vineyards to set up appropriate management strategies.

20. VELVET COMPLEX AND GLIOTOXIN BIOSYNTHESIS IN *TRICHODERMA AFRO-HARZIANUM* T6776. P. Crotti[1], L. Fiorini[1], S. Ferraboli[1], R. Baroncelli[2], S. Sarrocco[3], G. Vannacci[3], E. Gobbi[1]. *[1]Università degli Studi di Brescia, Piattaforma di Microbiologia Agro-alimentare, Dipartimento di Medicina Molecolare e Traslazionale, Viale Europa 11 - 25123 Brescia (BS), Italy. [2]Laboratorie Universitaire de Biodiversité et Ecologie Microbienne, Université de Bretagne Occidentale, Brest, France. [3]Università di Pisa, Dipartimento di Scienze Agrarie, Alimentarie Agro-ambientali, Via del Borghetto 80 - 56100 Pisa, Italy. E-mail: p.crotti002@studenti.unibs.it*

Nowadays intensive farming and the need of a massive agricultural production require the introduction of a sustainable alternative that can replace the use of chemical products, such as bio-fertilizers and biostimulants. Among them, *Trichoderma afro-harzianum* strain T6776 has been shown to act as a biocontrol agent and as a growth promoter in plants. The Velvet complex genes are reported in several fungal species as a regulatory system with a role in self-growth and reproduction, hydrophobicity and production of secondary metabolites such as gliotoxin (GT). GT was identified for the first time in *Trichoderma virens* and is a molecule with an antimicrobial/antibiotic activity and a role in plant growth promotion. Homologous genes sequences of the Velvet complex corresponding to *vel*A, *vel*B, *vos*A and *fac*B and of the GT biosynthesis gene, *gli*P have been identified in the *T. afro-harzianum* strain T6776 genome and evaluation of their role in T6776- host interaction, by phenotypical characterization of corresponding knock-out mutants will be presented.

21. EXPLOITING PATHOGEN CONFUSION STRATEGY TO ACHIEVE *XYLELLA FASTIDIOSA* BIOCONTROL. G. D'Attoma[1,2], M. Saponari[1], M. Morelli[1], A. Giampetruzzi[2], V.N. Savino[2], D. Boscia[1], P. Saldarelli. *[1]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP-CNR), Via Amendola 122/D - 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, Italy. E-mail: giusy.dattoma@ipsp.cnr.it.*

The recent findings of the plant pathogenic bacterium *Xylella fastidiosa (Xf),* infecting several plant species in Italy and France, raised major concerns for its potential impact on the EU and Mediterranean agriculture. In the current EU outbreaks, olive is the predominantly affected crop, in which the bacterium has been consistently associated with a new severe syndrome, denoted "Olive Quick Decline". So far, no effective treatments are available to cure infected plants. However, several approaches have been explored, mainly in grapevine and citrus, to reduce bacterial movement and multiplication or directly targeting *Xf*-cells for lysis.

Current knowledge shows that the virulence of the pathogen relies on a fine balance between more motile bacterial forms, able to move and proliferate within xylem vessels, and sticky cells forming a biofilm, which are responsible for vessels blockage and insect acquisition. This different behavior is regulated in a cell density-dependent manner by a diffusible signaling factor (DSF), produced by *rpfF*-gene, that initiates a transduction cascade resulting in up- or down-regulation of several genes. The aim of our investigation is to explore "pathogen confusion" strategy, by altering DSF level *in planta*, for reducing the impact of *Xf*-infections in olives. To this end, a plant viral-based vector, harboring the *rpfF*-gene, has been engineered to induce transient DSF production. Experiments will verify if, upon DSF accumulation, the bacterium will be less motile and more adhesive to the surface of xylem vessels, thus showing a decreased virulence in infected plants.

22. BIOCONTROL ALTERNATIVES FOR THE MANAGEMENT OF ROOT-KNOT NEMATODES. G. d'Errico[1], N. Lombardi[2], S. Lanzuise[1], A. Sacco[1], R. Quarto[1], R. Panza[1], L. Prasad[3], J. Strakowska[4], M. Lorito[1,2], S.L. Woo[1,2]. *[1]Università degli Studi di Napoli Federico II, Dipartimento di Agraria, Via Università 133-80055 Portici (NA), Italy. [2]Consiglio Nazionale delle Ricerche - Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), Via Università 133-80055 Portici (NA), Italy. [3]Indian Agricultural Research Institute Plant Pathology, New Delhi-110 012, India. [4]Polish Academy of Sciences, Institute of Plant Genetics, Strzeszynska 34, 60-479 Poznan, Poland. E-mail: giada.derrico@unina.it*

The root-knot nematode (RKN), *Meloidogyne* spp., is one of the most damaging agricultural pests for a wide range of crops. In intensive cropping systems, they are traditionally managed with chemical nematicides. However, the negative impact on the environment, animal and human health has led to a total ban or restricted use of many products. Thus, there is great interest in diversifying tools used for nematode control, such as the use of biological control agents (BCAs) or integrated pest management. Fungal antagonists of *Trichoderma*, are recognized as broad-spectrum plant protection agents and growth promoters, that are promising as alternatives to synthetic nematicides. Further, many *Trichoderma* spp. produce a variety of secondary metabolites (SMs), which may contribute to the biocontrol arsenal. The activity of *Trichoderma* and secondary metabolites, alone or in combinations, were tested *in vitro* and *in vivo* on *M. incognita* to determine their effects. In solutions, the immobility of nematodes augmented with increasing concentrations and longer exposure to *Trichoderma* and SMs. The assays mainly demonstrated paralysis reversibility of *M. incognita* (nematostatic effect). In controlled growth conditions, *Trichoderma*-SMs combinations significantly promoted growth of tomato plants, and inhibited *M. incognita* development, in comparison to water controls. The combination of BCAs and their SMs may produce a synergistic effect for improved plant growth, induce systemic resistance to pathogen attack, plus reduce nematode infestation. Novel bioformulates based on selected strains and/or SMs are promising for the development of next generation agricultural products.

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23. SUPPRESSIVITY OF COMPOSTS MADE FROM THE 2nd-GENERATION BIOETHANOL CHAIN CO-PRODUCTS AND AGRO-INDUSTRIAL RESIDUES AGAINST SOIL-BORNE DISEASES UNDER GREENHOUSE CONDITIONS. **U. De Corato[1], C. Pane[2], I. De Bari[1], M. Zaccardelli[2].** *[1]ENEA - Dipartimento Tecnologie Energetiche - Divisione Bioenergia, Bioraffineria e Chimica Verde, Ufficio Territoriale di Bari c/o Consorzio Universitario per la Formazione e l'Innovazione Uni. Versus, Viale Japigia 188 - 70126, Bari, Italy. [2]Consiglio per la Ricerca e l'analisi dell'economia agraria, Centro di ricerca per l'orticoltura, Via dei Cavallegeri 25 - 84098, Pontecagnano, Salerno, Italy. E-mail: ugo.decorato@enea.it*

Compost is a stabilized organic matter deriving from bio-oxidation of undecomposed feedstock. Its utilization in combination with other substrates may give several benefits for the plants, including the suppression of soil-borne plant diseases. However, not all composts are suppressive and their level vary, as well as the range of the suppressed pathogens. One feedstock of 2nd-generation ethanol chain co-products mixed with agro-industrial residues was investigated for finding novel suppressive composts able to control soil-borne diseases. The *in vivo* suppressiveness of three composts (C_M, C_A, C_{WS}) sourcing from the mixtures of crude steam-explosion liquid waste of miscanthus ($SELW_M$), giant reed ($SELW_A$) and wheat straw ($SELW_{WS}$) with the agro-wastes available in Southern Italy (Apulia and Campania) was studied against the *Phytium ultimum*/Cucumber, *Phytophthora nicotianae*/Tomato, *Rhizoctonia solani*/Bean, *Sclerotinia sclerotiorum*/Lettuce, *Fusarium oxysporum* f. sp. *melonis*/Melon, *F. oxysporum* f. sp. *lycopersici*/Tomato and *Verticillium dahliae*/Eggplant pathosystems, by comparing their suppressiveness with those of one reference compost (C_C) obtained from the bio-wastes. *In vivo* tests performed under greenhouse conditions showed multi-suppressive activity of all composts: C_{WS} ($SELW_{WS}$ + woodchips + tomato-waste) suppressed most efficiently *P. ultimum*, *R. solani*, *P. nicotianae*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *lycopersici*, *V. dahliae*; C_M ($SELW_M$ + coffee-grounds + artichoke-waste) was capable to suppress *P. ultimum*, *R. solani*, *P. nicotianae*, *S. sclerotiorum*, *V. dahliae*; C_A ($SELW_A$ + defatted olive marc + fennel-waste) was suppressive against *R. solani*, *P. nicotianae*, *V. dahliae*. Instead, C_C was mostly suppressive against *F. oxysporum*. Autoclaving composts reduced their suppressiveness in all pathosystems demonstrating crucial role played by microflora.

24. BIOLOGICAL CONTROL OF TRACHEO FUSARIOSIS IN WILD ROCKET BY *TRICHODERMA* spp. **L. De Martino[1], S. Sarrocco[1], L. Sigillo[2], V. Senape[2], G. Serratore[2], G. Vannacci[1].** *[1]Università degli Studi di Pisa - Dipartimento di Scienze Agrarie, Alimentari e Agro-Ambientali, Plant Pathology & Mycology lab Via del Borghetto 80, 56127 Pisa. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di sperimentazione e certificazione delle sementi - sede di Battipaglia, S.S. 18 km 77,700, 84091 - Battipaglia (SA), Italy. E-mail: sabrina.sarrocco@unipi.it*

Fusarum oxysporum f. sp. *raphani* (For) is one of the causal agents of fusarium wilt in wild rocket (*Diplotaxis tenuifolia*); this *forma specialis* is the most widespread in the ready-to-use vegetable produced in Piana del Sele (Salerno, Italy) where soil fumigation and solarisation are the treatments of choice. Aim of this work was to apply a screening procedure to select one or more antagonistic *Trichoderma* isolates effective against For in experimental glasshouse conditions set up to simulate real farming environment. Ninety-one *Trichoderma* isolates, from the collection of the Plant Pathology and Mycology Lab, were tested. Trials were run in 2014 and 2015. Wild rocket growing medium was inoculated with 5% of bran fermented by the *Trichoderma* isolates and with 1×10^4 cfu ml^{-1} of For (strain 10223, from CREA collection) grown in Potato Dextrose Broth. Twelve seeds of wild rocket were sown per pot in three replicates (pots) in the same day. Symptoms of wilting were assessed for 18 days by using a disease scale and data were analysed by ANOVA. The response of the 91 isolates was highly variable, but 26 of them gave interesting results. These 26 isolates were tested again in a second round of screening and two isolates gave a disease index significantly lower than inoculated control, with the isolate T8203 of *T. viride* ranking among the best in both screening rounds. This work paves the way to develop a bio-based plant protection product to limit the tracheofusariosis in wild rocket.

25. MOLECULAR ANALYSIS OF THE GENETIC DIVERSITY OF *PHYTOPHTHORA CITROPHTHORA SENSU LATO* USING NUCLEAR AND MITOCHONDRIAL DNA MARKERS. **M. Evoli[1], I. Puglisi[1], A. Pane[1], L. Schena,[2] T. Jung[3], M. Horta Jung[3], J. Bakonyi[4], G. Magnano di San Lio[2], S.O. Cacciola[1].** *[1]Università degli Studi di Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A) Via Santa Sofia, 100 - 95123 Catania, Italy. [2]Università degli Studi Mediterranea di Reggio Calabria, Feo di Vito - 89122 Reggio Calabria, Italy. [3]Centre for Mediterranean Bioresources and Food (MeditBio), University of Algarve, Campus de Gambelas 8005 - 139 Faro, Portugal. [4]Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary. E-mail:olgacacciola@unict.it*

In the present study, both mitochondrial and nuclear DNA markers were used to study the genetic variability of isolates of *Phytophthora citrophthora sensu lato* from different hosts and geographic origins. Overall, 125 isolates mainly sourced from citrus were included in this study. Mitochondrial (Mt15F-5R primers) and nuclear (SSRs markers I13F-I14R) sequence chromatograms were analyzed, aligned and checked for single nucleotide polymorphisms (SNPs) or indels using MEGA 6 and MUSCLE. Heterozygous SNPs identified in the nuclear coding regions by the presence of double peaks were marked with standard degeneracy codes. Haplotypes were identified in mitochondrial sequences with ElimDupes. Intraspecific genetic variability was detected not only among isolates of different geographic origin or host, but also among isolates with the same geographical origin (e.g. Vietnam and Taiwan), as already shown in *P. nicotianae*, *P. infestans, P. sojae* and *P. ramorum*. No consistent association of haplotypes or genotypes with the geographic origin, host species or year of isolation was observed. Results indicate these markers are an effective DNA barcoding method to revise and reorganize the taxonomy of *Phytophthora* Clade 2a. They can be used to clearly separate already known species whose ranges of morphometric data and morphological characters presently overlap, very probably leading to the hypothesis that they could be new species or hybrids that in the present classification are included in complex taxonomic entities and thus deserve further analyses.

26. BIOCONTROL POTENTIAL OF NON-FERMENTING YEAST STRAINS AGAINST OCHRATOXIN A PRODUCING ASPERGILLI. **M.G. Farbo, P.P. Urgeghe, A. Marcello, S. Jaoua, Q. Migheli.** *Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39 - 07100 Sassari, Italy. E-mail: mgfarbo@uniss.it*

Ochratoxin A (OTA) contamination in processed beverages is caused primarily by *Aspergillus* spp. grape infection. Aim of this study is to evaluate the biocontrol ability of selected yeast strains against different OTA producing *Aspergillus* species. In a previous report, two non-fermenting (*Cyberlindnera jadinii* 273 and *Candida friedrichii* 778) and two low-fermenting (*Candida intermedia* 235 and *Lachancea thermotolerans* 751) yeast strains have shown a

significant antagonistic behaviour against a virulent strain of *A. carbonarius* on grape berries as well as in *in vitro* experiments, while the filtered and autoclaved culture broth of the yeast strains had no significant effect on pathogen growth. Here we report on the *in vitro* biocontrol potential of the yeast strains against *A. carbonarius*, *A. ochraceus*, and *A. westerdijkiae*. Among the tested yeasts, *C. intermedia* and *C. jadinii* displayed the highest inhibition of *Aspergillus* spp. growth and sporulation. The biological effect is likely mediated by volatile organic compounds (VOCs), since growth inhibition was observed without contact between yeast and *Aspergillus* spp. *Aspergillus* colonies exposed to yeast VOCs did not sporulate, and were characterised by a white mycelium; the colony border was undefined, with elongated and scattered hyphae compared to unexposed control. Single hyphal tips and mycelium fragments were then transferred on PDA and after 5 days of growth at 25°C, typical dark sporulating colonies were evident, suggesting that the anti-sporulating effect is reversible. These results suggest that VOCs released by the selected yeasts are able to reduce conidial germination and possibly OTA production in preventive food safety strategies.

27. BLACKLINE AND OCCURRENCE OF *CHERRY LEAF ROLL VIRUS* (CLRV) ON WALNUT TREES IN VENETO REGION. L. Ferretti[1], B. Corsi[1], L. Luongo[1], C. Dal Cortivo[2], A. Belisario. *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale (PAV), Via C.G. Bertero, 22 - 00156 Rome, Italy. [2]Nogalba, Consorzio Produttori di Noce, 45010 Pettorazza, Rovigo, Italy. E-mail: alessandra.belisario@crea.gov.it*

Cherry leaf roll virus (CLRV) on common walnut trees grafted onto black walnut rootstocks is the cause of a serious disease known as "blackline". This disease affects the plant tissues involved in the transport of nutrients and water between the rootstock and scion. The black line symptom usually appears at the graft union and can completely girdle the tree, causing the death of the scion. In the spring 2014, blackline symptoms were observed for the first time in a commercial orchard in Veneto region (North-eastern Italy), one of the most important Italian walnut-producing area, on common walnut trees grafted onto 'Paradox' (*J. hindsii* × *J. regia*), originated from USA. In order to ascertain the occurrence and distribution of CLRV on walnut in this geographic area, an extensive 2-year field survey was carried out. A total of 1,684 walnut trees, belonging to different varieties (Chandler, Tulare, Lara) and rootstock-scion combinations were sampled and analysed by DAS-ELISA. CLRV was detected only on symptomatic and symptomless trees of cvs Chandler and Tulare grafted on Paradox imported from USA, and on a nearby symptomless tree of cv. Lara grafted on *J. regia*, imported from France. Results strongly suggest a possible relationship between infection and the origin of the propagative plant material. This hypothesis is also supported by the patchy distribution of the disease in the field. Molecular analyses will provide a further characterization of CLRV isolates.

28. EVALUATION OF CHICKPEA (*CICER ARIETINUM* L.) LOCAL GERMPLASM FROM CENTRAL ITALY FOR GENETIC RESISTANCE TO MAIN FUNGAL PATHOGEN OF THE CROP IN THE MEDITERRANEAN BASIN. M. Fierro[1], F. De Curtis[1], D. Vitullo[1], D. Palmieri[1], J. Rubio[2], T. Millán[3], G. Lima[1]. *[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis snc - 86100 Campobasso, Italy. [2]IFAPA Centro "Alameda del Obispo", Biotechnology and Plant Breeding Area, Avda. Menendez Pidal Apdo. 3092 - 14080 Córdoba, Spain. [3]Department of Genetics, University of Córdoba, Campus Rabanales Edificio C5 2a Planta - 14071 Córdoba, Spain. E-mail: marco.fierro@studenti.unimol.it*

Chickpea (*Cicer arietinum* L.) is an important source of nutrients for human as well as animal feeding. In Italy this crop is mainly cultivated in little farms where local ecotypes usually take name from the location. In a market prone to valorize traditional products, the use of chickpea ecotypes could represent a favourable alternative in crop turn over. Due to the low genetic diversity which can occur inside chickpea populations, high loss of production can be frequently caused by pathogens as *Fusarium oxysporum* f. sp. *ciceris* and *Ascochyta rabiei*, which are widespread in the Mediterranean area. Against this pathogens it is crucial to develop appropriate integrated control strategy mainly based on crop genetic resistance. The aim of this work was to search and valorize genetic resources from local ecotypes to be used to preserve genetic biodiversity. We evaluated the resistance of 18 Italians ecotypes against i) *Fusarium oxysporum* f. sp. *ciceris* race 5 (Foc5) and ii) *Ascochyta rabiei*, the causal agents of Fusariosis and Ascochyta blight (AB), respectively. Molecular analysis for markers associated with fungal disease resistance were also conducted. Our results showed that the 18 local ecotypes have different level of resistance to both pathogens. The high level of resistance was found in the ecotypes "Longano" and "S. Elia a Pianisi" towards Foc5 and in "Cercemaggiore", "Cece Nero di Cercemaggiore" and "Capracotta" to AB. Our results open the way to valorize and preserve this local chickpea biodiversity for local cultivation and as a source of plant pathogen resistance genes.

29. PHYLOGENETIC ANALYSIS AND RELATIONSHIPS AMONG *TOMATO SPOTTED WILT VIRUS* ISOLATES DETECTED IN PEPPER PLANTS. A. Fontana[1], A. Manglli[2], L. Tomassoli[2], G. Albanese[1]. *[1]Università degli studi Mediterranea di Reggio Calabria, dipartimento di agraria, Reggio Calabria, Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale, via C.G. Bertero, 22, 00156, Roma, Italy. E-mail: anna.fontana@unirc.it*

Tomato spotted wilt virus (TSWV), the type member of the genus *Tospovirus*, affects a wide range of host plants, including 1090 plant species worldwide, causing serious agronomic losses especially to vegetable crops such as tomato and pepper. In this study a total of 9 TSWV isolates detected in seven pepper (*Capsicum annuum* L.) plants from Calabria (southern Italy) and two from Latium (central Italy) were genetically analyzed. Total RNA extracts were subjected to reverse-transcription polymerase chain reaction (RT-PCR) using specific primers of TSWV to amplify the complete nucleocapsid (NP) protein gene. The PCR products were purified, sequenced in both directions and assembled. Obtained nucleotide sequences were aligned by CLUSTALW, and used to construct phylogenetic tree using neighbor-joining method with 1000 bootstrap replicates implemented in MEGA6. The analysis showed a phylogenetic distribution of the 9 isolates into three distinct clusters showing a different origin, pathway of introduction or evolution. In particular, according to phylogenetic analysis, isolates from Latium showed the closest nucleotide identity with isolates from South Korea in the clade e with an Asiatic origin. Further, three isolates from Calabria were in the clade g which is again a group associated with the Old World populations whereas the others were in the North American populations clustering into clade d. To better understand the structure and evolution of our TSWV isolates in pepper, the three ssRNA segments (L, M and S) remain to be investigated.

30. A NEW RACE OF *FUSARIUM OXYSPORUM* f. sp. *LACTUCAE* THAT CAUSES FUSARIUM WILT OF LETTUCE. **S. Franco Ortega[1], G. Gilardi[1], P.C.J. van Rijswick[3], G. Ortu[1], M.L. Gullino[1,2], A. Garibaldi[1].** *[1]AGROINNOVA, Centre of Competence for Agro-Environmental Innovation, University of Torino, Largo Paolo Braccini 2, 10095, Grugliasco, Torino, Italy. [2]DISAFA, University of Torino, Largo Paolo Braccini 2 - 10095 Grugliasco, Torino, Italy. [3]National Plant Protection Organization, National Reference Centre, P.O. Box 9102 - 6700 HC Wageningen, The Netherlands. E-mail: sfrancoo@unito.it*

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

31. INVESTIGATIONS ON COLLETOTRICHUM SPECIES ON *LUPINUS ALBUS* IN APULIA REGION. **S. Frisullo[1], L. Prudente[1], S.M. Mang[2], H. Elshafie[2], I. Camele[2].** *[1]University of Foggia, Department of Agro-Environmental Sciences, Chemistry and Plant Protection, Via Napoli, 25, 71121 Foggia, Italy. [2]University of Basilicata, School of Agricultural, Forestry, Food and Environmental Sciences, Viale dell'Ateneo lucano, 85100, Potenza, Italy. E-mail: salvatore.frisullo@unifg.it*

In Spring 2013 on leaves, pods and stems of white lupin (*Lupinus albus* L.) plants which occupied about 200 ha out of the total cultivated 600 ha located in Lecce Province, typical anthracnose symptoms were observed. Disease incidence ranged from 60% to 80%. The infected organs, leaves and apical stems, initially showed small, circular, brown spots 2-3 mm in diameter that evolved in larger sunken necrotic lesions with central orange conidial masses. In order to isolate the likely pathogen, small pieces of symptomatic plant tissues were previously surface-disinfested with 1% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water and then plated on PDA Petri dishes. Plates were incubated at 25°C and, after ten days, the grown colonies were transferred to PDA to obtain pure cultures. The obtained conidia were observed under light microscopy. The ITS1-5.8S-ITS2 region of 40 representative isolates was amplified with ITS5/ITS4 primers and sequenced. The obtained sequences were highly similar (99%) to *Colletotrichum acutatum* (AJ749674 and JN543068) or *C. lupini* from lupin (JN943454 and JN943480) present in GenBank. The ITS sequences of two *C. acutatum* (LN877887 and LT160697) and two *C. lupini* (LN877886 and LN877888) isolates were deposited in GenBank. On the basis of colony and conidia morphology and of sequences analyses, the isolates were identified as *C. acutatum* (J.H. Simmonds) or *C. lupini* [(Bondar) Nirenberg, Feiler & Hagedorn]. *Colletotrichum acutatum* was reported for the first time on lupin in Italy.

32. IDENTIFICATION AND CHARACTERIZATION OF *GRAPEVINE PINOT GRIS VIRUS* IN LAZIO. **A. Gentili[1], E. Di Lucca[1,2], M. Luigi[1], F. Faggioli[1].** *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per la patologia vegetale, via C.G. Bertero - 22 - 00156, Roma, Italy. [2]Dipartimento per la Innovazione nei sistemi biologici, agroalimentari e forestali (DIBAF), Università degli studi della Tuscia, Via San Camillo de Lellis snc - 01100 Viterbo, Italy. E-mail: andrea.gentili@crea.gov.it*

A new virus, recently discovered and named *Grapevine Pinot gris virus* (GPGV), is proving very widespread in Italian north regions causing several damages in grapevine. The symptomatic plants show mottled and deformed leaves, lower growth and lack of production, particularly in white grape cultivars. GPGV has been found also in asymptomatic vines. In order to determine the presence of GPGV also in vineyards of Lazio region, field and molecular investigations were carried out on grapevine samples collected from several symptomatic and asymptomatic plants. Specifically, one hundred and thirty samples, belonging mainly to local but also to national and international varieties, were collected in six different vineyards located in the three principal viticultural areas of Lazio (Castelli romani, Viterbese and Maccarese). Total RNAs extracted from leaves were amplified with specific primers targeting the virus movement protein (MP) gene; several plants of 'Vermentino', 'Sagrantino' and 'Sauvignon' showing either suspected symptoms or no specific symptoms (all from Maccarese area) were found positive in RT-PCR. Molecular characterization, by nucleotide sequence analysis of coat protein (CP), movement protein (MP) and replicase (Rep) genes of three GPGV isolates, was carried out in order to verify the similarity with the others GPGV isolates retrieved from the NCBI database.

33. MOLECULAR SCREENING OF THE NOVEL TYPE A TRICHOTHECENE MYCOTOXIN NX-2 IN *FUSARIUM GRAMINEARUM* ISOLATED FROM WHEAT WORLDWIDE. **V. Ghionna, A. Susca, A.F. Logrieco, A. Moretti.** *Institute of Sciences of Food Production, National Research Council of Italy, Via Amendola, 122/O - 70126 Bari, Italy. E-mail: veronica.ghionna@ispa.cnr.it*

Fusarium head blight (FHB) is an important disease of wheat worldwide, causing a significant reduction in grain yields and global concern for human health due to mycotoxin contamination on kernels at harvest. Members of the *Fusarium graminearum* species complex (FGSC) are the main agents of the disease. Fusarium mycotoxins include trichothecenes, terpens compounds characterized by a 12, 13epoxytrichothec-9-ene ring system. They are potent inibithors of proteic synthesis, and divided in type A and B according with the absence/presence of a carbonyl function group at C-8 of the ring. Every species of FGSC is capable of producing B-trichothecenes *in planta*. Recently, a novel type A trichothecene termed NX-2 has been detected from *F. graminearum* (*sensu strictu*) cultures isolated in northern United States. NX-2 is similar to 3-ADON, lacking the carbonyl group in C-8. Genetic analysis revealed a different *TRI1* allele in the isolates, responsible for the difference in hydroxylation at C-8. In this study, we used *TRI1* gene to develop specific PCR-based assays, for the detection of NX-2 genotype. A total of 95 strains of *F. graminearum* isolated from samples of wheat collected in Austria, Germany, China and different geographical areas of Italy were amplified by using specific primers in order to investigate their potential capability of producing this novel trichothecene type A.

34. LOQUAT DECLINE IN ITALY CAUSED BY BOTRYOSPHAERIACEOUS FUNGI. **S. Giambra[1], G. Piazza[2], A. Alves[3], V. Mondello[4], M. Berbegal[5], J. Armengol[5], S. Burruano[1].** *[1]Dipartimento di Scienze Agrarie e Forestali, Università di Palermo, Viale delle Scienze 4 - 90128, Palermo, Italy. [2]Scuola Superiore Sant'Anna, Istituto Scienze della Vita, Piazza Martiri della Libertà 33, 56127, Pisa, Italy. [3]Departamento de Biologia, CESAM, Universidade de Aveiro, Campus Universitario de Santiago, PT-3810-193 Aveiro, Portugal. [4]Département Laboratoire Stress, Défenses et Reproduction des Plantes - Université De Reims Champagne - Ardenne, France.* **[5]***Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain. E-mail: santella.burruano@unipa.it*

Branch cankers on loquat (*Eriobotrya japonica* Lindl.), caused by species belonging to the family Botryosphaeriaceae, have been recently reported in the main producing areas of Spain. In spring 2014 and 2015, in a Sicilian loquat field where autochthonous and allochthonous cultivars were introduced, similar symptoms were observed. About fifty percent of allochthonous Algerie and Bueno trees surveyed showed the declining syndrome. Different fungal colonies were isolated from branch cankers and were identified according to morphological macroscopic (color, texture and radial growth of mycelium) and microscopic features (shape, color and dimension of conidia). The obtained botryosphaeriaceous isolates were identified as *Diplodia seriata, Neofusicoccum parvum*, *N. vitifusiforme* and *Diplodia* sp. The species identity was confirmed by analysis of the internal transcribed spacer (ITS) region and partial translation elongation factor 1-α (EF1-α) sequences. Moreover *Diplodia* species were associated with loquat cv. Bueno whereas *Neofusicoccum* species with loquat cv. Algerie. The isolates were inoculated *in planta* on stem with mycelium plugs (2-year-old loquat plants of cv. Sanfilipparo). Apart from two isolates of *N. vitifusiforme*, the other isolates caused vascular discoloration, ranging from 21.0 to 83.8 mm. Significant lesion length differences were revealed among the four species and, occasionally, among isolates of the same species. All inoculated fungi were re-isolated, fulfilling Koch's postulates.

35. CHEMICAL AND BIOLOGICAL MANAGEMENT OF POWDERY MILDEW OF ZUCCHINI AND OF PHOMA LEAF SPOT OF LEAF BEET UNDER A CLIMATE CHANGE SCENARIO. **G. Gilardi, U. Gisi, M.L. Gullino, A. Garibaldi.** *AGROINNOVA, Centre of Competence for Agro-Environmental Innovation, University of Torino, Largo Paolo Braccini 2, 10095, Grugliasco, Torino, Italy. E-mail: giovanna.gilardi@unito.it*

Although there is ample evidence that the climate is changing, there is still no consensus on the nature and magnitude of the long-term impacts and on the geographical distribution of such changes. This study has been carried out by simulating climate changes under phytotron conditions and it provides new information on the effect of combining an increase in temperature and CO_2, on four fungicides and one microbial treatment, using zucchini-*Podosphaera xanthii* and leaf beet-*Phoma betae* pathosystems as models. Six CO_2 and temperature combinations have been tested to establish their effect on chemical and biological control measures. Four experimental trials have been conducted for each pathosystem. Penconazole and sulphur resulted in a significant powdery mildew control, corresponding to 84.4 to 92.5%. Penconazole has provided the best protection against zucchini powdery mildew with 800 ppm of CO_2. Increases in CO_2 have significantly improved the efficacy of *Ampelomices quisqualis*. The effectiveness of both mancozeb and azoxystrobin against Phoma leaf spot has been affected by high levels of CO_2. Mancozeb improved disease control from 74.9% in 450 ppm of CO_2 to 86.2% in 850 ppm of CO_2 at 18-22°C and from 73.5% in 450 ppm of CO_2 to 88.8% in 850 ppm of CO_2 at 22-26°C. Azoxystrobin gave a better Phoma leaf spot reduction at 22-26°C with an efficacy from 42.3% in 450 ppm of CO_2 to 63.2% in 850 ppm of CO_2. More attention should be paid to evaluating the efficacy of chemical and biological control measures considering the predicted climate changes.

36. DEVELOPMENT OF PRACTICAL TOOLS FOR THE MONITORING AND THE CONTROL OF THE INVASIVE PLANT PATHOGEN *HETEROBASIDION IRREGULARE* IN CENTRAL ITALY. **L. Giordano[1,2], G. Lione[1], F. Sillo[1], 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. E-mail: paolo.gonthier@unito.it*

The North American forest pathogen *Heterobasidion irregulare* was accidentally introduced in central Italy in 1944. The fungus is invasive and is currently distributed in pine and oak stands over about 105 km of coast around Rome, often in association with significant mortality of *Pinus pinea* trees. Since the complete eradication of the pathogen appears an unrealistic management option, an integrated disease management program would be crucial in order to minimize the risk of spread of *H. irregulare* outside the current zone of infestation and reduce infection rates. Within the EMPHASIS project (Effective Management of Pests and Harmful Alien Species – Integrated Solutions), funded by the European Commission in the frame of Horizon 2020 Research and Innovation Program, practical experiments for the optimization of both local eradication, through uprooting and destumping, and biological and chemical control against the invasive pathogen will be carried out. A target monitoring method for the rapid and sensitive detection of *H. irregulare* based on LAMP technology will be also designed. Research activities, organized with the support of local stakeholders, will be performed in some selected sites located in the Lazio Region (central Italy) including: i) the pine stand "La Gallinara", ii) the oak stands of Anzio and Nettuno and iii) the oak-pine mixed stand of Castelfusano.

37. ELICITATION OF RESISTANCE TO FIRE BLIGHT BY A MICROBIAL CONSORTIUM: SEARCH FOR AN EVIDENCE THROUGH A TRANSCRIPTOMIC APPROACH. **D. Giovanardi[1], V. Catalano[1,2], E. Verzelloni[1,2], L. Dondini[3], E. Stefani[1].** *[1]Dept. of Life Sciences, University of Modena & Reggio Emilia, Via Amendola 2 - 42122, Reggio Emilia, Italy. [2]CCS Aosta Srl, Fraz. Olleyes 9 - 11020, Quart (AO), Italy. [3]Dept. of Agricultural Sciences, University of Bologna, Viale Fanin 46 - 40127, Bologna, Italy. E-mail: davide.giovanardi@unimore.it*

Fire blight is the most destructive bacterial disease of pome fruits: it is caused by *Erwinia amylovora* and its management remains cumbersome. Nowadays, treatments with several beneficial microorganisms can lead to an affective integrated pest management (IPM): nevertheless, their specific activity in enhancing plant defence mechanisms is only partially understood. An extensive 3-years study in a commercial pear orchard was carried out, to verify the efficacy of a commercial microbial consortium (Micosat F®, CCS Aosta srl, Italy) to control a fire blight outbreak. In parallel, we have used a dHPLC (denaturing High Performance Liquid Chromatography) and automated DNA fragment collection using the WAVE System to analyse and recover cDNA-AFLP fragments. This transcriptomic approach was applied to understand which complex transcriptional changes these microorganisms may

have elicited in the plant-pathogen interaction. In the commercial orchard, the beneficial effects of microbial consortium were confirmed by a significant disease reduction starting from the first year of application, as compared with other treatments commonly used by orchardists in IPM strategies. Among the eighty-five transcript-derived fragments (TDFs) collected and identified through the cDNA-AFLP-dHPLC approach, fourteen were found involved in systemic acquired resistance (SAR) according to the available literature. The transcriptomic approach developed in this study has been a robust and user-friendly mRNA fingerprinting method for the identification of differentially expressed genes, where prior knowledge of specific gene sequences is not a prerequisite. Finally, we confirmed the activity of such the microbial consortium as resistance inducer.

38. DEVELOPMENT OF A STABLE TRANSFORMATION PROTOCOL TO INVESTIGATE THE ROLE OF A LECTIN GENE IN THE SUSCEPTIBILITY OF STRAWBERRY FRUITS TO *COLLETOTRICHUM ACUTATUM*. **M. Guidarelli[1], E.G. Nagpala[1], R. Cappelletti[2], S. Sabbadini[2], P. Bertolini[1], B. Mezzetti[2], E. Baraldi[1].** *[1]Università degli Studi di Bologna, Dipartimento di Scienze Agrarie (DipSA), Viale Fanin 46 - 40127, Bologna, Italy; [2]Università Politecninca delle Marche, Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Via Brecce Bianche 10 - 60131, Ancona, Italy. E-mail: elena.baraldi@unibo.it*

The fungal pathogen *Colletotrichum acutatum,* the causal agent of strawberry (*Fragaria×ananassa*) anthracnose, can infect strawberry fruit hosts at pre-harvest unripe stages becoming quiescent until fruit ripen, causing anthracnose symptoms only on red ripe fruit. In order to understand the molecular basis of the low susceptibility of white unripe fruits, the role of a gene encoding for a lectin protein that becomes overexpressed in white fruits upon *C. acutatum* inoculation, has been investigated by developing an *Agrobacterium*-mediated stable transformation protocol, for producing strawberry plants silencing and overexpressing this gene. Exploiting the potential of plant species to silence genes when recognize intron-containing constructs encoding self-complementary 'hairpin' RNA (ihpRNA), a construct containing the partial sense and corresponding antisense sequences of *FaMBL* (*Fragaria×ananassa Mannose Binding Lectin)* gene separated by an intron was generated. On the other hand, to overexpress *FaMBL*, a vector containing the gene under the control of 35S promoter was constructed. Since the regeneration of transformed strawberry plants is strongly dependent from the genotype, genetic transformation was performed in two strawberry cultivars, Calypso and Sveva. The different steps of the protocol related to the leaf tissue *Agrobacterium* infection, selection of regenerating adventitious shoots, as well as the identification of selected lines capable to proliferate on the selective agent (kanamycin), will be described.

39. NEW REPORTS OF *PHYTOPHTHORA* spp. ON WOODY PLANTS. **A. Haegi, S. Vitale, L. Luongo, M. Galli, A. Belisario.** *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22 - 00156 Rome, Italy. E-mail: alessandra.belisario@crea.gov.it*

In the recent years, new findings of *Phytophthora* species causing diseases on woody plants have been reported in Italian commercial nurseries. *Phytophthora megasperma* has been isolated from declining and dead European hackberry (*Celtis australis*) and English (Persian) walnut (*Juglans regia*) trees. In commercial fruit orchards, also *P. gonapodyides* was detected as causal agent of sudden death of well-developed 7-year-old English walnut trees. Although this species is mainly known as a minor pathogen, there are reports indicating that some isolates can be highly virulent as in the present case. In addition, *Phytophthora* species were obtained from soil, root baiting and collar of declining *Albizia julibrissin* plants grown in soil in a commercial nursery. On the basis of morphological and molecular features *P. tropicalis* was identified together with an undescribed species, named *P.* taxon *albizia,* still under investigation. It is becoming increasingly evident that natural interspecific hybridization is a casual event in heterothallic *Phytophthora* evolution which could lead to an increase of virulence. The rapid increase of plant international trade, due to the market globalization, has allowed the introduction of several alien *Phytophthora* species which represent a continuous threat to the sanity of nursery plant material. On this context, climatic changes favour the spread and the settlement of the alien pathogens.

40. VOLATILE ORGANIC COMPOUNDS ANALYSIS FOR THE IDENTIFICATION OF DIFFERENT *FUSARIUM* spp. PRODUCER OF MYCOTOXIN IN CEREALS. **A. Infantino[1], C. Costa[2], M. Aragona[1], M. Reverberi[3], M. Beccacioli[3], C. Taiti[4], S. Mancuso[4].** *[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 per la ricerca in agricoltura e l'analisi dell'economia agraria, Unità di ricerca per l'ingegneria agraria (CREA-ING) - Via della Pascolare 16 - 00015 Monterotondo scalo, Rome, Italy. [3]Università degli Studi di Roma "La Sapienza", Dipartimento Biologia Ambientale, P.le Aldo Moro 5 - 00185 Roma. [4]Università degli Studi di Firenze, Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente - Viale delle Idee, 30 - 50019 Sesto Fiorentino, Florence, Italy. E.mail: alessandro.infantino@crea.gov.it*

Volatile organic compounds (VOCs) are a large group of chemicals with low molecular weight and high vapor pressure of different chemical origin produced during the primary and secondary metabolism of microorganisms such as fungi or bacteria. Proton-Transfer-Reaction Time-of-Flight (PTR-ToF-MS) technology is an innovative technique for VOCs detection in several substrates, including mycotoxins-infected foodstuff. Recently, it allowed for discriminating wheat samples with DON concentration values above/below the legal limit. In the present study, 18 isolates belonging to 9 different *Fusarium* species, were identified by means of Elongation Factor specific primers and characterized for the volatile profile. Two *Trichoderma* spp. and two *Pyrenochaeta lycopersici* isolates were used as outgroup. The mycotoxin profile of the *Fusarium* spp. isolates has been analyzed by a multi components approach in LC-MS/MS. Each fungal sample has been grown on Potato Dextrose Agar in glass Petri dishes (6 cm diameter) and subsequently introduced in a glass jar (250 ml) respectively connected with the PTR and zero air generator for VOC analysis. A PLS-DA multivariate modelling approach was applied to discriminate among the different species tested (13 groups). The overall results showed the ability of PTR-ToF MS to positively classify *Fusarium* spp. at 79.4% in the independent internal test showing problems of classification for three out of 9 species tested. The PTR-ToF-MS represents a new alternative tool for fungal identification and detection in complex environments. Chemotyping based on VOC emissions and the correlation with mycotoxyns produced could be utilized as a rapid and diagnostic tool for early fungal infection stages on naturally contaminated cereal foodstuff.

41. MICROBIOME ANALYSIS OF BIOFUMIGATED SOILS FOR THE CONTROL OF CORKY-ROOT OF TOMATO IN ITALY. **A. Infantino[1], S. Mocali[2], A. Haegi[1], S. Vitale[1], C. Chiellini[2],**

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Biofumigation performed with the use of *Brassica* spp. is an effective and interesting tool for the control of soil borne pests, pathogens and nematodes. The high content of glucosinolate in several brassicas biomasses could provide a natural and practical alternative to chemical fumigants. Nevertheless, few studies focusing on the effect of biofumigation on total soil microbiota are available. Next-generation sequencing (NGS) technology provides new insights for the analysis of microbial species diversity in cultivated soils. In particular NGS techniques could help in understanding the role of biofumigation in shaping the microbial community, shedding light on its role in the control of soil-borne diseases. In the present work, biofumigation was used for the control of *Pyrenochaeta lycopersici* (corky-root), an important soil-borne pathogen of tomato. The efficacy of fresh biomass incorporation as green manure, the use of pellets and of liquid formulations were compared in greenhouse where tomatoes for fresh market were grown in 2015. Fungal and bacterial communities were evaluated through amplicon (16S, ITS) sequencing of soils samples collected in different times. The overall results showed a significant effect of green manure on the total yield and on the reduction of disease severity. Changes in microbial community structure after biofumigation using non-metric multidimensional scaling (NMDS) of NGS data were positively correlated with yield parameters.

42. SEPTORIA DISEASE COMPLEX ON DURUM WHEAT CULTIVARS GROWN IN ORGANIC FARMING.

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A study was carried out on six durum wheat varieties, cultivated in organic farming in different Italian experimental fields, to analyze their resistant or susceptible behaviour against Septoria Disease Complex (SDC). This complex includes two major septoria diseases: Septoria tritici blotch and Stagonospora nodorum blotch caused by *Zymoseptoria tritici* and *Parastagonospora nodorum*, respectively. Data were obtained from eleven years of trials. Heavy SDC attacks were observed in the locations of Northern Italy in particular in the years: 2007, 2008, 2009 and 2013. In those years all tested cultivars had attacks of more than 50%, except one that showed slightly lower infections. In the fields of Central Italy, the complex was present with higher attacks in 2008, 2010 and 2015. Much less was the presence of SDC in Southern Italy and the Islands, where generally the diseases pressure caused by different biotic stresses is lower. Infected leaves from different fields were collected and microscopic analysis were carried out in order to identify the two pathogens. In conclusion, the cultivars showed a similar behaviour against the SDC; it was evident that all cultivars were moderately or completely susceptible to one or both pathogens of the complex in the years with epidemic presence of fungi.

43. EFFECTS OF ORGANIC FEEDSTOCKS AND THEIR BIOCHARS ON GROWTH OF PHYTOPATHOGENIC FUNGI, SOILBORNE BACTERIA, AND CROP PLANTS.

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A number of recent studies show that biochar may significantly reduce the incidence of plant diseases caused by airborne and soilborne pathogens. In this work we investigated how biochars affect plant growth and microbial activity in soil. Specific aims were i) to detect the chemical changes occurring in four feedstocks (e.g. wood chips, urban organic waste, *Zea mais* leaves and stems, and *Medicago sativa* hay) when pyrolyzed at two temperatures (300 and 550 °C) by using ^{13}C NMR spectroscopy; and ii) to assess how biochars influence growth of fungi, bacteria, and crop plants. As pyrolyzation temperature increased, organic matter chemistry of all four feedstocks significantly changed, with a progressive loss of O-alkyl C, di-O-alkyl C, and methoxyl and N-alkyl C, coupled with an enrichment in aromatic C. We found that undecomposed urban waste and *Medicago* hay cause a severe inhibition of *Lepidium*, *Lactuca* and *Lycopersicon* plant root growth, while no inhibitory effects were found for the other feedstocks. However, the inhibitory effect largely decreased after pyrolyzation. In contrast to the higher plants, fungi and bacteria throve on most of the unprocessed organic materials but showed a steep decline or a complete inhibition of growth on biochars obtained at 300°C and 550°C. Twelve out of fourteen tested microbes, with the exception of two basidiomycetes, showed a remarkably similar pattern of correlations between growth and organic material chemistry. These results suggest that biochars can be useful in plant disease control.

44. UNDERSTANDING THE ROLE OF THE *ASPERGILLUS FLAVUS* CLUSTER 32 DURING THE PATHOGENESIS PROCESS ON MAIZE.

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Aspergillus flavus is an opportunistic and saprophytic crop pathogen mostly known as a strong producer of aflatoxins, a highly carcinogenic mycotoxins. In fungi, the genes involved in secondary metabolism are clustered. We focused our studies on the cluster 32, and specifically within this cluster, on a salicylate hydroxylase (SalOH) and on a necrosis and ethylene inducing protein (nepA) to clarify their role within the process of pathogenesis. Trying to understand the role of *SalOH* and *nepA* genes during the pathogenesis process, we inoculated maize kernels *in vitro* with a suspension of asexual spores of *A. flavus* 3357 (WT) and we followed the progress of infection from 14 hours to 7 days. So we are analyzing the expression profiles of these genes during the process of colonization of inoculated corn kernels; and, in parallel, we are carrying out histological studies to follow the progression of the fungus into the host tissues in order to understand if the change in the expression pattern of these genes is prodromal for invading living tissues (e.g. aleurone) of maize kernel.

45. THE USE OF PLANT BIOSTIMULANTS TO IMPROVE THE SUSTAINABILITY IN AGRICULTURE.

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The improvement of sustainability in agriculture is very important to protect the environment and human and animal health. Adequate practices including the use of plant biostimulants can contribute to achieve this objective. Plant biostimulants are defined as products stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant: nutrient use efficiency, tolerance to abiotic stress, or crop quality traits. This definition is reported in the proposal for a new regulation, that will lay down rules on the making available on the market of CE marked fertilizing products. This regulation, including the category of plant biostimulants, will repeal the existing Fertilisers Regulation No 2003/2003. It will remove the market fragmentation and will harmonize the situation on these substances that are currently subject to national rules in Europe. This type of substances are also used in non-European countries: in USA they are regulated at the state level and there are not a harmonized legislation, in Canada they fall into the category of supplement defined as any substance or mixture of substances, other than a fertilizer, that is manufactured, sold or represented for use in the improvement of the physical condition of soils or to aid plant growth or crop yields and in South Africa they fall into "Group 3 Fertilizers" defined as natural or synthetic substance or organism/s that improve/s the growth or yield of plants or the physical, chemical or biological condition of the soil.

46. EVALUATION OF ANTIFUNGAL AND ANTI-OOMYCETE ACTIVITIES OF ESSENTIAL OILS. A. La Torre, F. Caradonia, L. Righi, V. Battaglia. *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA-PAV), Via C.G. Bertero 22 - 00156 Roma, Italy. E-mail: anna.latorre@crea.gov.it*

The objective of this study was to evaluate the effect of essential oils of *Lavandula officinalis*, *Tagetes minuta* and their major components in laboratory tests (mycelial growth and spore germination) against *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *lycopersici* and *Phytophthora infestans*. The activity of two essential oils was also carried out under greenhouse condition against Fusarium wilt in tomato plants cv. Cuore di bue sel. Albenga. Tomato seedlings were transplanted into pots containing pasteurized soil after the roots had been dipped in a conidial suspension of *F. oxysporum* f. sp. *lycopersici.* Immediately after transplanting, the treatments were carried out in the soil surrounding the roots of tomato plants with the essential oils at various concentrations. Control treatments were also carried out. The obtained results indicated that essential oils and their main components were able to control mycelial growth and spore germination. The best inhibitory effect were achieved by lavender oil followed by its major components, while the least inhibitory activity on *P. infestans* and on all tested fungi was observed by ocimene, the main constituent of *T. minuta.* Greenhouse pot experiments showed antifungal effect of the two essential oils. In conclusion, the essential oils may have an important role in management of phytopathogenic fungi and oomycetes. Their use for disease control could represent an environmentally safe alternative to synthetic pesticides in agriculture.

47. INSECTICIDAL SECONDARY METABOLITES FROM THE FUNGAL ENDOPHYTE *TALAROMYCES PINOPHILUS*. F. Lacatena[1], R. Nicoletti[2], F. Borrelli[3], S. Finizio[3], B. Romano[3], O.A. Parisi[3], M. Pascale[1], M.C. Digilio[1], M. Giorgini[4], M. Lorito[1,4], S.L. Woo[1,4]. *[1]Università degli Studi di Napoli Federico II, Dipartimento di Agraria, Via Università 100 - 80055 Portici (NA), Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria. [3]Università degli Studi di Napoli Federico II, Dipartimento di Farmacia, Via D. Montesano 49, Napoli, 80131, Italy. [4]Istituto per la Protezione Sostenibile delle Piante (IPSP-CNR), Via Università 133 -80055 Portici (NA), Italy. E-mail: federica.lacatena@unina.it*

The biodiversity of the endosymbiont-plant system represents an important source of microorganisms able to produce bioactive compounds that can induce beneficial effects on cultivated plants, and be useful in agriculture. Fungal endophytes were obtained from the strawberry tree (*Arbutus unedo*) microbiome. From the fungus, *Talaromyces pinophilus* (TP1), total extracts from the culture filtrate were produced and tested for possible biological effects to insect pests, such as the pea aphid (*Acyrthosiphon pisum*). The natural compounds produced by TP1 were analyzed by using a metabolomic approach with a LC-MS QTOF system, then the separated compounds were screened and identified by comparison to a fungal metabolite database. The main compound identified was 3-O-methylfunicone (OMF), which was then purified, fully characterized by spectrometric methods (UV-Vis, MS, mono and bi dimensional NMR), then tested for insecticidal activity to aphids in a *Vicia faba* bioassay system. The experiments were carried out by spraying a 1 ml OMF (conc. 0.5 mg/ml) to 20 aphid nymphs (3rd-instar), from a synchronized parthenogenetic female population, placed on circular leaf plugs and incubated at 20-22°C with 16/8 h day/night. Treatments of OMF produced a *A. pisum* mortality of 23, 25 and 48% greater than the control, when observed at 24, 48 and 72 h, respectively. Results indicate that the mutualistic interactions between endophytic fungi and plants can provide beneficial compounds with still unexplored biological activities.

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48. RNA-SEQ APPROACH TO UNVEIL MOLECULAR MECHANISMS INVOLVED IN TRICHODERMA GROWTH. S. Lanzuise[1], A. Sacco[1], M. Ruocco[3], R. Quarto[1], M. Tucci[2], M. Salzano[2], M. De Palma[2], G. Manganiello[1], A. Pascale[1], M. Senatore[1], M. Lorito[1]. *[1]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 133 - 80055 Portici (NA), Italy. [2]Istituto di Bioscienze e BioRisorse (IBB-CNR), via Università 133 - 80055 Portici, Napoli, Italy. [3]Istituto per la Protezione Sostenibile delle Piante (IPSP-CNR), Via Università 133 - 80055 Portici (NA), Italy. E-mail:stefania.lanzuise@unina.it*

Soil-plant root-beneficial microbe interactions are fundamental for plant productivity and soil health. Fungi belonging to the genus *Trichoderma* are extremely interactive with the plant and other soil microbiota, and many strains are important biological control agents in agriculture. Molecular mechanisms underlying *Trichoderma* growth are still not completely known. In order to have an in-depth understanding of the genetic mechanisms involved, particularly of genes expressed, a transcriptome profile was developed by means of RNA-seq analysis. *T. harzianum* strain T22 was grown in liquid culture, the fungal biomass was collected at three different time points (24h, 48h, 72 h), RNA extracted, sequenced and the transcriptome changings were evaluated. After processing reads, conducting the quality checks and all statistical analysis, differentially expressed genes (DEGs) were found in the time comparisons: 147 DEGs in 48 h *vs* 24 h, 603 DEGs in 72 h *vs* 24 h, and 41 DEGs in 72 h *vs* 48 h. As expected the highest number of DEGs was found in the contrast 72 h *vs* 24 h. Further analysis indicated that only seven DEGs were in common in the three time points, while 125 and 32 DEGs were in common in the 48 *vs* 24 and 72 *vs* 48, respectively. Generally, 604 out of 791 DEGs resulted as not annotated, while most of the 187 annotated DEGs encoded for hydrolytic enzymes. Further analyses and a deeper investigation of RNAseq data will shed light on the complex mechanisms regulating *Trichoderma* growth.

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49. ADVANCEMENT ON CERTIFICATION PROCESS OF GRAPEVINE CLONES IN CALABRIA. G. Leo[1], A. Gentili[2], S.B. Grande [1], G. Albanese[1], F. Faggioli[2]. *[1]Università degli Studi Mediterranea di Reggio Calabria, Dipartimento di agraria Feo di Vito, 89122 Reggio Calabria, Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero, 22 - 00156 Roma Italy. E-mail: giovanna.leo@unirc.it*

A sanitary selection of virus-free grapevine clones in Calabria region (southern Italy) has been conducted since 2010 on various autochthonous varieties. Serological (ELISA) and molecular (Multiplex RT-PCR) tests were used to detect the virus species included in the Italian certification program: *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll associated virus-1* (GLRaV-1), *Grapevine leafroll associated virus-2* (GLRaV-2), *Grapevine leafroll associated virus-3* (GLRaV-3), *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine fleck virus* (GFkV). Diagnostic analysis revealed the presence of viruses in 201 samples out of 209 tested (96.2%), mostly in mixed infection of different viruses. With respect to all found infections (352) the frequency of infection of each virus was 37.8%, 31.2%, 12.5%, 8.5%, 7.1%, 2.5% and 0.3% for GVA, GLRaV-3, GFLV, GFkV, GLRaV-1, GLRaV-2 and GVB respectively; whereas ArMV was never detected. Three putative clones of 'Arvino', and two of the rootstock '17-37' were free from all eight viruses. The healthy sanitary status of the five clones has been confirmed also by the biological assays on the woody indicators *V. vinifera* 'Cabernet franc' and 'Kober 5BB'. In parallel to the sanitary selection all healthy clones were analyzed also for their morphological and molecular characterization. Obtained genetic and sanitary results will be submitted for their official approval as "homologate clones" by the Italian Ministry of Agriculture. It is worth noting that five putative clones of 'Pecorello', four of 'Lacrima' and two of 'Magliocco Dolce' were found infected only with GFkV, virus tolerated in the *Vitis vinifera* by certification program; for this reason biological assays are currently underway to determine the definitive sanitary state of the putative clones, in order to initiate the process of approval for their homologation.

50. *SARDINIELLA URBANA* GEN. *ET* SP. NOV., A NEW PATHOGEN OF *CELTIS AUSTRALIS*. B.T. Linaldeddu[1], A. Alves[2], A.J.L. Phillips[3]. *[1]Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, Viale Italia 39, 07100 Sassari, Italy. [2]Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal. [3]Biosystems and Integrative Sciences Institute (BioISI), Faculty of Science, University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal. E-mail: ben@uniss.it*

European hackberry (*Celtis australis* L.) is a deciduous tree tolerant to dry and poor soils and is widely used for reforestation and as shade trees in parks and roadside plantings in southern Europe. In 2013, during a survey carried out throughout the main streets of Sassari (Sardinia, Italy) aimed at clarifying the causes of a decline affecting European hackberry, a collection of botryosphaeriaceous fungal strains was isolated from 14 trees with sunken cankers, wedge-shaped necrotic sectors and a progressive dieback of shoots and branches as well as collar rot and stem exudates. Although morphologically similar to *Diplodia* and *Dothiorella*, these strains differed in their colony appearance and conidial shapes from all known species of *Botryosphariaceae*. A multi-gene phylogenetic analysis based on combined LSU, ITS and EF1-α sequence data showed that these strains form a distinct lineage in *Botryosphariaceae*. Based on molecular phylogeny and morphology, a new genus named *Sardiniella*, will be introduced to accommodate the new *taxon Sardiniella urbana*. Pathogenicity was verified by wound inoculation of 3 year-old seedlings of European hackberry with four different strains. All strains caused cankers and necrotic lesions on inoculated seedlings and were successfully re-isolated, fulfilling Koch's postulates. Results from the pathogenicity test suggest that this new species is directly involved in the etiology of the observed decline in European hackberry trees as well as representing a potential risk to public safety in urban environments.

51. IMPROVING INTEGRATED PEST MANAGEMENT IN STRAWBERRY PRODUCTION USING BENEFICIAL MICROORGANISMS AND THEIR METABOLITES. N. Lombardi[1], L. De Vitto[2], S. Lanzuise[2], G. d'Errico[2], G. Manganiello[2], M. Senatore[2], P. Lombari[2], M. Lorito[1,2], S.L. Woo[1,2]. *[1]Consiglio Nazionale delle Ricerche-Istituto per la Protezione Sostenibile delle Piante (CNR -IPSP), Via Università 133 - 80055 Portici (NA), Italy. [2]Università degli Studi di Napoli Federico II-Dipartimento di Agraria, Via Università 100 - 80055 Portici (NA), Italy. E-mail: nadia.lombardi@ipsp.cnr.it*

Worldwide production of strawberry is around 2.5 million tons, whereby Europe produces 1.5 million tons, and Italy contributes about 130,000 tons. Strawberries are susceptible to numerous phytopathogens, in particular fungi that attack roots, foliage and fruits. Consequently, the main problem is the widespread and indiscriminate use of chemical pesticides, that leave residues on the fruit, cause environmental pollution and damage to human health. The biological control agents *Trichoderma* spp. are recognized for their capacity to control disease, function as fertilizers and plant-stimulants. The aim of the present work was to test Integrated Pest Management (IPM) treatments using selected *Trichoderma* and secondary metabolites (SMs), to improve the production of cultivated strawberry, *Fragaria* × *ananassa* cv. Sabrina. Plantlings were treated by a root-dip at transplant, followed by irrigation or spray treatments every 15 days until the harvest. *Trichoderma* strains and a single SM produced a growth promotion effect, increasing vegetative biomass compared to control, that led to a significant increase in fruit production. The irrigation treatments increased yield 15 to 39%, whereas the spray method increased yield 25 to 37%. A SM spray application also augmented the sugar content (degree Brix) in the harvested fruit. Results indicate that selected beneficial microbes and their bioactive compounds can be incorporated in an IPM regime to produce enhanced benefits for the cultivation of strawberries. This biological diversification in crop protection is important in light of recent European and national legislation that imposed less chemicals for agricultural production.

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52. COMPARISON OF THREE ARTIFICIAL METHODS FOR THE RE-INOCULATION OF BACTERIAL ENDOPHYTES IN MICROPROPAGATED *MALUS DOMESTICA* (BORKH) PLANTLETS. S. Lòpez-Fernàndez[1,2], F. Pedrazzoli[3], A. Campisano[1], L. Covelli[1]. *[1]Fondazione Edmund Mach, Research and Innovation Centre. San Michele all'Adige (TN), Italy. [2]Technische Universität Braunschweig. Institute of Microbiology, Braunschweig, Germany. [3]Fondazione Edmund Mach, Technology Transfer Centre. San Michele all'Adige (TN), Italy. E-mail: lauratiziana.covelli@fmach.it*

Apple proliferation (AP) is a devastating phytoplasmal disease in Europe. The etiological agent, *Candidatus (Ca.)* Phytoplasma mali, is mainly vectored by the psyllids *Cacopsylla melanoneura* and *C. picta.* Healthy, symptomatic and infected but symptomless plants can be present in the field at the same time. Some AP-infected symptomatic plants show a spontaneous remission of symptoms ("Recovery" phenomenon), whose mechanism remains to be

determined. Factors involved could include host genotype, strain specificity and plant microbiome.

Endophytes are harmless bacteria that dwell inside the plant and protect it from pathogens. Given that artificial inoculation of endophytes has been successful in other plants, we decided to investigate their potential as biocontrol agents of AP disease. We thus compared the effectiveness of three methods of endophyte re-introduction in micropropagated *M. domestica* plantlets. We inoculated the bacterium *Pantoea vagans* PaVv7 strain bearing the GFP-encoding plasmid pMP4655 through injection with a hypodermic syringe, micro-spraying and drop-soaking of the roots.

Our results suggest that both the injection and spray inoculation were the more effective methods, with a recovery of 3.78×10^4 CFU/ml and 2.4×10^3 CFU/ml respectively, in contrast to the drop-soaking, where just few cells were recovered. Extraction from plants and plating of the bacterium in selective media and microscopic observations confirmed the re-isolation of the strain and PCR tests confirmed the presence of the pMP4655 plasmid. Our findings show these methods as alternatives to check performance of apple associated bacterial endophytes as plant protection agents against *Ca.* P. mali and to control the disease in AP-infected ones.

53. PRELIMINARY CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* HR-NEGATIVE STRAINS. S. Loreti, A. Pompi, A. Gallelli, N. Pucci, S. Talocci, A. L'Aurora, C. Morone[1], A. Brunetti, M. Pilotti. *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA) - Centro di ricerca per la Patologia Vegetale, V. C.G. Bertero 22, 00156 Roma. [1] Servizio Fitosanitario Regione Piemonte, via Livorno 60, 10144 Torino. E-mail: stefania.loreti@crea.gov.it*

Strains of *Pseudomonas syringae* pv. *actinidiae* (Psa), causal agent of bacterial canker of kiwifruit, grouped in several biovars that differed for biochemical, genetic and pathogenicity characteristics, but all resulted positive at the hypersensitivity of tobacco (HR). HR is one of the LOPAT test used for the identification of pathogenic *P. syringae* pathovars. Twenty-three isolates with the same characteristics of Psa, but HR-negative, were recovered in several Italian regions. These isolates responded as the reference Psa strains ISF8.43 and CREA-PAV1530 (biovar 3) at LOPAT and other nutritional-biochemical tests and revealed a similar genomic-fingerprinting by rep-PCR analysis; conversely, by *in vitro* inoculation in leaf-disc assay did not show the developing of symptoms, with respect Psa reference strains. These results highlighted that Psa strains deficient in both HR and pathogenicity can be recovered from bacterial canker-affected kiwifruit. The ability to cause HR is a requirement for virulence of phytopathogenic Pseudomonads and pathogenicity expression of *P. syringae* depends on a functional type-III secretion system. While further studies are under way, it could be supposed that a spontaneous HR-deficient variant may be advantageous in host tissue, because it does not challenge the plant-defence mechanism and also have a better chance than the HR-positive strains for survival during decline of the population in a late stage of plant colonization. Therefore the possibility of identifying Psa HR-negative strains, has to be considered also for diagnostic purposes, since the HR tests is one of the assays suggested by the Standard EPPO PM 7/120 (1) for the identification of this quarantine pathogen.

54. TRANSCRIPTIONAL PROFILES OF TOMATO PLANTS INFECTED WITH TYLCSV OR EXPRESSING THE CENTRAL TYLCSV REP PROTEIN DOMAIN UNCOVER CHANGES IMPACTING PATHOGEN RESPONSE AND SENESCENCE. A. Lucioli[1], C. Perla[1,2], A. Berardi[1], F. Gatti[1], L Spanò[2], M. Tavazza[1]. *[1] ENEA, SSPT-BIOAG, Via Anguillarese 301 - 00123- Rome, Italy. [2] Università degli Studi dell'Aquila, Department of Physical and Chemical Sciences, Via Giovanni di Vincenzo - 67100 L'Aquila, Italy. E-mail: mario.tavazza@enea.it*

To establish a successful infection viruses need to overcome plant immune responses and redirect host gene expression for their benefit. *Tomato yellow leaf curl Sardinia virus* (TYLCSV) is a geminivirus that causes significant economic losses in tomato. The replication associated protein of geminivirus (Rep) plays a key role during viral infection. In particular, the Rep central domain spanning from aa 120 to 180 is known to interact with host factors. Here, we used longSAGE to analyse the transcriptional profiles of transgenic tomato plants expressing the first 210 amino acids of TYLCSV Rep (Rep210) and TYLCSV-infected wild-type tomato plants (Wt-Ty). We identified 118 and 203 differentially expressed genes (DEGs) in Wt-Ty and Rep210 plants, respectively. Of note, 55% of Wt-Ty DEGs were in common with Rep210, and no ones showed opposite expression. TYLCSV- and Rep210-repressed but not induced genes overlapped with the leaf senescence process. TYLCSV upregulates expression of genes involved in the negative regulation of programmed cell death (PCD), several of which were also regulated by ABA whereas Rep210 upregulated genes related to defence response, immune system processes and negative regulation of PCD. Importantly, comparison of Wt-Ty and Rep210 RNA profiles with those of transgenic Rep130 tomatoes highlighted a negligible overlap among DEGs, thus indicating the pivotal role of the central Rep domain in redirecting host plant gene expression.

55. EPIDEMIOLOGICAL STUDY ON *ONION YELLOW DWARF VIRUS* IN ONION CULTIVATION. A. Manglli[1,2], L. Tomassoli[2], G.E. Agosteo[1], A. Tiberini[1], G. Albanese[1]. *[1] Università degli Studi Mediterranea di Reggio Calabria, Dipartimento di AGRARIA, Località Feo di Vito - 89122 Reggio Calabria, Italy. [2] Consiglio per la ricerca in agricoltura e lo studio dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22 - 00156 Roma, Italy. E-mail: manglliariana@gmail.com*

A recent survey on viral diseases of onion cv. Rossa di Tropea in Calabria (Southern Italy) showed that *Onion yellow dwarf virus* (OYDV, *Potyvirus*) is the most important virus in terms of spread and damage for this crop. Therefore, according to the biennial onion life cycle, disease monitoring was performed during two growing seasons: from seed to bulb production and from bulb to seed production. OYDV-infection was 1.8% in seedlings, increased up to 6.5% during the growing stage of onion plants, and reaching 37.5% near the bulbs harvesting. A representative number of bulbs coming from such cultivation were tested, resulting with an infection rate of 17.85%. In the next crop growing for true seed production, originated from the above checked stock of bulbs, rate infection increased becoming 100% at the time of seed-collection. As compared to late OYDV-infected plants, early OYDV-infected plants significantly reduced in number and weight of seeds per inflorescence. According to the data collected during this investigation, some control measures need to contrast OYDV spread and incidence. Anyway, the production area of 'Rossa di Tropea' onion is under severe threat by this viral disease which could compromise this IGP product for quantity and quality behaviors. To improve knowledge on this issue a research (SI.ORTO-RBSI149LD5 granted by Italian Ministry of Education, University and Research in the 'Scientific Independence of young Researchers' program) has been purposely activated to study OYDV effect on nutraceutical compounds of 'Rossa di Tropea' onion using -omics approaches.

56. THE DEACETYLATION OF CHITIN IN BOTRYTIS CINEREA INFECTION CUSHIONS COULD MASK THE FUNGUS FROM THE ACTION OF PLANT CHITINASES. **R. Marcato[1,2], M. Choquer[2], C. Bruel[2], L. Sella[1], F. Favaron[1], N. Poussereau[2].** *[1]Dipartimento Territorio e Sistemi Agro-Forestali (TeSAF), Research group in Plant Pathology, Università di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. [2]Equipe de Génomique Fonctionnelle des Champignons Pathogènes des Plantes (FungiPath) (Labo. Mixte Université LYON 1 / CNRS / BAYER CropScience). BAYER S.A.S., 14 impasse Pierre Baizet, BP 99163, 69263 Lyon Cedex 09, France. E-mail: francesco.favaron@unipd.it*

The ascomycete *Botrytis cinerea* represents a model organism for the study of necrotrophic fungal pathogens. As all the higher fungi, *B. cinerea* possess a complex and dynamic multilayered cell wall involved in crucial aspects of fungal life and pathogenicity. During plant infection, polysaccharide components functioning as PAMPs are released from the fungal cell wall. Here we report the immunolocalization of several polysaccharides (β-glucan, α-glucan, mannoproteins, chitin and chitosan) in the wall of *B. cinerea* hyphae and infection cushions. β-glucan, α-glucan and mannoproteins seem present in both hyphae and infection cushions. On the contrary, chitin and chitosan seem to have a different localization: chitin is present prevalently along vegetative hyphae and is prevalently abundant at apex tip and septa, while chitosan, the deacetylated form of chitin, is mainly present in infection cushions. In comparison to chitinase, chitosanase activity is rarely reported in plants and docking experiments show that chitinase has higher affinity for chitin than for chitosan. Therefore, the prevalence of chitosan in infection cushions may reduce the fungal cell wall degradation by plant chitinases and the release of chitin oligomers biologically active in triggering the defence responses.

57. INHIBITORY EFFECT OF SIXTEEN NATURAL COMPOUNDS AGAINST *BOTRYTIS CINEREA*. A STUDY OF STRUCTURE-PROPERTY-ACTIVITY RELATIONSHIP. **R. Marcato, L. Sella, F. Favaron.** *Dipartimento Territorio e Sistemi Agro-Forestali (TeSAF), Research group in Plant Pathology, Università di Padova, Viale dell'Università 16 - 35020 Legnaro (PD), Italy. E-mail: francesco.favaron@unipd.it*

Natural compounds can be used as alternative to synthetic fungicides, mostly to control pathogens that damage fruit and vegetables during postharvest stage. Among 16 plant natural compounds (eugenol, isoeugenol, thymol, carvacrol, cinnamaldehyde, vanillin, quercetin, catechin, emodin, ferulic acid, caffeic acid, p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, veratric acid and estragole) assayed against *B. cinerea*, only eugenol, thymol, cinnamaldehyde, isoeugenol, and carvacrol strongly inhibited the fungal mycelium growth and conidial germination. Furthermore, these compounds cause the release of cellular material from *B. cinerea* mycelium, indicating possible damages of cell membrane. A QSAR (Quantitative structure–activity relationship) analysis conducted on these more effective molecules reveals that parameters such as Hy (*Hydrophilic factor*), tPSA (*topological polar surface area*), AMR (*molar refractivity*) and HBD (*hydrogen bond donors*) are correlated to biological activity of these compounds. Eugenol, thymol, cinnamaldehyde, isoeugenol, and carvacrol have negative value of Hy, relative low value of tPSA and a HBD value of one. These parameters suggest that these active compounds have affinity for lipophilic structure of fungal cell and they might have their target in the plasmalemma. Furthermore, the absence of synergistic effects and similar values of AMR suggest that eugenol, thymol, cinnamaldehyde, isoeugenol, carvacrol could specifically interact with the same target proteins.

58. EFFECTS OF BENEFICIAL MICROBES AND THEIR BIOACTIVE COMPOUNDS ON FITNESS AND METABOLIC PROFILES OF OLIVES. **R. Marra[1], F. Vinale[1], M. Senatore[2], C. Gigliotti[3], F. Grasso[1], S. Bolletti Censi[4], A. Sicari[4], F. Fedele[4], M. Lorito[1,2].** *[1]Consiglio Nazionale delle Ricerche-Istituto per la Protezione Sostenibile delle Piante (CNR -IPSP), Via Università 133 - 80055 Portici (NA), Italy. [2]Università degli Studi di Napoli Federico II, Dipartimento di Agraria, Via Università 100-80055 Portici (NA), Italy. [3]Cosvitec S.C.A R.L., via Galileo Ferraris 171 - 80142 Napoli, Italy. [4]Linfa S.C.A R.L., Zona Industriale Snc- 89900 Porto Salvo Vibo Valentia (VV), Italy. E-mail: roberta.marra@ipsp.cnr.it*

Italy represents a central point in the Mediterranean area for olive production because of its optimal geographical and environmental conditions. Research was focused on the development of novel biological tools to improve the production of this woody crop. Based on the application of beneficial microbes, such as fungi of the genus *Trichoderma* and their secondary metabolites (SMs), the olive trees were evaluated for the effects on growth, development, productivity and resistance to fungal infections in the field. Further, the plant response to the biological treatments was determined by analyzing the production of compounds that can influence plant and fruit quality. Experiments were conducted on 2 yr-old trees, treated with *Trichoderma* spore suspensions or fungal SMs solutions at time of transplant, and repeated every 15-30 days, for a total of 6 applications. Every 30 days, biometric evaluations and leaf samples were collected from each treatment, consisting of 15 replicates. Our results indicate that different *Trichoderma* isolates, as well as their SMs, produced beneficial effects to olive trees in terms of increased leaf number and branch length. Metabolomic analysis of olive leaves by HPLC-ESI-QTOF-MS approach was also performed. Significant differences in the accumulation pattern of phenolic compounds were noted depending upon the various treatments, and about 30 different compounds were chemically identified.

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59. GENOME DRAFTs OF FLUORESCENT PSEUDOMONAS BIOCONTROL STRAINS ISOLATED FROM HYDROPONIC CULTURES. **M. Martini, S. Moruzzi, C. Polano, N. Loi, G. Firrao, P. Ermacora.** *Università degli studi di Udine, DI4A, Via delle Scienze 206 - 33100 Udine, Italy. E-mail: paolo.ermacora@uniud.it*

Fluorescent pseudomonads are common and most studied biocontrol agents. The genus *Pseudomonas* is highly diverse, yet biocontrol strains that can effectively protect plants from phytopathogens appear to belong to taxonomically defined groups. In particular, the recently described species *P. protegens* appears to be prominent, so that the metabolic and genomic ground of this assertion are increasingly better understood. A group of *P. protegens*-related strains were isolated for their ability of inhibiting *in vitro* the growth of fungal pathogens in hydroponic cultures of *Valerianella locusta* (L.) Laterr., *Rhizoctonia solani* in particular. The strains varied in the degree of biocontrol, with Pf-4 having the highest and Pf-11 one of the lowest activity. Following second generation sequencing (Illumina) the genomes of Pf-11 and Pf-4 were assembled. The draft genome sequence of Pf11 is 7,053,517 nts in total and consists of 125 contigs with N50 = 1,036,338 nts. The Pf4 is 6,832,152 nts in total and consists of 36 contigs with N50 = 688,889 nts. The two genomes were annotated using RAST (http://rast.nmpdr.org/) and compared between themselves and with the few *P. protegens* genomes available in the database using OMA (http://omabrowser.org/standalone/). The comparison suggests that there are relatively minor differences between strains isolated from the rhizosphere of soil vs. hydroponic grown plants and that the differences in biocontrol activity can be ascribed mainly to secondary metabolism.

60. AWARENESS OF TREE PESTS AND PATHOGENS AMONGST EUROPEAN TREE PROFESSIONALS. **M. Marzano[1], N. Dandy[1], I. Papazova-Anakieva[2], D. Avtzis[3], T. Connolly[1], R. Eschen[4], M. Glavendekić[5], B. Hurley[6], Å. Lindelöw[7], D. Matošević[8], R. Tomov[9], A.M. Vettraino[10].** *[1]Forest Research, Northern Research Station, Roslin & Alice Holt Lodge, Farnham, UK. [2]Faculty of Forestry, Ss. Cyril and Methodius University in Skopje, Skopje, Macedonia. [3]Forest Research Institute, NAGREF, Vassilika, Thessaloniki, Greece. [4]CABI, Delémont, Switzerland. [5]Faculty of Forestry, University of Belgrade, Belgrade, Serbia. [6]Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Cnr Lynnwood and University Roads, Hatfield, Pretoria, South Africa. [7]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden. [8]Department for Forest Protection and Game Management, Croatian Forest Research Institute, Jastrebarsko, Croatia. [9]University of Forestry, Sofia, Bulgaria. [10]Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Viterbo, Italy. E- mail: vettrain@unitus.it*

Recently there has been a significant increase in the number of invading plant pathogens in Europe, closely linked to global trade in plants for planting and wood products. To effectively manage an invading pathogen population its early detection is a crucial step. This requires the awareness of pests and a deep knowledge of their biology and epidemiology. Therefore, a lack of awareness of pests and diseases among key stakeholders is a critical biosecurity problem and driver of biological invasions. In order to investigate the awareness levels of tree professionals, including workers in forestry, agronomists, landscape architects, horticulturalists, conservationists and researchers, a questionnaire survey was carried out across nine European countries. The aim was to gauge the awareness levels of a range of tree pests and pathogens, knowledge of how they spread, willingness to undertake specific management actions and to identify the sources utilised to gain information. Results show that awareness of pests and diseases is relatively modest amongst tree professionals and it varies in relation to the respondent's demographic. Barriers to changing behaviours include financial and resource pressures but also the perceived behaviour of others. Arguably, increasing awareness of pest and disease threat remains, therefore, an area that urgently needs to be addressed, not only for current 'known' pests but including those which pose a future risk.

61. ACTIVITY OF SELECTED FUNGICIDES ON THE GROWTH OF *FUSARIUM* SPECIES AND *MICHRODOCHIUM NIVALE* INVOLVED IN FUSARIUM HEAD BLIGHT OF CEREALS. **M. Masiello, S. Somma, V. Ghionna, A.F. Logrieco, A. Moretti.** *[1]Institute of Sciences of Food Production, Research National Council (ISPA-CNR), Via Amendola 122/O - 70126 Bari, Italy. E-mail: mario.masiello@ispa.cnr.it*

Fusarium species can be casual agents of a wide range of plant diseases with consequent productive and economic losses. Many *Fusarium* species can also produce mycotoxins, harmful metabolites for plant, human and animals. This ability represents a serious problem due to the possible accumulation of toxic secondary metabolites in the colonized tissues. Fusarium Head Blight (FHB) is a disease of cereals to which several *Fusarium* species (*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. crookwellense*, *F. sporotrichioides*, *F. tricinctum*, *F. acuminatum*, *F. equiseti*, *F. langsethiae*) and *Michrodochium nivale* are associated. The response to 5 fungicides registered for seed treatments and 5 fungicides registered for foliar spraying, was evaluated by colony-growth assay. The dose recommended by manufacturers, for tanning fungicides, and the dose recommended and two further lower decimal dilutions for other fungicides were tested. De-Methylation Inhibitors molecules have shown excellent performances, arresting completely colony growth up to 10 days of incubation. After 7 days, a moderate decrease of sensitivity was observed for pyraclostrobin (average of inhibition ranging between 78 and 100%), fluxapyroxad (average of inhibition ranging between 33 and 100%) and fludioxonil that could not inhibit *F. tricintum* and showed less effective against other species (average of inhibition ranging between 44 and 100%). A different sensitivity among the species tested was observed: *F. tricintum*, *F. poae*, and *M. nivale* were less sensitive to tanning fungicides than other species. The data on the response to fungicides obtained in this screening has provided useful information for the FHB control management in the field.

62. *IN VITRO* RESPONSE OF DIFFERENT FUNGICIDES ACTIVE AGAINST *ASPERGILLUS FLAVUS* AND *FUSARIUM* SPECIES CAUSING EAR ROT DISEASE OF MAIZE. **M. Masiello, S. Somma, V. Ghionna, A.F. Logrieco, A. Moretti.** *Institute of Sciences of Food Production, Research National Council (ISPA-CNR), Via Amendola 122/O - 70126 Bari, Italy. E-mail: mario.masiello@ispa.cnr.it*

Maize plants can be attacked by several toxigenic fungal species such as *Aspergillus* and *Fusarium* species. The so called "Fusarium maize ear rot" disease, is caused by several species of the *Fusarium* genus such as *F. graminearum* Schwäbe that produces deoxynivalenol, and *F. proliferatum* (Matsushima), Nirenberg and *F. verticillioides* (Saccardo) Nirenberg that both produce fumonisins. Also, a pathogen associated to infection of maize kernels is *Aspergillus flavus*, an Aflatoxin B1 producing species. Nowadays, there is poor knowledge on the effectiveness of fungicides to control their contamination. The sensitivity to different fungicides, by colony growth and conidial germination assays, of *F. graminearum*, *F. proliferatum*, *F. verticillioides* and *A. flavus* was evaluated. Three different concentrations (the dose recommended by manufacturers and two further lower decimal dilutions) of De-Methylation Inhibitors (DMIs) tebuconazole, propiconazole, metconazole, difenoconazole, prothioconazole, prochloraz, of Succinate dehydrogenase inhibitors (SDHIs) boscalid and isopyrazam, of PhenylPyrroles fludioxonil, MethylBenzimidazole Carbamates thiophanate-methyl and the multi-site fungicide folpet were tested. DMIs showed excellent performances, inhibiting *Fusarium* and *Aspergillus* mycelial growth up to 10 days of incubation and conidial germination and germ tube elongation at the highest dose and, in almost all cases, also at the two lower concentrations. Among the SDHIs, isopyrazam showed a higher effectiveness than boscalid, which was not able to control *Fusarium*. Isopyrazam could inhibit completely colony growth and conidial germination. *A. flavus* showed an higher sensitivity than *Fusarium* species to both SDHIs and fludioxonil. Studies are in progress in order to explain molecular mechanisms associated to different sensitivity among fungal genera and the different response of two SDHI fungicides belonging to same chemical group.

63. PHYLOGENETIC ANALYSIS AND MYCOTOXINS PROFILE OF *ALTERNARIA* STRAINS ISOLATED FROM DURUM WHEAT KERNELS SHOWING BLACK POINT DISEASE SYMPTOMS. **M. Masiello[1], S. Somma[1], A. Susca[1], A.F. Logrieco[1], G. Meca[2], A. Moretti[1].** *[1]Institute of Sciences of Food Production, Research National Council (ISPA-CNR), Via Amendola 122/O - 70126 Bari, Italy. [2]Department of Preventive Medicine, Nutrition and Food Science Area, University of Valencia (Spain), Avenida-Vicent Andres Estelles s/n, 46100 Burjassot, Valencia, Spain. E-mail: antonio.moretti@ispa.cnr.it*

Black point is a complex fungal disease of wheat mainly associated to *Alternaria* species, that shows the risk of possible

accumulation of *Alternaria* mycotoxins in the kernels. Ninety-two *Alternaria* strains isolated from wheat in Northern Italy were characterized by sequencing portions of the allergen alt1a, glyceraldeyde-3-phosphate dehydrogenase and translation elongation factor 1α genes, according with a multi-locus gene sequencing approach. The analysis of the combined sequences of the 3 genes resulted in a Maximum Parsimony tree showing 2 well defined groups: *A. infectoria* and *A. alternata*. The *A. infectoria* reference strain clusterized with 8 out of 92 strains tested, while the remaining 84 strains clusterized with *A. alternata*, *A. tenuissima* and *A. arborescens* reference strains. In *A. alternata* group, 4 different clades were defined: Clade A, including 42 strains and both *A. alternata* and *A. tenuissima* reference strains; Clade B including 18 strains and the *A. arborescens* reference strain; Clades C and D, including 12 and 12 strains, respectively. A high level of genetic variability among and within the clades was observed. The capability of the *Alternaria* strains to produce Alternariol (AOH) and Methyl Ether Alternariol (AME) on sterilized rice was evaluated. Eighty-nine strains produced AOH (1-8060 µg/g), while ninety strains produced AME (1-14340 µg/g), with a high variability among strains belonging to the same species. On the other hand, a low capability to produce both AOH (mean value 52 µg/g) and AME (mean value 84 µg/g), was observed in *A. infectoria* strains.

64. COMMUNICATING EUROPEAN PROJECTS: A DIFFERENT APPROACH FOR RESEARCH INNOVATION. A. Masino[1] A. Bertin[2], M.L. Gullino[1]. *[1]Centro AGROINNOVA, Università di Torino, Italy. [2]SPIN-TO srl, Via Roma 366, Torino, Italy. E-mail: andrea.masino@unito.it*

Since 2004 Agroinnova gained a broad expertise in the coordination of complex and multidisciplinary European projects and initiatives in the sustainable agriculture sector. EU projects aim at updating and improving the environmental knowledge of European decision makers and experts, as well as public and students. Moreover, one of the objectives of European Commission Program Horizon 2020 is to bring researchers closer to the public and to increase awareness of research and innovation activities, with a view to supporting the public recognition of researchers. Communication often combines sciences with entertainment, especially when addressing young audience. It can take various forms: educational activities (e.g. summer school and training courses) hands-on experiments and science shows (*European Researchers Night*), debates and conferences (*Raccontare la Salute delle Piante*, *Incontri Fitoiatrici*, *Designing the Circular Economy, Open Day*), artistic performance (*EMPHASIS for the Environment*), etc. Agroinnova will focus to keep the following goals in the near future: increasing awareness among the general public of the importance of research and innovation and more favourable general attitude towards its funding; better understanding of the key benefits that research brings to society; reducing in the stereotypes about researchers and their profession. The key to success in communication, as with science and research, is to continue developing skills through focused practice. This extra effort will pay off in the long run.

65. DEVELOPMENT OF *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF THE BIOCONTROL AGENT *CRYPTOCOCCUS LAURENTII* LS28. C. Miccoli[1], G. Ianiri, R. Castoria. *[1]Università degli Studi del Molise, sede Campobasso, via Francesco De Sanctis, 1 - 86100, Campobasso, Italy. E-mail: castoria@gmail.com*

Penicillium expansum, the causal agent of blue mold of stored pome fruits, produces patulin, a mycotoxin that contaminates both fruits attacked by this pathogen and derived products. Infections by *P. expansum* are mainly controlled by using synthetic fungicides, but alternative approaches such as biological and/or integrated control are needed, both for the onset of resistant fungal strains, and the toxicological and environmental risks posed by fungicides. This has recently led EU to require the decrease of chemical input in agriculture (EU Directive 2009/128). Likewise the biocontrol agent (BCA) *Rhodosporidium kratochvilovae* LS11, *Cryptococcus laurentii* LS28 is capable of reducing the incidence of blue mold and to detoxify PAT to less toxic compounds (ascladiol and deoxypatulinic acid). With the aim of preparing tools to study the mechanisms of action of LS28 at a molecular level, random mutagenesis based on *Agrobacterium tumefaciens*-mediated transformation (AMT) has been developed for this BCA. Different selective markers (auxotrophy fro Uracil and resistance to antibiotics) were assessed and Hygromicin-resistant transformants, which harbor *hygR* gene (Hygromycin B Phosphotransferase), were successfully selected. Molecular characterization of these transformants is being carried out. Furthermore, screening of transformants is also in progress for identification of phenotypes of interest through incubation with patulin and with different stressors that are known to affect biocontrol activity of LS28.

66. CHARACTERIZATION OF ALIVE FUNGAL POPULATION IN WHEAT FLOUR. S.A. Minutillo, D. Ruano-Rosa, F. Garganese, M.G. Li Destri Nicosia, G.E. Agosteo, L. Schena. *Università Mediterranea, Dipartimento di Agraria, località Feo di Vito, 89122 Reggio Calabria, Italy. E-Mail: serena.minutillo@unirc.it*

Mycotoxigenic fungi constitute a threat for humans but little is known about their presence in flour, raw material of baked goods. The aim of this work was the characterization of fungal population in wheat flour samples (types "*0*", "*00*" and wholemeal) from four mills and one shopping centre (commercial flours). The extent of fungal contamination (expressed in CFU/g of flour) was determined using a conventional plating method and potato dextrose agar as culturing medium. Single representative colonies were identified according to morphological features and by sequencing the ITS regions of the rDNA. Wholemeal flours were found the most contaminated (653-1840 CFU/g) while type "*0*", on average, showed the lowest values (54-87 CFU/g). Intermediates values were observed for type "*00*" (87-247 CFU/g). Within each typology, except for the wholemeal one, commercial flours presented values of contamination lower than mill flours. *Penicillium* spp. was the most frequent genus (in all samples) and was particularly abundant in wholemeal flours with 593-1840 CFU/g. *Cladosporium* spp. and *Aspergillus* spp. were found in types "*0*" and "*00*" (7-33 and 7-20 CFU/g respectively) but not in wholemeal. The genus *Alternaria* was isolated from all sample (7-47 CFU/g), while *Fusarium* spp. was found in a single sample of type "*00*" and with a low concentration (7 CFU/g). Other non-identified fungi accounted for an average of 7-73 CFU/g. Data of the present study highlights an abundant presence of alive fungal propagules in wheat flour and a relevant potential threat for consumers since most detected genera are potential mycotoxin producers.

67. STORAGE UNDER N2 CONTROLLED ATMOSPHERE REDUCES CEREALS GRAIN QUALITY AND QUANTITY LOSSES. L. Moncini[1], S. Sarrocco[2], G. Pachetti[1], G. Vannacci[2]. *[1]Centro Ricerche Strumenti Biotecnici nel settore Agricolo-forestale (CRISBA), c/o ISIS "Leopoldo II di Lorena" Cittadella dello Studente, 58100, Grosseto, Italy. [2]Università degli Studi di Pisa - Dipartimento di Scienze Agrarie, Alimentari e Agro-Ambientali, Plant Pathology*

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Cereals quality can be affected, during storage, by pests or mycotoxigenic fungi. The use of controlled atmosphere, with O_2 replaced by N_2, represents a promising tool to reduce cereals crop losses in post harvest. Aim of the present work is to evaluate a system – patented by *Eurosider* sas – to mantain qualitative and quantitative features of cereals grain by their storage under a highly purified N_2 controlled atmosphere. The project consists in different parallel lab-scale experiments aimed to assess the effect of N_2 atmosphere on: i) growth and mycotoxin production by mycotoxigenic fungi, such as *Fusarium verticillioides*, *Aspergillus flavus*, *Fusarium langsethiae* and *Fusarium graminearum,* on corn and wheat; ii) growth and reproduction of two of the most important post harvest pests such as *Sitophilus* sp. and *Tribolium* sp.; iii) qualitative parameters of corn. The use of highly purified N_2 controlled atmosphere significantly affects growth and sporulation of *F. verticillioides* and *A. flavus*, fumonisin and aflatoxin producers respectively, and of *F. graminearum* e *F. langsethiae*, DON and T2 and HT2 producers respectively. Atmosphere containing 95.0% and 98.5% N_2 caused, in wheat, the complete mortality of adult population of *Sitophilus* sp. after 12 and 6 days, respectively. This last result was confirmed for *Tribolium* sp. Qualitative parameters, and mainly umidity and *Total Antioxidant Capacity*, were positively affected in corn. These positive preliminary results prompt us to scale up the system, to assess its efficacy in controlling cereals grain crop losses in larger silos.

68. THE H2020 PONTE PROJECT WEB SITE: AN ONLINE RESOURCE FOR SCIENTIFIC DISSEMINATION ON EMERGING PEST DISEASES. M. Morelli[1], M. Saponari[1], D. Tavano[1], D. Boscia[1], A. Obradović[2]. *[1]CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), SS Bari, 70126 Bari, Italy. [2]University of Belgrade, Faculty of Agriculture, 11080 Belgrade, Serbia. E-mail: massimiliano.morelli@ipsp.cnr.it.*

The International Research Consortium POnTE (Pest Organism Threatening Europe) is being funded by the European Commission under the Horizon 2020 programme to investigate four pathogens (i.e. *Xylella fastidiosa*, *Candidatus* Liberibacter solanacearum, *Hymenoscyphus fraxineus* and *Phythophtora* spp.) representing a major threat to strategic crops and natural landscapes in the EU, and to identify integrated management strategies for their containment. The wide range of studies conducted within the Project tasks on key emergent pests and the rising request for accessing up-to-date references over the Internet, suggested the need to provide a larger variety of real-time information about the project and its targets for a much wider variety of end-users. A WordPress-based web portal (www.ponteproject.eu) has been created by the Coordination Team to support collaborative platform functions, enhance the project's visibility and provide in a flexible manner a rapid dissemination of valuable information, fostering raise of general knowledge and public awareness on relevant themes in plant pathology. Answering to the modern challenges, accounting for an effective web-based pest information system, the resource is intended as an open-access platform to share scientific achievements, upload promotional material and fact sheets, communicate conferences and training courses, report press review and legislative regulations. A social media presence on Twitter and Facebook channels was set up from the early stages in order to enable a two-way communication with a web-active audience and work towards a continuous engagement of the major plant pathology networking platforms and institutional accounts. To keep Project partners and interested parties always informed of the web site updates and encourage frequent visits, a newsletter is being released on a weekly basis.

69. WILTING LEAVES OF EGGPLANT CAUSED BY *PSEUDOMONAS SYRINGAE* IN ITALY. C. Moretti[1], G. Carannante[2], V.M. Stravato[2], R. Buonaurio[1]. *[1]Dipartimento di Scienze Agrarie, Alimentari e Ambientali (DSA3) - University of Perugia, Borgo XX Giugno 74 - 06121 Perugia, Italy. [2]GENISTA s.r.l., via San Vincenzo 13 - 04022 Fondi (LT), Italy. E-mail: chiaraluce.moretti@unipg.it*

In March 2015, eggplants (*Solanum melongena* L.) cv. Angela, grown in Terracina (Latium, Italy) under plastic, showed severe wilting symptoms with an incidence of about 40%. The symptoms, which started from the basal leaves, were sometimes unilateral and led to leaf blight. The plants showing such symptoms were stunted and no vascular discolorations were observed. Whitish bacterial colonies were consistently isolated from the diseased stem/leaf tissues. Two representative selected strains, which were gram negative, fluorescent on King's medium B, and had oxidative but not fermentative metabolism, were subjected to pathogenicity test by inoculating eggplant seedlings at 4^{th}-5^{th} leaf stage. Blackening of the stem above and, at less extent, under the inoculation point was observed 4-5 days after the inoculation, and bacteria with the same cultural features of the original strains were re-isolated from inoculated plants. For bacterial identification, the strains were subjected to LOPAT tests. They were levan positive, oxidase negative, potato rot negative, arginine dihydrolase negative, and tobacco hypersensitive response positive, indicating that they belonged to Lelliot's LOPAT group 1. When 16S rDNA gene sequences were compared by BLASTn with nucleotide sequences from GenBank, they showed 93% identity with the comparable sequences of *Pseudomonas syringae*. In a MLST approach, four housekeeping genes (*gltA*, *gap1*, *gyrB* and *rpoD*) were sequenced and the plylogenetic tree was built based on concatenated sequences. This analysis revealed that the two eggplant isolates and the strains PSC1B of *P. syringae* pv. *syringae* from corn were identical. Further analyses are in progress to confirm whether the bacterium belong to the *syringae* pathovar.

70. THE PLANT PROTECTION PRODUCTS DATABASE (Mi.PAAF). C. Morgia, F. Milano, A. Santacaterina, A. Matrone, S. Rosati, L. Donnarumma. *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero, 22 - 00156 Roma, Italy. E-mail:cinzia.morgia@crea.gov.it*

The Plant Protection Products Database of Ministry of Agriculture, Food and Forestry Policies (Mi.PAAF), managed by the CREA-Plant Pathology Research Center, has been active for more than 15 years and is a complete information system that provides quick upgrade to agricultural technicians and operators in crop protection. The Plant Protection Products and Active Substances Database is a reference tool for workers in the Rural Network, particularly in the areas of agriculture, agribusiness, floriculture: it provides detailed and updated information on plant protection products for field, vegetable, horticulture and fruit crops as well as ornamentals (more than 15.000 forms). Moreover, Maximum Residue Limits (MRLs) allowed on active substances authorized in agriculture at the national level are listed. The database management, the update in real time and the computer program based on, have been the object of an innovative action and expansion of the system for the best use. In the 2014-2015, it was put in place an effective audit and management of the database in accordance with the needs of users and the actions planned by the National Action Plan for integrated pest management. The work shows this innovative action founded by Mi.PAAF.

71. MONITORING OF *XYLELLA FASTIDIOSA* IN MARCHE REGION, CENTRAL-EASTERN ITALY. S. Murolo[1], V. Mancini[1], R. Foglia[1], J. Concas[1], A. Servili[1], S. Nardi[2], G. Romanazzi[1]. *[1]Department of Agricultural, Food, and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 10 - 60131 Ancona, Italy. [2]Regional Phytosanitary Service, A.S.S.A.M., Marche Region, Via Industria, 1 - 60027 Osimo Stazione (AN), Italy. E-mail: g.romanazzi@univpm.it*

Xylella fastidiosa it is a fastidious bacterium that is able to infect a long list of plants. It is well known for the economic impact on grapevine in California and on citrus in Brazil, and it was recently found in Apulia, southeastern Italy, where it is devastating for olive trees. Considering the potential damages to olive groves and to nursery productions of several ornamental plants that are sensitive to the bacterium, the quick spreading on short distance mainly by *Philaenus spumarius* and on long distance by trading of asymptomatic propagating materials, it was carried out a monitoring for the presence of *X. fastidiosa* in the Marche region, central-eastern Italy. The samples were collected from olive trees (n. 146) symptomless or showing desiccated areas of the canopy, and from ornamental plants (*Nerium oleander*, *Polygala myrtifolia*, *Myrtus communis*, *Rosmarinus officinalis*, *Citrus reticulata*, *Spartium junceum*, *Coffea* spp.) grown in nursery and in or around commercial fields (n. 244). DNA was extracted from the samples and molecular detection was carried out using *X. fastidiosa*-specific primers (RST31/RST33). None of the analyzed samples showed the specific amplicon (733 bp) present only in the positive sample. This first survey did not allow to record symptomatic plants, but taking into account the epidemiology of *X. fastidiosa* and the trade of plants, it is mandatory to keep a high level of attention and follow monitoring potential infection sources to apply soon the planned measures to control the pathogen.

72. ELUCIDATING STRAWBERRY FRUIT RESISTANCE TO FUNGAL PATHOGENS WITH TRANSCRIPTOMIC AND METABOLOMIC APPROACHES. E.G. Nagpala, M. Guidarelli, M. Gasperotti, D. Masuero, U. Vrhovsek, P. Sonego, M. Moretto, L. Zoli, P. Bertolini, C. Moser, K. Engelen, E. Baraldi. *Dipartimento di Scienze Agrarie, Università di Bologna, Viale Fanin 46 - 40127, Bologna, Italy. E-mail: ellainegrace.nagpal2@unibo.it*

The ontogenic resistance of strawberry fruits *(Fragaria* spp.) to pathogens such as *Colletotrichum acutatum* and *Botrytis cinerea* has been noted to decrease with ripening. Unripe fruits could get infected with fungal pathogens and remain symptom-free until the maturation of berries. During the asymptomatic stage of the infected fruits, both *C. acutatum* and *B. cinerea* are present in the berry in the dormant state. The quiescence of the pathogens in unripe strawberry fruits has been related to the present physico-chemical composition of the berry. In this study, the underlying factors that contribute to the varying degree of resistance of unripe and ripe strawberries were investigated through transcriptomic and metabolomic approaches. White and red strawberry fruits were inoculated with *C. acutatum* and *B. cinerea*. RNA-Seq results revealed a general up-regulation of defense-related genes, particularly secondary metabolites, hormones and lipids in white fruits of woodland strawberry after 24 h of inoculation with *B. cinerea.* Meanwhile, an increase in the concentration of proanthocyanidins, flavan-3-ols and ellagitannins were detected in white fruits of strawberry inoculated with *C. acutatum* and *B. cinerea* after 48 h of inoculation. The accumulation was in accordance with the results from the qRT-PCR of the genes leading to the synthesis of the mentioned phenolic classes. Results obtained from the study could be of significance for the improvement of the resistance of ripe strawberry fruits to pathogen infection.

73. METABOLOMICS OF *DIPLOCERAS HYPERICINUM*, AN AMPHISPHAERIACEOUS FUNGUS ASSOCIATED TO ST. JOHN'S WORT (*HYPERICUM PERFORATUM*). R. Nicoletti[1], B. Zimowska[2], F. Vinale[3], R. Marra[3], N. Lombardi[3], F. Lacatena[4], A. Bottiglieri[4], M. Lorito[3,4]. *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria. Current address: Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100 - 80055 Portici (NA), Italy. [2]Department of Plant Pathology and Mycology, University of Life Sciences, Leszczyńskiego 7 - 20069, Lublin, Poland. [3]Consiglio Nazionale delle Ricerche-Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), Via Università 133 - 80055 Portici (NA), Italy. [4]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100 - 80055 Portici (NA), Italy. E-mail: rosario.nicoletti@crea.gov.it.*

Among medicinal plants, St. John's wort (*Hypericum perforatum*) is cropped on reduced extensions in both Italy and Poland for the extraction of hypericin, hyperforin and other compounds of valuable pharmaceutical interest. Recently, the evidence that many endophytic fungi are able to synthesize important bioactive metabolites of plant origin has stimulated a notable research activity with the aim to find culturable strains to be employed for producing these compounds on a large scale. Until now, hypericin production has been reported only by an endophytic strain of *Thielavia subthermophila*. In this work, we investigated the production of secondary metabolites by two isolates of *Diploceras hypericinum*, an amphisphaeriaceous fungus which is known as both an endophyte and a pathogen of *H. perforatum*. Their culture filtrates were analysed by LC-MS-QTOF, and the major compounds were putatively identified by screening an internal database containing approximately 3,000 fungal compounds and the METLIN database that includes over 25,000 metabolites. The software generated automatically a search list for major metabolites, finding the most likely adducts and/or ion fragments. This approach allowed a rapid screening of fungal cultures, highlighting the most important chromatographic peaks corresponding to known or novel compounds. Metabolomic analysis revealed that no compounds known from *H. perforatum* were produced by these *D. hypericinum* strains. Besides a number of primary metabolites, the main peaks were identified as pyroglutamic acid, *cis*-fusarinine and brevioxime. Another definite peak (MW 440.0943) represents an unknown compound requiring further investigations in view of its structural elucidation.

74. GENETIC DIVERSITY AND CHEMOTYPING OF *FUSARIUM* SPECIES ASSOCIATED WITH FOOT AND ROOT ROT ON DURUM WHEAT IN TUNISIA. S. Oufensou[1,2], V. Balmas[2], B. Scherm[2], A. Marcello[2], M. Ben Attia[1], S. Gargouri[3], M. Pasquali[4], Q. Migheli[2]. *[1]Laboratoire de Bio-surveillance de l'environnement, Faculté des Sciences de Bizerte, Route de Tunis - 7021 Zarzouna, Université de Carthage, Tunisia. [2]Dipartimento di Agraria, Università degli Studi di Sassari, Via E. De Nicola 9, I - 07100 Sassari, Italy. [3]Laboratoire de Protection des Végétaux, Institut National de Recherche Agronomique de Tunis, Rue Hédi Karray - 2049 Ariana, Tunisia. [4]Department of Environmental Research and Innovation, Luxembourg Institute of Science and Technology, Belvaux, 41, rue de Brill, L-4422 Belvaux, Luxembourg. E-mail: safaoufensou@gmail.com*

Environmental conditions in Tunisia are conducive to Fusarium Foot and Root Rot (FRR) which represents the major devastating disease of durum wheat. To identify the *Fusarium* species associated with FRR, a total of 3500 plant samples were collected from approximately 70 durum wheat fields in Northern Tunisia (Beja; Bizerte; le Kef; Bousalem; Siliana) during the 2015 cropping season. A total of 174 single spore *Fusarium* sp. cultures were obtained and provisionally identified by observing the morphological traits. *F. culmorum* was detected as the predominant species (more than

85% of all isolates), while most of the other isolates belonged to the *F. incarnatum-equiseti* species complex (FIESC). The *TRI12* genotype was determined in order to define genetic chemotypes. Among the *F. culmorum* isolates, the 3-ADON chemotype was the most prevalent (97%), while the remaining 3% belonged to the NIV chemotype. Pathogenicity of *F. culmorum* was assessed on the Saragolla durum wheat variety. The statistical analyses showed that both the geographical site and the genetic chemotype of the isolates had a significant impact on the degree of virulence ($P<0.01$). Moreover, the interaction between these two factors has been established ($P<0.02$). To further characterize the population composition, a single nucleotide polymorphism (A/T), located 34 bp after the first exon of the elongation factor EF-1-α partial gene sequence is being analyzed. The results will shed light on the possible fitness advantage that has been previously hypothesized for the A-haplotype in the establishment of FRR.

75. GENE DISRUPTION APPROCH TO INVESTIGATE THE SYNERGISTIC EFFECT OF *FUSARIUM GRAMINEARUM* POLYGALACTURONASE AND XYLANASE ACTIVITIES DURING HOST INFECTION. **M.C. Paccanaro[1], L. Sella[1], C. Castiglioni[1], W. Schäfer[2], F. Favaron[1].** *[1]Dipartimento Territorio e Sistemi Agro-Forestali (TESAF), Università degli Studi di Padova, Viale dell'Università 16 - 35020 Legnaro (PD), Italy. [2]Biocenter Klein Flottbek, Molecular Phytopathology and Genetics, University of Hamburg, Ohnhorststr. 18 - 22609 Hamburg, Germany. E-mail: mariachiara.paccanaro@studenti.unipd.it*

Fungal polygalacturonases (PGs) and xylanases have been shown to play an important role during pathogenesis of some fungal pathogens, but little is known about their combined effect. Previously we have produced single gene disruption mutants of the *F. graminearum xyr1* and *pg1* genes encoding the major regulator of xylanase genes expression and the main PG isoform of the fungus, respectively. Targeted disruption of the *pg1* gene produced a mutant with a nearly abolished PG activity and a virulence reduced on soybean but not on wheat spikes; besides, the Δxyr mutant, dramatically impaired in xylanase activity, showed a virulence comparable with WT on both soybean and wheat. We therefore produced a ΔΔxyr/pg double mutant which showed a dramatic reduction of xylanase and PG activities when grown on xylan and pectin as carbon sources compared to the WT. The growth of the double mutant was affected only on xylan containing medium. Infection experiments on soybean seedlings and wheat spikes showed that the virulence of the double mutant strains was significantly reduced compared to the single mutants. A capacity of xylanase to increase the PG activity was verified measuring the uronides released from wheat cell walls treated with the purified PG and xylanase enzymes mixed together.

76. MECHANISMS INVOLVED IN THE BIOCONTROL ACTIVITY OF THE RHIZOSPHERE-COLONIZING BACTERIUM *RAHNELLA AQUATILIS* AGAINST *FUSARIUM OXYSPORUM*. **D. Palmieri[1], F. De Curtis[1], D. Vitullo[1], D. Turrà[2], A. Di Pietro[2], G. Lima[1].** *[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis snc - 86100 Campobasso, Italy. [2]Department of Genetics, University of Cordoba, Campus Rabanales, Ed. Gregor Mendel - 14071 Cordoba, Spain. E-mail: davide.palmieri@studenti.unimol.it*

The composition of plant root exudates is an important factor that influences the rhizosphere microbial community and determines the type of interactions occurring between host plants, soilborne pathogens and plant growth promoting rhizobacteria (PGPR). Plant roots, as well as root-associated microorganisms and pathogens can change the pH in the rhizosphere. The main goal of this investigation was to evaluate the role of pH in the tritrophic interaction among *Solanum lycopersicum/Rahnella aquatilis/Fusarium oxysporum* f. sp. *lycopersici*. The bacterium *R. aquatilis* was selected from the naturally occurring microflora of the chickpea rhizosphere due to its ability for mineral phosphate solubilization and biological control of chickpea and tomato fusariosis. Furthermore this bacterium showed a great capacity to acidify both in synthetic media and in tomato root exudates. Neutralizing the acidifying activity of *R. aquatilis* significantly decreased its biocontrol capacity against *F. oxysporum* on tomato. These results suggest that the biocontrol activity of *R. aquatilis* is related to its capacity to acidify the rhizosphere and to inhibit *F. oxysporum* during the early stages of root colonization.

77. EVALUATION OF ON-FARM COMPOSTS FOR SOILBORNE DISEASE SUPPRESSIVENESS REVEALING THE ROLE OF MICROBIAL COMMUNITIES. **C. Pane, R. Sorrentino, R. Scotti, G. Celano, A.M. Palese, A. Piccolo, R. Spaccini, M. Zaccardelli.** *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per l'orticoltura, Via dei Cavalleggeri 25, I-84098, Pontecagnano, Salerno, Italy. E-mail: catello.pane@crea.gov.it*

On-farm composting is a sound low-cost technology aimed at producing quality compost by recycling cropping biomasses in a broader view of environmental and economic sustainability of agricultural systems. In the framework of LIFE+ CarbOnFarm project, we evaluated a set of fifteen composts produced on-farm for suppressiveness to soil-borne phythopathogens. Composts may suppress plant pathogens mainly through biological control mechanisms implemented by resident microbial communities. In this work, compost suppressiveness was evaluated through two biological systems, *Rhizoctonia solani* and *Sclerotinia minor* against *Lepidium sativum*, on infected soil-peat amended with raw or heat-sterilized compost samples at 20% vol concentration. The *in vivo* assays showed differential patterns of disease control among composts. While, the net contribution of the biological component of the organic matter in suppressiveness were also measured by comparison with heated-compost. Compost microbial communities, affected by the quality and composition of molecular organic carbon, could have a role in compost control efficacy. The in-depth study of the composts living microflora by populations counting, metabolic profiling and biochemical activities revealed untargeted antagonistic structures that may explicate hypothetical mechanisms underlying general pathogen suppression. However, complexes carbon sources-consuming microorganisms developed on the transformed lignin-cellulosic feedstock may also be associated to a more specific action. To increase knowledge, assessment of compost microbiota diversity by analysis of terminal restriction fragment length polymorphisms (T-RFLPs) is ongoing. This study indicated the potential suppressiveness of on-farm composts, whose application in agricultural fields may contribute to reduce needs for chemical fungicides.

78. A COMPUTATIONAL INSIGHT INTO THE STRUCTURE-ACTIVITY RELATIONSHIP OF NATURAL PHENOLIC FUNGICIDES AND TRICHOTHECENE BIOSYNTHESIS INHIBITORS. **G. Pani, A. Dessì, R. Dallocchio, B. Scherm, E. Azara, G. Delogu, Q. Migheli.** *Università degli Studi di Sassari, Dipartimento di Agraria,Viale Italia 39 - 07100 Sassari, Italy. E-mail: gpani@uniss.it*

A molecular modelling approach was implemented to better understand the interactions existing between the TRI 5 trichodiene

synthase protein structure and a collection of phenolic compounds able to inhibit trichothecene biosynthesis and/or fungal vegetative growth in the wheat pathogen *Fusarium culmorum.* The molecules were selected and optimised based on molecular mechanics studies and energy minimisation by Density Functional Theory. Docking analyses of phenolic molecules were run on the 3D model of *F. culmorum* TRI5 based on homology modelling with the X-ray crystal of *F. sporotrichioides*. A set of 13 phenolic molecules – namely, carvacrol, 3-hydroxy cinnamic acid, ferulic acid, ferulic acid dimer, apocynin, caffeic acid, propyl gallate, octyl gallate, eugenol, eugenol dimer, Me-dehydrozingerone, magnolol, and ellagic acid – were subsequently tested *in vitro* and their biological activity was compared with molecular descriptors and interacting-structures obtained from computational analysis. The analysis highlighted three TRI5 protein sites, including the catalytic domain, which are involved in the interaction with the phenolic inhibitors of trichothecene biosynthesis. This study describes for the first time the interactions between TRI5 trichodiene synthase and phenolic ligands and provides an additional tool to discover new molecules with potential as fungicides or as trichothecene inhibitors that will help to guide the synthesis of novel *Fusarium*-targeted compounds by shortening the time of research and by reducing cost.

79. OCCURRENCE OF *TOMATO LEAF CURL NEW DELHI VIRUS* IN ITALY. S. Panno[1], A. Manglli[2], G. Iacono[1], L. Tomassoli[2], M. Davino[1], S. Drago[3], G. Stampone[3], S. Davino[4]. *[1]Università degli Studi di Catania, Dipartimento Di3A, via S. Sofia 100 - 95123 Catania, Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C. G. Bertero, 22 - 00156 Rome, Italy. [3]Binat Italia via Aquileia 34 - 90144 Palermo, Italy. [4]Università degli Studi di Palermo, Dipartimento SAF, viale delle Scienze Ed 5 - 90123 Palermo, Italy. E-mail: salvatore.davino@unipa.it*

In October 2015, during a survey in Trapani province (Sicily, Italy), severe symptoms not previously reported by growers were observed on zucchini squash (*Cucurbita pepo*) in open fields and in tunnels. Symptoms consisting in leaf curling, shortening of internodes and yellow mosaic were reminiscent of those caused by begomoviruses as recently reported on cucurbits in Spain. Total DNA extracts from young leaves of 12 plants were examined by PCR using degenerated primers for geminivirus detection. As positive, PCR was performed on a wider number of samples using specific primer sets to identify *Tomato leaf curl New Delhi virus* (ToLCNDV) both in DNA-A and DNA-B components. All samples (40) were positive. The nucleotide sequences of DNA-A and DNA-B components from three plants were determined. A BLAST analysis showed an identity >99% with sequences of DNA-A and DNA-B of ToLCNDV isolates from Spain on zucchini and tomato. The DNA-A and DNA-B sequences of one sample were deposited in GenBank. This is the first report of ToLCNDV in Italy, as well as the first identification of a bipartite begomovirus in Italian crops. The concern is high on possible new outbreaks and spread of ToLCNDV in other cucurbit growing areas. Therefore, alert has been issued and surveillance has been immediately activated in the Italian region where *Bemisia tabaci*, the vector, is present and the pest risk analysis showed a high probability of pathways for introduction of the virus.

80. FIRST REPORT OF *TOMATO SPOTTED WILT VIRUS* INFECTING *STEVIA REBAUDIANA* BERT. IN ITALY. G. Parrella[1], E. Troiano[1], G. Bozzano[2], M.G. Bellardi[3]. *[1]Istituto per la Protezione Sostenibile delle Piante del CNR, Bioagroalimentare, Via Università 133- 80055 Portici (NA), Italy. [2]Società Cooperativa L'Ortofrutticola, Albenga, Reg. Massaretti, 30/1, Albenga (SV) Italy. [3]Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-ambientali (DiSTA), Alma Mater Studiorum, Bologna, Via Zamboni, 33 - 40126 Bologna Italy. E-mail: mariagrazia.bellardi@unibo.it*

Stevia rebaudiana Bert. ('Sweet Weed' or 'Sweet Herbs'; Asteraceae family) is an herbaceous perennial plant native to the highlands of Paraguay, having actually a strong economically interest as sugar source since it is estimated to be 300 times sweeter than cane sugar. In "Piana d'Albenga" area (Liguria region) this species is cultivated as aromatic plant to be used in home gardens in mixed herb borders and/or for cooking. In June 2016, virus-like symptoms similar to those caused by *Tomato spotted wilt virus* (TSWV) (chloro-necrotic spots on the leaves) were observed on *S. rebaudiana* pot-plants propagated by cuttings at a Ligurian farm. Forty percent of plants showed this disease. TSWV was first detected by serological analysis (Lateral Flow Test) in all symptomatic plants and mechanically transmitted to herbaceous species belonging to Chenopodiaceae, Fabaceae and Solanaceae. To confirm TSWV presence symptomatic leaves were tested in RT-PCR using the BR60/BR65 universal primers, which amplify part of the nucleocapsid protein gene of several tospoviruses, and two new set of primers able to amplify the entire ORF of the NSs gene of TSWV. Target amplicons were produced for all samples tested. The resulting sequences showed high percentage of identity with several isolates of TSWV (96.0 to 99.5%). Our finding is thought to be the first report of natural occurrence of TSWV in *S. reabaudiana* in Italy. Considering that this virus infects several aromatic and ornamentals crops in this Ligurian area, thrips constitute the main responsible for TSWV spreading as well as increasing of its natural hosts.

81. USE OF *TRAMETES VERSICOLOR* FOR ELICITING DEFENCE REACTIONS IN WHEAT PLANTS AGAINST FOLIAR DISEASES. C. Pietricola[1], A. Iori[2], C. Fanelli[1], M. Scarpari[1], M. Reverberi[1], V. Scala[1]. *[1]Department of Environmental Biology, University of Rome "Sapienza", Rome, Italy; [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - QCE via Cassia 132 - 00100 Roma, Italy. E-mail: chiara.pietricola@uniroma1.it*

Durum and common wheat are amongst the most cultivated cereals worldwide. Recently Septoria Complex Disease, in which the prevalent causal agents are *Zymoseptoria tritici* and *Parastagonospora nodorum,* causes up to 20-30% of crop losses. The market of cereal fungicides increased in the last year accordingly to the fungicide resistance and new European regulation. Sapienza University and CREA started a project to find new molecules useful to stimulate the protection of wheat plant against *Z. tritici* and *P. nodorum*. In this study, we used purified and semi-purified Trametano (SPT), an exo-polysaccaride extracted from basidiomycete *Trametes versicolor* under *in vitro*, *in planta* and in open field tests.

Under *in vitro* analysis, SPT is able to inhibit the pathogen' growth whereas Trametano significantly delays the germination of the spores of both *P. nodorum* and *Z. tritici*. We assessed that SPT and Trametano can induce the plant defences. In particular, we monitored some of the biochemical and structural induced defences, such as PR proteins and cell wall thickening. The enhancement of plant defences reduced *de facto* the severity of fungal infection of about 40%. It is highly probable that SPT as well as Trametano may activate an induced systemic resistance based on jasmonates pathways, even if this aspect is still under investigation.

These promising results prompted us to try SPT, produced lowering the cost compared to pure Trametano, in formulation with half-dose of standard fungicides such as demethylase inhibitors (DMI).

82. GENOME MINING, PATHOGENECITY AND SECONDARY METABOLISM OF THREE STRAINS OF *FUSARIUM FUJIKUROI*, THE CAUSAL AGENT OF BAKANE DISEASE ON RICE. E. Piombo[1], H. Banani[1,2], I. Siciliano[2], P. Abbruscato[3], A. Acquadro[1], M.L. Gullino[1,2], D. Spadaro[1,2]. *[1]Università degli Studi di Torino, Centro AGROINNOVA, Largo Braccini 2 - 10095 Grugliasco (TO), Italy. [2]Universita degli Studi di Torino, DISAFA, Largo Braccini 2 - 10095 Grugliasco (TO), Italy. [3]Bioeconomy Unit, Parco Tecnologico Padano, via Einstein, 26900 Lodi, Italy. E-mail: davide.spadaro@unito.it*

Bakanae is an important seedborne disease of rice, caused by *Fusarium fujikuroi*. This pathogen can produce a wide range of secondary metabolites, including fumonisins, gibberellins and fusaric acid. In order to gain insight into secondary metabolites (SM) synthesis in *Fusarium fujikuroi,* we sequenced the genome of three strains named Augusto2, CSV1 and I1.3, identified the allelic variants in the genes responsible for SM production, and compared the virulence on rice and the SM production *in vitro* and on rice. Sequence analysis was conducted by *de novo* genome assembly. Three genomes of 42.8 Mb on average were obtained. The gene clusters responsible for fumonisin, gibberellin and fusaric acid production, formed by 15, 7 and 12 genes, respectively, were analyzed and aminoacidic differences were identified for *fum1, fum13* and *fum21*. *In vitro* colony diameters significantly increased with time and the three *F. fujikuroi* strains exhibited distinct differences in colony morphology and growth kinetics. We further compared the virulence and fumonisin production of the three strains on rice 'Galileo'. At 3 weeks post germination, *F. fujikuroi* strain I1.3 showed statistically higher virulence compared to Augusto2 and CSV1. Augusto2 was the major producer of fumonisins both *in vitro* and *in vivo*, followed by CSV1and I1.3. CSV1 was unable to produce gibberellins *in vivo* and *in vitro* on Petri dish, confirming the different symptomatology of CSV1 on rice, characterized by dwarfing and chlorosis, but lack of stem elongation.

This work permits to add a new tile to the complex puzzle of rice-F. fujikuroi interactions.

83. A REVISED AND EFFECTIVE PIPELINE BASED ON RELATIVE COVERAGE FOR THE GENOME RECONSTRUCTION OF PHYTOPLASMA AND OTHER FASTIDIOUS PROKARYOTES. C. Polano, P. Ermacora, M. Martini, R. Musetti, N. Loi, G. Firrao. *Università degli Studi di Udine, DI4A, Via delle Scienze 206 - 33100 Udine, Italy. E-mail: paolo.ermacora@uniud.it*

An alternative to the difficult, inefficient and time consuming methods that require purification of the pathogen DNA for its genomic analysis is to sequence a large library of DNA extracted from diseased plants and then select pathogen specific sequences. However, pathogen sequence selection is not trivial and many genome drafts published so far are incomplete.

The procedure developed here exploits the differential coverage of sequences originating from pathogen and those from host, due to the size difference between the prokaryote and the plant genomes and the relative abundance of pathogen even in samples with less than 10% pathogen DNA.

In brief, the procedure requires a reference genome for the uninfected plant, and the sequence reads obtained from an Illumina MiSeq. The procedure starts assembling the mixed genome reads and the reference genome reads if necessary. A perl script then calculates the coverages and then groups the contigs accordingly. The following steps are looped to find the optimum cutoff. The reads of the contigs with coverage higher than the cutoff are aligned against the healthy assembly. The non-mapping sequences are assembled with the A5 pipeline, to obtain the non-mapping-on-healthy contigs list. Another perl script queries a database from the reference genome contigs file to check the effectiveness of the cutoff and filtering parameter used.

Using this pipeline we have recently obtained very high quality draft assemblies of the phytoplasma strains associated with Lime Witches' Broom in Brazil, with the Cassava Frogskin Disease, with Chicory Phyllody and of a *Spiroplasma citri* strain.

84. SURVEY FOR THE PRESENCE OF *XYLELLA FASTIDIOSA* subsp. *PAUCA* STRAIN CoDiRO IN THE NATIVE FLORA OF THE SALENTO PENINSULA. O. Potere[1], L. Susca[1], F. Civita[1], S. Marullo[1], G. Loconsole[1], M. Saponari[2], D. Boscia[2], V.N. Savino[1,2], P. La Notte[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A - 70126 Bari, Italy. [2]Istituto per la Protezione Sostenibile delle Piante, CNR, SS di Bari, Via Amendola 165/A - 70126 Bari, Italy. E- mail: oriana.potere@uniba.it*

Xylella fastidiosa subsp. *pauca* strain CoDiRO was identified as associated with the "Olive Quick Decline Syndrome", a devastating disease first observed in October 2013 in the southeastern Apulia. At least 350 plant species belonging to 75 families are reported as hosts of *X. fastidiosa.* These provide a source of inoculum for the vectors (xylem sap-feeding leafhoppers), thus playing a major epidemiological role and facilitating the entrenchment of the pathogen in the affected area. To investigate the CoDiRO strain host range in Salento, monthly samplings of the native flora of two heavily infected olive groves and of the side of adjacent roads were conducted from January 2014 onwards. One of the groves was grass-covered, whereas periodic tillage was performed in the other. Overall, more than 200 species of 50 families were sampled, observed for the presence of symptoms, photographed and identified. In the spring, *Philaenus spumarius* the main vector of the Salentinian *X. fastidiosa* strain was abundantly present on the herbaceous flora and shrubs at all sites. All samples, in pools of no less that 3 to 5 plants, were tested by DAS-ELISA and uncertain/positive results were verified by conventional and real time PCR. Bacterial isolates were obtained in axenic culture from some positive species. In a two-year survey, only *Euphorbia terracina* proved to be *Xylella*-positive among the herbaceous hosts, whereas some shrubs and subshrubs i.e. *Asparagus acutifolius*, *Cistus creticus*, *Myrtus communis*, *Phillyrea latifolia*, *Rhamnus alaternus* and *Rosmarinus officinalis* were infected. These results provide as strong indication that, rather than weeds, are the perennial shrubs that play a major role in the epidemiology of the *X. fastidiosa* in this area.

85. GENETIC DIVERSITY AND VIRULENCE OF STRAINS OF *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* ISOLATED FROM *ACTINIDIA DELICIOSA* IN PIEDMONT. S. Prencipe[1], A. Garibaldi[2], D. Spadaro[1]. *[1]Università di Torino, DISAFA, Grugliasco, Italy; - [2]Università di Torino, AGROINNOVA, Grugliasco, Italy. E-mail: angelo.garibaldi@unito.it*

Pseudomonas syringae pv. *actinidiae* (*Psa*), the causal agent of bacterial canker of kiwifruit, is responsible for significant economic losses, both in yield and quality. During the first severe outbreak (2008-2010) and few years afterwards 40 strains of *Psa* were isolated. To analyse the genetic diversity of the pathogen REP, RAPD-PCR, and MLST of six housekeeping and effector genes were performed. RAPD technique, compared to REP-PCR showed an increased level of resolution in evaluating the genetic diversity within the pathovar *actinidiae* of *P. syringae*, with the sets of primers used in this study. The molecular fingerprinting, *Na*, *Ne*, *H*, *I*, polymorphic loci, and AMOVA showed a high level of variability and genetic

diversity in the population of *Psa* isolated in 2014 compared to the population of 2010. MLST showed SNPs in two of the effector genes analysed, while no mutation was found in the housekeeping genes. Furthermore, *in vitro* and *in vivo* virulence was evaluated, displaying a higher disease index for the strains isolated in 2014, compared to the older population. This study shows the evolution of genetic diversity and differences in the degree of virulence of *Psa* in the same geographical area, from the first epidemic outbreak to the endemic and established population. The high degree of the intraspecific variability found in *Psa* could be used as a model to study the evolution of bacterial pathogen diversity.

86. 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 appropriately respond 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. The role of 6 candidate genes in SA/JA crosstalk and of 12 candidate genes in ABA/JA crosstalk was confirmed by T-DNA insertion lines. 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.

87. MICROBIAL COMMUNITIES INVOLVED IN COMPOST-MEDIATED SUPPRESSION OF FUSARIUM WILT OF LETTUCE. M. Pugliese[1,2,3], M.L. Gullino[1,2,3], A. Garibaldi[1,3]. *[1]Centro AGROINNOVA, Università di Torino, Grugliasco (TO), Italy. [2]DISAFA, Università di Torino, Grugliasco (TO), Italy. [3]AgriNewTech srl, Torino, Italia. E-mail: massimo.pugliese@unito.it*

Compost suppressiveness depends primarily on microbiological composition and antagonists can be isolated from high quality composts. The objective of the present work was to evaluate the suppressive effect of compost against *Fusarium oxysporum* f. sp. *lactucae*, and evaluate microbial communities involved. A compost from municipal biowastes that showed a good suppressive activity in previous trials was added at 1% to steamed sandy inoculated with the pathogen. Compared to the same soil not treated, compost showed a 40% disease control of *Fusarium* wilt of lettuce. Microbial activities, bacterial and fungal concentrations were quantified and correlated in a Principal Component Analysis in order to clarify the correlation between the variables and compost suppressiveness. Samples taken from the rhizosphere of plants grown in suppressive media had highest total enzymatic activity and highest concentration of total fungi. The comparison of DGGE profiles of microbial populations revealed a greater diversity in the fungal community than that of bacteria. *Pseudomonas* sp., among bacteria, and *Simplicillum lamellicola*, among deuteromycetes, were detected only in the rhizophere of plants treated with 1% compost, indicating that they may play an active role in disease suppressiveness.

88. A DECLINE OF LENTIL (*LENS CULINARIS*) CROPS IN THE USTICA ISLAND CAUSED BY *PHYTOPHTHORA* species. I. Puglisi[1,2], F. Aloi[1], F. La Spada[1], M. Evoli[1,2], A. Pane[1], G. Di Miceli[3], L. Schena[2], G. Magnano di San Lio[2], S.O. Cacciola[1]. *[1]Università degli Studi di Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente, via S. Sofia 100 - 95123 Catania, Italy. [2]Università degli Studi Mediterranea, Dipartimento di Agraria, Loc. Feo di Vito, 89122, Reggio Calabria, Italy. [3]Università degli Studi di Palermo, Dipartimento di Scienze Agrarie e Forestali, viale delle Scienze Ed. 4 - 90128 Palermo, Italy. E-mail: olgacacciola@unict.it*

Ustica is a small island some 38 Km north of Palermo. A major crop in this island is a local variety of lentil (*Lens culinaris*) that is cultivated with organic farming systems and has been recently recognized as a Slow Food Presidium. In the last years, chlorotic patches, stunting, wilting and final death of plants as a consequence of root and basal stem rot have been observed in lentil fields across the island and the disease is becoming a limiting factor to the cultivation of lentil. In a survey of symptomatic fields, three *Phytophthora* species have been found to be associated to this decline, *P. megasperma, P. cryptogea* and *P. nicotianae*. Isolates were identified on the basis of DNA sequence data from the internal transcribed spacer regions (ITS1 and ITS2) and 5.8S gene of the rRNA operon and the mitochondrial *cox*1 gene in combination with morphological and physiological characteristics. Koch's postulates were fulfilled by artificial root-inoculation of potted lentil plants with representative isolates of the three *Phytophthora* species and re-isolation of the pathogens from roots of symptomatic plants. It is the first report of these *Phytophthora* species on lentil worldwide. Repeated monoculture and soil waterlogging were major factors favoring the disease. Cultivation on mounds was proposed as a way to prevent it.

89. WIDESPREAD OCCURRENCE OF *PHYTOPHTHORA MULTIVORA* IN EASTERN SICILY. I. Puglisi[1,2], F. Aloi[1], F. La Spada[1], A. De Patrizio[1], D. Ruano-Rosa[2], A. Pane[1], G.E. Agosteo[2], L. Schena[2], M. Horta Jung[3], T. Jung[3], S.O. Cacciola[1]. *[1]Università degli Studi di Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente, Via S. Sofia 100 - 95123 Catania (CT), Italy. [2]Università degli Studi Mediterranea, Dipartimento di Agraria, Loc. Feo di Vito, 89122, Reggio Calabria (RC), Italy. [3]Centre for Mediterranean Bioresources and Food (MeditBio), University of Algarve, Campus de Gambelas 8005-139 Faro, Portugal. E-mail: olgacacciola@unict.it*

Phytophthora multivora was recently described as a new species in the *P. citricola* complex and has a widespread distribution across natural ecosystems in Western Australia. In a previous large-scale survey conducted in 23 European countries, this polyphagous *Phytophthora* species was not found in Continental regions and was rare in Mediterranean and Atlantic regions. On the basis of this and other direct and indirect evidence, including high aggressiveness to native European plants and widespread occurrence in healthy undisturbed natural ecosystems in Australia, a non-European origin of this species was supposed. However, in a recent survey carried

out between 2013 and 2015, *P. multivora* was found to be common in volcanic soils of foothills of Mount Etna and coastal areas of north-eastern side of Catania province (eastern Sicily) from sea level to 900 m a.s.l. DNA sequences from the internal transcribed spacer regions (ITS1 and ITS2) and 5.8S gene of the rRNA operon and the mitochondrial *cox*1 gene were used in combination with morphological characteristics to identify the isolates. The pathogen was associated to chronic decline of holm oak (*Quercus ilex*) trees in forest stands and to root rot and trunk gummosis of several woody host-plants in nurseries and amenity gardens. In the botanical garden of Catania, it was found associated to trunk gummosis on two new non-native hosts, *Pistacia atlantica* and *Sterculia diversifolia*. In the surveyed area this homothallic *Phytophthora* species is favored by a mild and very wet climate in winter and survives to summer drought by producing oospores.

90. DECIPHERING *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* VIRULENCE: BIOVAR-SPECIFIC RESPONSES AND COMMUNICATION WITH HOST PLANT. M.R. Puttilli[1], A. Regaiolo[1], T. Colombo[1], C. Foresti[1], V. Venturi[2], G. De Vescovo[2], A. Polverari[1], E. Vandelle[1]. *[1]Università degli Studi di Verona, Dipartimento di Biotecnologie, Ca' Vignal 1, Strada Le Grazie 15 - 37134 Verona, Italy. [2]International Centre for Genetic Engineering and Biotechnology, Loc. Padriciano, 99 - 34149, Trieste, Italy. E-mail: mariarita.puttilli@univr.it*

Kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) is a recently emerged quarantine plant disease that is threatening global kiwifruit industry, where Italy plays a leading role worldwide. Currently, control measures are mainly based on the use of copper, for which the European Union has concerns due to its accumulation in the soil and its possible negative environmental effects. Moreover, the possible development of Psa resistance to copper is an event already emerging in several Countries. Such a scenario would rapidly leave kiwifruit growers with no chance to efficiently control the pathogen. We are currently attempting to decipher molecular signals involved in Psa virulence induction and promotion. This will allow the identification of targets for designing new control strategies based on the inhibition of the infection process, instead of killing bacteria, thus averting the appearance of new antibiotic resistances, in accordance with the new trend of bacterial disease control also developed in medical field. To that purpose, we produced a Psa-specific microarray chip carrying whole genomes of Psa strains of all currently known biovars (1, 2 and 3) to investigate in deep biovar-specific responses to provide insights into molecular mechanisms possibly controlling their different aggressiveness. Moreover, to decrypt the molecular signals governing bacteria-plant communication, the promoter of interesting target genes (involved in Psa virulence) have been fused to GFP-encoding gene to monitor the expression of the targets during Psa growth in presence of kiwifruit extracts.

91. THE SEAWEEDS *ASCOPHYLLUM NODOSUM* COMMERCIAL EXTRACT INDUCE RESISTANCE IN GRAPEVINE AND PROTECT AGAINST *BOTRYTIS CINEREA*. M. Quaglia[1], C. Moretti[1], T. Frioni[1], S. Tombesi[2], A. Palliotti[1]. *[1]Università degli Studi di Perugia, Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Perugia, Italy. [2]Università Cattolica del Sacro Cuore, Dipartimento di Scienze dell Produzioni Sostenibili, Piacenza, Italy. E-mail: mara.quaglia@unipg.it*

In the vineyard, the control of fungal pathogens is a key step to obtain high quality grapes. The Integrated Pest Management, mandatory in the European Community, along with the market demanding products from organic or biodynamic agriculture, places limits to the use of synthetic pesticides. Effective means alternative to chemicals are thus needed. Extracts from seaweeds are used in agriculture as biostimulants, due their ability to promote plant growth and increase yield. Furthermore, they can protect plants against biotic and abiotic stress. Extracts act directly against plant pathogens through their antimicrobial compounds and/or indirectly, increasing host resistance by means of their polysaccharides and other compounds. Here, *Ascophyllus nodosum* extract (Acadian Seaweed Marine Dry Powder, Biogard, Italy; 1.5 kg ha^{-1}) was sprayed on 3-years-old potted vines of cv. Sangiovese six times during the season 2015, from 3 June with weekly intervals, and berries were sampled at 0 and 24 h after the last treatment (hpt). Eva Green-based qPCR performed on defense-related genes showed a significant accumulation of the *VvPR1* (*Pathogenesis-related protein 1*) and *VvCaS2* (*Callose Synthase 2*) genes in berries harvested 24 hpt from treated vines, with respect to the untreated ones. At the same time, not significant differences were observed between treated and untreated samples for the *VvLOX* (*lipoxygenase*). In ripe berries detached from treated vines and artificially inoculated with *Botrytis cinerea*, the Index Percentage of Infection was significantly lower respect to the control. Our results support the role of *A. nodosum* extract in plant defense.

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92. *FUSARIUM GRAMINEARUM* SNODPROT PROTEINS PROTECT FUNGAL CELL WALL POLYSACCHARIDES FROM ENZYMATIC DEGRADATION. A. Quarantin[1], A. Glasenapp[2], W. Schäfer[2], F. Favaron[1], L. Sella[1]. *[1]Dipartimento Territorio e Sistemi Agro-Forestali (TESAF), Università degli Studi di Padova, Viale dell'Università 16 - 35020, Legnaro (PD), Italy. [2]Biocenter Klein Flottbek, Molecular Phytopathology and Genetics, University of Hamburg, Hamburg, Germany. E-mail: alessandra.quarantin@gmail.com*

The genome of *Fusarium graminearum*, a necrotrophic fungal pathogen causing Fusarium head blight (FHB) disease of wheat, barley and other cereal grains, contains five genes putatively coding for proteins similar to cerato platanins, which contribute to the virulence of the fungal pathogens *Botrytis cinerea* and *Magnaporthe grisea*. Two of them (FGSG_10212 and FGSG_11205) belong to the class of SnodProt proteins, with reported phytotoxic activity. To verify the contribution of the two *F. graminearum* SnodProt proteins to the infection process, single and double gene knock-out mutants were produced but no reduction in symptoms severity was observed compared to the wild type strain on both soybean and wheat spikes. Histological analysis performed by fluorescence microscopy on wheat spikelets infected with mutants constitutively expressing the dsRed confirmed that the *F. graminearum* SnodProt proteins do not contribute to fungal virulence. In particular, the formation of compound appressoria on wheat glumes was unchanged. Looking for other functions of these proteins, the double mutant was characterized by *in vitro* experiments. Wild type and mutants were similarly inhibited by salt and H_2O_2 stress. Surprisingly, the mutant grew better than wild type on carboxymethyl cellulose, while no difference was observed on glucose. Furthermore, conidia and mycelium of the mutant were more affected by treatments with chitinase and β-1,3-glucanase, thus indicating that the *F. graminearum* SnodProt proteins could protect fungal cell wall polysaccharides from enzymatic degradation.

93. GENOTYPING FLAVESCENCE DORÉE PHYTOPLASMA TO TRACE EPIDEMIOLOGY OF THE DISEASE AT

THE VINEYARD SCALE. M. Ripamonti[1], M. Rossi[1], M. Pegoraro[1], F. Veratti[1], D. Beal[2], D. Bosco[1,2], C. Marzachì[1]. *[1]CNR, Istituto per la Protezione Sostenibile delle Piante, Torino, Italy. [2]Università degli Studi di Torino, Largo Braccini 2, 10095 Grugliasco, (TO), Italy. E-mail: cristina.marzachi@ipsp.cnr.it*

Flavescence dorée is a quarantine disease of grapevine with serious impact on grape production in north-western Italy. The disease is present in Piemonte from 1998 and, despite many efforts for implementation of control measures, this dramatic threat to viticulture is still actively spreading. In an effort to evaluate the involvement of the natural compartment (*Scaphoideus titanus* vector and wild areas nearby vineyards) in the epidemiology of the disease, molecular markers for FD phytoplasma (FDp) were developed. The FDp belong to two 16Sr ribosomal groups and both are present in the study area. Seven FD infected vineyards were identified, all possessing a surviving population of *S. titanus* despite a known history of insecticide treatments, and alternative FD host plants along their edges and surrounding landscape. Available genome drafts were searched to identify suitable housekeeping genes for FDp genotyping from infected grapevines, alternative FD plant hosts (wild and abandoned grapevines, *Clematis vitalba*) and *S. titanus* from the vineyard and the wild compartment. Two housekeeping genes were selected upon screening of more than 20 targets: *dna*K and *mal*G. Preliminary genotyping identified several *mal*G types, often present as mixed infections in both plant and vectors at six sites. A homogeneous population was present at the remaining site. In particular, the genetic variability of FDp in *S. titanus* collected within the wild compartment was high and comparable to that of the cultivated grapevines. Moreover, FD genotypes from infected *C. vitalba* were not found in any other component of the cultivated and the wild compartments.

94. SHELF LIFE EXTENSION OF FRESH STRAWBERRIES USING POMEGRANATE EXTRACT. D. Rongai[1], F. Milano[2], P. Pulcini[2], C. Di Marco[1], N. Sabatini[1]. *[1]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, v. le Petruzzi, 75 - 65013 Città Sant'Angelo (Pescara), Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegeale (CREA-PAV), Via C. G. Bertero, 22 - 00156 Roma, Italy. E-mail domenico.rongai@crea.gov.it*

Punica granatum, commonly known as pomegranate, is an important source of bioactive compounds. The antimicrobic activity is linked to an high level of phenolic and flavonoid content in the pomegranate fruit peel. Pomegranate extract may be useful to maintain fruit quality and extend strawberry shelf life. This work describes the antifungal activity of pomegranate extract on the development of gray mold of strawberry caused by *Botrytis cinerea*, the main agent of fruit breakdown. To determine the effectiveness of the extract, strawberries were soaked in the pomegranate aqueous extract at 1.25, 2.50 and 5.00% (w/v). Strawberries soaked in sterile distilled water were used as a control. Disease incidence (DI), disease severity (DS) and FT-NIR (Fourier Transform Near-Infrared) spectra were recorded. The findings showed significantly differences between control and treated fruits. At the lowest dose, DI and DS of treated fruits were significantly lower than the control. No significantly differences were recorded between doses. The NIR data showed a significantly different spectrum between the untreated control and treated fruits. The effectiveness of pomegranate extract has been also confirmed by chemometric predictive model built using DS and NIR spectra $R^2 = 98.8\%$ and F value = 99.5 were registered by Principal Component Regression analysis.

95. ANTIMICROBIAL ACTIVITY OF POMEGRANATE EXTRACT: EFFECT ON CONSERVATION OF PROCESSED BLACK OLIVES. D. Rongai[1], C. Di Marlo[1], B. Lanza[1], N. Sabatini[1]. *[1]Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, v. le Petruzzi, 75 - 65013 Città Sant'Angelo (PE), Italy. E-mail domenico.rongai@crea.gov.it*

Several and different methodologies of preparing table olives are used in olive-producing countries. In all Italian olive regions, the black olives from *Olea europaea* L. cv. *Leccino* are prepared in accordance with the "Trade Standard Applying to Table Olives" as "dehydrated and/or shriveled black olives". The fruits are disposed in alternating layers with dry coarse salt (equivalent to 10-20% w/w olives) and several spices in special containers that allow drainage of the vegetable water drawn out by the salt. Processing time is around four to six weeks. The resulting olives are shriveled in appearance and have a salty-bitter taste. Salt acts as a preservative but the residual humidity of the product promotes the fungal proliferation. The purpose of our work is to treat the olives with pomegranate extract to prevent the proliferation of molds and extend the shelf life of the product. Three different doses have been used to test its antifungal activity. Seven days after incubation at 24°C and 90% RH, disease incidence (DI) and disease severity (DS) were evaluated. FT-NIR (Fourier Transform Near-Infrared) spectra, were also recorded during the experiment. At the dose of 1.25%, DI and DS were 52% and 6% respectively, significantly lower than 100% and 33% of the control. No significantly differences were recorded between doses. The effectiveness of pomegranate extract has been confirmed by NIR test. Principal Component Regression analysis demonstrated a correlation trends between spectra data and DS values ($R^2 = 90.43\%$ and F value = 19).

96. HETEROLOGOUS EXPRESSION OF A PLANT VIRUS PROTEIN CHANGED THE NATURE OF ACETIC ACID-INDUCED CELL DEATH IN *SACCHAROMYCES CEREVISIAE*. L. Rubino[1], N. Guaragnella[2], A. Antonacci[1], S. Giannattasio[2]. *[1]Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy. [2]Consiglio Nazionale delle Ricerche, Istituto di Biomembrane e Bioenergetica, Bari, Italy. E-mail: luisa.rubino@cnr.it*

A highly conserved feature of positive-strand RNA viruses is the association of the viral replication complex with specific intracellular membranes, which are induced to proliferate and are extensively rearranged to form vesicles, where virus replication occurs. Virus-encoded proteins are responsible for the intracellular localization of the replication complex and for the formation of vesicles. *Carnation Italian ringspot virus* (CIRV, genus *Tombusvirus*, family *Tombusviridae*) replication takes place in membranous structures, originating from the vesiculation of the mitochondrial outer membrane. CIRV p36 is required for targeting and anchoring CIRV replication to the outer membrane of mitochondria in plant and yeast cells. Since many DNA and RNA virus proteins have been reported to behave as proapoptotic or antiapoptotic viral factors, either by direct or indirect interaction with the mitochondrial outer membrane, *Saccharomyces cerevisiae* was used as a model to study the possible role of CIRV p36 on cell survival and death. It was shown that p36 expression affected cell growth rate, but did not promote cell death. p36 could change the nature of acetic acid-induced cell death in yeast from apoptosis-like to necrosis. Acetic acid treatment did not impair the strict association of p36 with mitochondrial membranes, thus confirming the independent interaction of p36 with mitochondria. Yeast proved to be a model organism for studying the mitochondrial determinants of cell death mode, as well as the

molecular pathogenesis of (+)RNA viruses and the mechanisms for the induction of necrosis in infected plants.

97. CORRECT DIAGNOSIS FOR GOOD DISEASES MANAGEMENT: THE CASE OF THE HYPERSENSITIVE REACTION OF *Sw-5* GENE IN TOMATO FRUITS. M. Ruocco, E. Troiano, A. Cozzolino, A. Aliberti, G. Parrella. *Istituto per la Protezione Sostenibile delle Piante del CNR, S.S. di Portici, Via Università, 133 - 80055 Portici (NA), Italy. E-mail: giuseppe.parrella@ipsp.cnr.it*

During spring-summer 2015, a severe disease was observed in tomato hybrids cv. Sir Elyan, cultivated in plastic greenhouses in different locations of Campania region (Southern Italy). Symptoms on the fruits consisted of concentric necrotic rings of 0.5 up to 2.5 cm of diameter. Tomato crops were unsuccessfully treated with fungicides against early blight disease (*Alternaria solani*). Therefore, several samples of symptomatic fruits from different locations were collected in order to isolate and identify the pathogen. From concentric necrotic rings, surrounding undamaged areas and asymptomatic tomatoes, procedures for the pathogen isolation were performed. No colonies were obtained from all the sampled tomatoes. Since cv. Sir Elyan contain *Sw-5* gene that confers resistance to several tospoviruses, we hypothesize that the symptom could be a hypersensitive response (HR) of the tomato fruit to *Tomato spotted wilt virus* (TSWV) vectored by thrips. To prove our hypothesis biological, serological and molecular tests were conducted on necrotic rings and healthy tissues. Results confirmed the presence of TSWV only within the necrotic lesions. Sequences obtained from RT-PCR showed 99.8% of identity with the TSWV isolate PED-1 (Accession number FN424155), a non resistance-breaking of the *Sw-5* gene. *Sw-5* confers resistance to TSWV in tomato by HR and usually in leaves *Sw-5* elicits pinpoint necrotic lesions preventing the spread of the virus from the infection site through the plant. Here we demonstrate that on fruits the HR reaction is less efficient and could develop large concentric necrotic rings, which can be confused with other common tomato disease.

98. OCCURRENCE AND DISTRIBUTION OF CITRUS VIROIDS AFFECTING DECLINING SWEET ORANGE TREES GRAFTED ON CITRANGE. M. Russo[1], G. Licciardello[1], M.C. Bazzano[1], A. Catara[2], G. Scuderi[1]. *[1]Agrobiotech ZI. Blocco Palma I, Str.le V. Lancia 57- 95121 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia ZI. Blocco Palma I, Str.le V. Lancia 57- 95121 Catania, Italy. E-mail: mrusso@agrobiotech.it*

The development of bioindexing on Etrog citron (*Citrus medica* L.), the selectivity of sPAGE analysis and the specificity of PCR tests have contributed to clarify the biological effects of the most important viroids on citron and trifoliate orange [*Poncirus trifoliata* (L.) Raf.], whereas the effects on citranges and other hybrids remain still unclear. As matter of fact the number of declining trees grafted on citranges is increasing and a better understanding appears relevant since, after the recent spreading of *Citrus tristeza virus* (CTV) in Italy, citranges appear the most suitable candidates to the replacement of sour orange rootstock. The occurrence and distribution of citrus viroids on sweet orange trees grafted on Troyer and Carrizo citranges and their association (if any) with low performance, dwarfing and bark scaling has been investigated in Eastern Sicily. A short testing has been carried out as well on some candidate source plants to understand the role of the propagation material on the local viroids spread. RT-PCR and real time RT-PCR analysis show the average infection by *Citrus exocortis viroid* (CEVd), *Hop stunt viroid* (HSVd) or *Citrus dwarfing viroid* (CDVd) is over 50%. None out of hundreds trees tested was infected by *Citrus bent leaf viroid* (CBLVd), *Citrus bark crack viroid* (CBCVd), *Citrus viroid V* (CVd V) or *Citrus viroid VI* (CVd VI). In general, declining trees showing bark scaling were infected by at least one viroid, a few showing reduced size were not infected, whereas others were asymptomatic despite CEVd infection.

99. *PENICILLIUM* spp. AND PATULIN CONTAMINATION ASSOCIATED TO BLUE MOULD OF POME FRUIT MARKETED IN SOUTHERN ITALY. S.M. Sanzani[1], A. Susca[2], S. Mastrorosa[2], M. Solfrizzo[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, - 70126 Bari, Italy. [2]Istituto di Scienze delle Produzioni Alimentari, CNR, Via Amendola 122/O - 70126 Bari, Italy. E-mail: simonamarianna.sanzani@uniba.it*

Blue mould is one of the most important postharvest diseases of pome fruit. Its main causal agent, *Penicillium expansum*, is also producer of the mycotoxin patulin, with mutagenic, immunotoxic, and neurotoxic properties. The present study aimed at identifying *Penicillium* isolates associated to blue mould of pome fruit marketed in Apulia (Southern Italy), and verify their ability to produce patulin. Twenty-nine *Penicillium* spp. isolates were recovered from apples and pears with blue mould symptoms. The fruits were analysed for patulin content and results compared with related fungal *in vitro* toxin production. In general, patulin was detected more abundantly *in vivo* than *in vitro*, although the stronger *in vivo* content did not match with the stronger *in vitro* producer. Isolates were morphologically and molecularly identified by amplification with *P. expansum*-specific primers and DNA sequencing of β-tubulin gene. Furthermore, isolates were tested for the occurrence of *patN* gene, coding isoepoxydon dehydrogenase (IDH), an enzyme involved in patulin biosynthetic pathway. All 26 strains identified as *P. expansum* were positive to *patN* and produced patulin. Three isolates belonging to other species of *Penicillium* were obtained from pears. They were positive for the *patN* gene, but only two actually produced patulin. It can be concluded that blue mould of pome fruit marketed in Apulia is mainly associated with toxigenic *P. expansum* strains, thus its rapid detection is important to prevent patulin contamination above regulatory limits. On the other hand, *patN* gene alone cannot be considered a reliable predictive assay; an evaluation of its expression level has to be carried out.

100. SINGLE-TUBE NESTED REAL TIME PCR ASSAY TO DETECT *PENICILLIUM* spp. IN WINES AND MUSTS. S.M. Sanzani[1], M.M. Miazzi[1], V. di Rienzo[1,2], V. Fanelli[1,2], G. Gambacorta[1], M.R. Taurino[3], C. Montemurro[1,2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via G. Amendola 165/A - 70126 Bari, Italy. [2]Sinagri s.rl. Spin-off, Università degli Studi di Bari Aldo Moro, Via G. Amendola 165/A - 70126 Bari, Italy. [3]Agro.Biolab Laboratory s.r.l., SP240 km 13,800 - 70018 Rutigliano (BA), Italy. E-mail: simonamarianna.sanzani@uniba.it*

Wine and fermenting musts are among the grape products more widely consumed. Since the presence of contaminating fungi may greatly compromise their quality characteristics and safety, there is a need for "user friendly", relatively rapid quantitative assays to measure the levels of fungal rot contamination, both in grapes delivered to wineries and in final products. Although other fungi are frequently involved in grape deterioration, secondary infections by *Penicillium* spp. are quite common, especially in cool areas with high humidity. In this work, the single-tube nested real-time PCR approach, already successfully applied to hazelnut and peanut

allergens detection, was used to trace *Penicillium* spp. in musts and wines. The method consists of two sets of primers specifically designed to target β-tubulin gene, with the aim of lowering the limit of detection of the conventional real-time PCR. The assay was able to detect up to 1 fg of *Penicillium* DNA. Related wine/must samples were almost all contaminated by patulin. Although further large-scale trials are needed, these results support the suitability of newly developed methods for the detection of *Penicillium* DNA in processed grapes.

101. COMPARATIVE GENOMICS OF *CITRUS TRISTEZA VIRUS* IN A NON-ERADICATIVE AREA OF EASTERN SICILY REVEAL WIDE GENETIC DISTANCES AND PHENOTYPE REACTIONS OF THE ISOLATES. G. Scuderi[1,2], R. Ferraro[2], M. Russo[1,2], M.C. Bazzano[1], A. Catara[2], G. Licciardello[1,2]. *[1]Agrobiotech Z.I. Blocco Palma I, Str.le V. Lancia 57- 95121 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia ZI. Blocco Palma I, Str.le V. Lancia 57- 95121 Catania, Italy. E-mail: glicciardello@agrobiotech.it*

Citrus tristeza virus (CTV) is a phenotypically complex virus causing serious economic losses to citrus industry worldwide, depending on the isolate and the variety/rootstock combination. Therefore, molecular-based strategies to control the disease, require an insight on the genetic assessment of the local virus population structure, supported by phenotype/genotype analysis. As result of an intensive biological and immunological testing of isolates collected in a non-eradicative area of eastern Sicily, selected isolates were analyzed by a new lab-on-chip (LoC) device running on an In-Check platform, enabling a multiplex RT-PCR with six pairs of primers and microarray hybridization of the amplicons with a set of 44 probes representative of the CTV strains in GenBank, which revealed three strains (VT, T30 and T36) with many isolates. Six of them were deep-sequenced and the genetic distance of their genomes analyzed in comparison with 42 genomes present in GenBank. Four isolates belong to the VT genotype and are largely divergent from the genome of SY568 isolate. Two show 98% homology sequence with T318A, and induce seedling yellow on sour orange, but not on lemon, whereas the remaining two have a mild cross protective phenotype. The T30-like and T36-like isolates fit 97-98% with the genomes of the respective strains. As expected, the former has a mild phenotype whereas the latter affects sour orange. The divergent results of phenotype/genotype analysis show that a multiple approach is required to assess the best management strategy of tristeza disease for each specific circumstance.

102. EFFECTS OF CERTIFIED MATERIAL ON RECONVERSION OF SICILIAN CITRUS ORCHARDS. G. Sorrentino, S. Di Silvestro, M.C. Strano, M. Guardo. *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CREA-ACM), Corso Savoia 190, 95024, Acireale (CT), Italy. E-mail: guido.sorrentino@crea.gov.it*

In 2007 CREA-ACM started a monitoring of citrus tristeza virus (CTV) and viroids in field-grown plants, collecting results on citrus grafted first on Sour Orange and subsequently (2014-2015) on Citrange Carrizo and Troyer. The spread of CTV in 2013 led to a reconversion of citrus orchards. Sour Orange was the most distributed rootstock in Sicily and for its high sensitivity to CTV it was replaced with Citrange Carrizo and Troyer, both susceptible to viroids infections. In 2007 62,5% of samples, grafted on sour orange, collected in commercial citrus orchards, were infected by viroids. In particular 59.4% of infected samples showed ISA1-CT-I mild strain of citrus exocortis viroid (CEVd) in combination with either citrus viroid III (CVd-III), citrus viroid IV (CVd-IV) or hop stunt viroid (HSVd) and 40.6% in single infection. In 2014 in Catania province, new citrus crops, originated from certified material, showed that 12% of samples were infected by CEVd ISA1-CT-I alone, 1% in combination with CVdIII and 2.4% with HSVd. In particular CEVd in combination with CVdIII shows symptoms like plants decaying and cracking or peeling of the bark. In 2015 in others citrus orchards viroids infections were caused by CEVd (1%), HSVd (8%) and CVdIII (28%). In greenhouses young plants were infected with CEVd (24%) and HSVd (37%). By using the certification system, viroid infection decreased in orchards, however it was still detectable in young plants grown in greenhouses. That is dangerous for the risks of field infection that the symptomatic contemporary presence of CEVd with CVdIII induces.

103. PLANT HEALTH AND SOCIOECONOMIC DIAGNOSIS OF FRUIT IN THE DA MATA RONDONIA AREA. G. Souza Gudin, D. Gomes da Silva, G. Lima Duarte. *Universidade Federal Rondônia, Rolim de Moura (RO), Brazil. E-mail: geovanes805@gmail.com*

The survey was conducted in the municipalities of Rondonia Territory forest area. With a questionnaire seeking to understand the economy of 20 farmers, photographic records were made of the main symptoms of diseases of fruit, a check post in the literature. Economic issues were treated as the producer monthly income and other sources of income. The fruits are sold in agricultural business and free markets. There is the use of pesticides on crops in 15 properties. There are symptoms of *Moliniophthora pernicious* in cupuaçu crops, *Didymella bryoniae* in melon, *Mycosphaerella fijiensis* and *M. musae* in banana, *Cladosporium herbarum* in passion fruit, in guava *Meloidogyne enterolobii*, *Alternaria* spp., *Citrus Leprosis* vírus, *Phytophthora nicotianae* var. *parasitica* and *Phytophthora citrophthora*, *Guignardia citricarpa*, *Elsinoë fawcetti* in Citros. Two producers said they do not use personal protective equipment (PPE). Use much monoculture, seeds / seedlings are proper, conferences and labor market influence the advancement of fruit production in the region, but low prices are the problems faced by farmers in marketing. Therefore, the fruit still has a long way to go, additional technical assistance is required and monitoring, as well as new technologies to increase production.

104. EVALUATION OF A SAMPLING METHOD FOR *XYLELLA FASTIDIOSA* DETECTION IN OLIVE TREES. L. Susca[1], O. Potere[1], V. Roseti[1], F. Civita[1], G. Loconsole[1], D. Boscia[2], V.N. Savino[1,2]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A - 70126 Bari, Italy. [2]Istituto per la Protezione Sostenibile delle Piante, CNR, SS di Bari, Via Amendola 165/A - 70126 Bari, Italy. E-mail: leonardo.susca@uniba.it*

To assess the presence of the xylem-limited bacterium *Xylella fastidiosa* subsp. *pauca* strain CoDiRO in olive trees, a specific sampling method was evaluated. Symptomatic and symptomless plants were randomly selected in four olive orchards located in the province of Lecce (Southern Italy). The crown of each plant was subdivided into a lower and an upper portion; four samples were collected from each layer in the main four cardinal directions. A total of eight samples per plant, composed of one- or two-year-old asymptomatic twigs, were collected next to branches showing leaf-scorch symptoms. In this preliminary study, the null hypothesis was tested. i.e. there is no difference between the lower and the upper portions of the tree canopy and across the four cardinal directions.

Samples (472), collected from 60 plants belonging to 11 different olive cultivars, were tested by qPCR. Out of 236 samples taken from the upper and lower parts of the canopy only 38.1% of lower samples, in contrast to 56.8% taken from the upper crown layer, were positive to the bacterium,. The McNemar test determined that there is a statistically significant difference in the proportion of positive samples between the upper and lower crown ($p < 0.001$). The Cochran's Q test was performed to evaluate differences in the four cardinal directions. The null hypothesis suggesting there is no difference across cardinal directions was confirmed ($p = 0.097$). Based on these preliminary results, it appears that sampling should be directed to the upper part of the canopy. However, further studies are needed to improve the efficiency of the sampling technique.

105. FAST PURIFICATION OF *PEPINO MOSAIC VIRUS* USING MONOLITHIC CHROMATOGRAPHIC SUPPORTS. **A. Taglienti, P. Pulcini, L. Tomassoli.** *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA-PAV). Via C.G. Bertero, 22 - 00156 Rome (Italy). E-mail: anna.taglienti@crea.gov.it*

Obtaining purified virus suspensions is a key process to implement plant virology applications, such as the production of virus antisera, transmission and interaction studies, and isolation of purified nucleic acid and coat protein (CP); moreover, it considerably simplifies the data analysis in Next Generation Sequencing (NGS) to characterize a viral genome. Plant viruses are usually purified *via* clarification and multiple ultracentrifugation steps, a complex and time-consuming procedure (about 4 days). In recent times, monolithic supports for High Performance Liquid Chromatography (HPLC) have been developed for separation and purification of macromolecules and supramolecular adducts, thus representing a cheaper and easier alternative for obtaining pure pDNA, protein and virus suspensions. In this work, we report the purification of *Pepino mosaic virus* (PepMV). PepMV (*Potexvirus*, *Alphaflexiviridae*) is a filamentous virus of 510 nm size with a ssRNA genome of 6.4 kb and a single 26kDa CP protein. It occurs everywhere tomato is grown, causing damage mainly to the quality of the fruit product. Raw extract of artificially infected tomato plants, after a brief pre-treatment, was subjected to chromatographic run using a convective monolithic column functionalized with quaternary amine (QA-CIM®). The presence of the virus in all the collected chromatographic fractions was monitored by RT-PCR; virus-rich fractions were also examined by RT-qPCR. From these fractions, CP protein was separated by acetone precipitation and reconstituted in water; total protein concentration was determined by Qubit fluorometric quantitation, and the presence of CP protein was confirmed by ELISA.

106. GENOME SEQUENCING OF SEVERAL *GRAPEVINE PINOT GRIS VIRUS* ISOLATES FROM SYMPTOMATIC AND ASYMPTOMATIC GRAPEVINES. **G. Tarquini[1], G.L. Bianchi[2], F. De Amicis[2], M. Martini[1], A. Loschi[1], N. Loi[1], R. Musetti[1], P. Ermacora[1].** *[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze, 206, I-33100 Udine, Italy. [2]ERSA, Servizio fitosanitario e chimico, ricerca, sperimentazione ed assistenza tecnica, via Sabbatini, 5, I-33050 Pozzuolo del Friuli, Udine, Italy. E-mail: tarquini.giulia@spes.uniud.it*

Grapevine Pinot gris virus (GPGV) is a newly discovered virus included in the genus *Trichovirus* in the family *Betaflexiviridae.* The virus has been detected on grapevine plants of different cultivars, showing symptoms of stunting, chlorotic mottling, mosaic and deformation of leaves, but also on asymptomatic plants. Despite the increase of reports of GPGV around the world, data on the genetic variability of the virus population are still scarce. The aim of this work was to give some preliminary results about the genetic variability among different GPGV isolates obtained from symptomatic and asymptomatic *Pinot gris*, *Pinot white Glera* and *Friulano* grapevines sampled in Friuli Venezia Giulia region (north-east Italy). Isolates were chosen considering detection variability using two different real-time PCR methods. Viral cDNA was obtained using 5'-RACE and subsequently cloned. To sequence the complete genome of the selected isolates, ten primer pairs were designed based on the consensus sequences of GPGV isolates available in GenBank in order to obtain overlapping amplicons spanning the entire genome of the virus. Each primer pair was initially tested on the GPGV isolates, resulting in amplification of all the viral genomic regions with just few exceptions. The purified PCR products were sent for sequencing to Genechron laboratory (Rome, Italy). The obtained sequences were mapped on the reference GPGV genome (FR877530) and assembled with *Geneius 7.1.6*. Our study will allow acquiring preliminary knowledge on the genetic variability inside the GPGV population, and defining a possible correlation between genome characteristics and symptom disease expression on grapevines**.**

107. REFERENCE GENE EVALUATION AND VALIDATION FOR NORMALIZATION OF QUANTITATIVE qPCR GENE EXPRESSION STUDIES IN cv. ROSSA DI TROPEA ONION INFECTED BY *ONION YELLOW DWARF VIRUS*. **A. Tiberini[1], L. Tomassoli[2], G. Albanese[1].** *[1]Università degli Studi Mediterranea di Reggio Calabria, Dipartimento di agraria, Località Feo di Vito - 89122 Reggio Calabria, Italy. [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero, 22 - 00156 Rome, Italy. E-mail: antonio.tiberini@unirc.it*

Among activities of the project "SI.ORTO" funded by Italian Ministry of Education, University and Research in the frame of 'Scientific Independence of young Researchers' program, *Onion yellow dwarf virus* (OYDV) relative quantification is provided by a Comparative C_T Method - $\Delta\Delta C_T$ to relate OYDV titer to secondary metabolites accumulation. In view of above, data normalization by an appropriate reference gene (RGs) is needed and required. Ideal RGs should be specific, stable in various plant development stages and portions, growth conditions, and should not be affected by pathogens infection. As no single internal control gene is universal to be used as an RG, in this study multiple stably expressed reference genes reported in literature were selected. In particular, elongation factor (Elf), protein phosphatase 2A (PP2A), helicase (HELI), 5.8S rDNA, ubiquitin (UBQ) and β-Actin (β-Act) genes, were compared in different leaf and bulb tissues, time points and biotic stress conditions (healthy and OYDV-infected). The assays were performed by a preliminary screening by RT-qPCR assay using SYBR green reagent, in triple replicates, evaluating Ct values and melting curves. Moreover, stability in the expression of the references genes in the samples sets was independently determined by three software (geNorm, NormFinder and Bestkeeper). UBQ and PP2A were the most stable genes, while Elf and HEL were the less stable. Melting curve analysis showed multiple peaks in UBQ amplification plot, so PP2A had been selected to be applied in the future OYDV relative quantification by $\Delta\Delta C_T$ normalization method. These results can be useful for better elucidating molecular interaction in the system OYDV/ 'Rossa di Tropea', and for RGs selection in virus-plant pathosystems.

108. A CASE OF STUDY: HOW A NEW-TREND IN HOT PEPPER CROP COULD REPRESENT A PHYTOSANITARY RISK

FOR INTRODUCTION OF NON-ENDOGENOUS VIRAL PATHOGENS. A. Tiberini[1], A. Ahmad[2], A. Manglli[3], U. Cassia[4], M. Barba[3], L. Tomassoli[3]. *[1]Università degli Studi "Mediterranea" di Reggio Calabria, Feo di Vito, 89121 Reggio Calabria, Italy. [2]Plant Virology Laboratory, Department of Plant Pathology, PMAS-Arid Agriculture University. Rawalpindi, Pakistan. [3]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero, 22 - 00156 Rome, Italy. [4]Associazione Pepperfriends. Email: antonio.tiberini@unirc.it*

Hot pepper (*Capsicum* spp.) is a worldwide cultivated crop, integrated into dietary habits of billion of people. Recently, the identification of several nutraceutical compounds has been increasing the crop value. It is possible to assist to an explosion of monothematic fairies and expositions, all over the peninsula, where to retrieve hundreds of cultivars from all over the world, and where it is possible to exchange "freely" germplasm. This introduction and exchange of seeds and plantlets are *de facto* introducing phytosanitary risks, facilitating the possibility of new outbreaks. During a two years of surveys, chilli from commercial and core collection crops has been investigated for viral diseases. A great variety of symptoms was observed including yellowing, brittleness, mosaic and necrosis associable to several pathogenic agents. Serological and molecular analysis were not always decisive in the identification of the etiological viral agent endemic to Italy (*Tomato spotted wilt virus*, *Alfalfa mosaic virus*, *Broad bean wilt*, *Potato virus Y*, *Pepper mild mottle virus*). However, additional diagnostic tests allowed to identify two alien viruses as *Pepper vein yellows virus* (PeVYV) and *Chili veinal mottle virus* (ChiVMV), belonging to *Polerovirus* and *Potyvirus* genus, respectively. In particular, the accidental occurrence of ChiVMV, an aphid-transmitted virus in stylet-borne, was related to the presence of an Asiatic community growing chilli in family garden close to the surveyed hot pepper collection area. This preliminary and not intensive survey showed how high is the risk to introduce dangerous pathogens and how much is difficult to establish measures and apply rules to control germplasm exchanges.

109. PRELIMINARY STUDIES ON THE IDENTIFICATION OF VIRULENCE GENES BY DNA METHYLATION ANALYSIS IN THE CEREAL PATHOGEN *FUSARIUM GRAMINEARUM*. F. Tini[1], G. Beccari[1], D.M. Gardiner[2], M. Bocchini[1], G. Marconi[1], E. Albertini[1], L. Covarelli[1]. *[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74 - 06121, Perugia, Italy. [2]CSIRO Agriculture, Queensland Bioscience Precinct, Brisbane - 4067 QLD, Australia. E-mail: lorenzo.covarelli@unipg.it*

The study of DNA methylation represents an important tool to determine epigenetic modifications in living organisms and to explain the dynamics of their adaptations to environmental changes. At this purpose, a study based on the analysis of DNA methylation was conducted to identify virulence genes of the cereal pathogen *Fusarium graminearum*. A selected highly virulent strain of this fungus was consecutively subcultured for 50 times by transferring a mycelium plug every week into new plates containing a rich medium such as Potato Dextrose Agar. To assess the effect of subculturing on fungal virulence, wheat (cv. A416) seedlings and heads were inoculated with subcultures 1, 5, 9, 15, 23, 34, 42 and 50. Seedling virulence assays were performed by evaluating the necrotic area caused by the different subcultures on the leaves surrounding the stem base while head assays were carried out by observing the blighted spikelets. The DNA isolated from the different subcultures was subject to methylation analysis by a combination of NGS and methylation-sensitive enzymes. Seedling stem base assays showed a progressive virulence decline, which was about 46% in the last subculture with respect to the first one. Head blight assays showed a virulence reduction of all tested subcultures of about 27%, on average, with respect to the first one. Virulence was restored to subculture 1 level after transferring subculture 50 onto wheat heads for three times. The effect of DNA methylation, following prolonged subculturing on a rich medium, on fungal aggressiveness as a possible strategy to identify novel virulence genes in fungal pathogens is discussed.

110. OPTIMIZATION AND VALIDATION OF A DIAGNOSTIC REAL-TIME PCR FOR *TILLETIA INDICA* KARNAL BUNT. M.T. Valente, L. Riccioni. *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero, 22 - 00156 Rome, Italy. E-mail: mariateresa.valente@crea.gov.it*

Tilletia indica Mitra is the agent of the wheat disease commonly known as Karnal bunt: it causes a qualitative damage on grain wheat making it unmarketable. A strong quarantine action exists to control the presence of *T. indica* on wheat grain imported in Europe and in many Countries worldwide. A multiplex real-time PCR (qPCR), included in the ISPM (International Standards For Phytosanitary Measures) 27 Diagnostic Protocols, targeting the ITS1 region in rDNA was optimized and validated for the identification of *T. indica* from wheat grains following the guidelines for diagnosis of infectious diseases [EPPO pm 7/98(2)]. The original protocol was modified for the DNA extraction, from the pellet obtained in the "washing test" of 50 grams of wheat seed sample, and the pre-amplification PCR step, aiming to increase the amount of amplicon production to be used as template in the qPCR. Comparative quantification of the PCR product was carried out using both the original and the modified pre-amplification protocol, confirming a significantly increased yield. The assay had an analytical sensitivity of 3 teliospores present in the pellet. Analytical specificity was tested on *T. horrida* and *T. walkeri*, two morphologically similar species, and there was no cross reaction. A test performance study was organized with 9 participating laboratories to assess diagnostic sensitivity, diagnostic specificity, accordance and concordance. This work represents the optimization of a diagnostic protocol for *T. indica*, especially useful for the detection of low number of spores.

111. COST ACTION FP1406: PINE PITCH CANKER – STRATEGIES FOR MANAGEMENT OF *GIBBERELLA CIRCINATA* IN GREENHOUSES AND FORESTS (PINESTRENGTH). A.M. Vettraino[1], J. Martín-García[2,3], R. Ioos[4], E. Vainio[5], R. Vasaitis[6], M. Fernández[1,7], J. Hantula[5], P. Capretti[8], S. Woodward[9], R. Raposo[10], A. Vannini[1], T. Dogmus[11], A. Alves[12], V. Vasic[13], M. Vasconcelos[14], J.J. Diez[2,3]. *[1]DIBAF, University of Tuscia, Viterbo, Italy. [2]Sustainable Forest Management Research Institute, University of Valladolid-INIA, Avda. Madrid 44, Building E - 34004, Palencia, Spain. [3]Department of Vegetal Production and Forest Resources, Higher Technical School of Agrarian Engineering, University of Valladolid, Avda. Madrid, s/n, 34004 Palencia, Spain. [4]ANSES. Laboratoire de la Santé des Végétaux, Unité de Mycologie. Domaine de Pixérécourt, Bât. E 54220 Malzéville, France. [5]Vantaa Research Unit, Finnish Forest Research Institute, PO Box 18 - 01301 Vantaa, Finland. [6]Swedish University of Agricultural Sciences, Box 7026, SE - 75007, Uppsala, Sweden. [7]Department of Agroforestry Sciences, Higher Technical School of Agrarian Engineering, University of Valladolid, Avda. Madrid, s/n, 34004 Palencia, Spain. [8]Department of Agri-Food Production and Environmental Sciences, University of Firenze, P.le delle Cascine 28, I - 50144 Firenze, Italy. [9]Department of Plant and Soil Science, Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, UK. [10]The Spanish National Institute for Agricultural and Food Research and Technology (INIA). C. Coruña km 7.5. 28040 Madrid, Spain. [11]Suleyman Demirel University. Faculty*

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Fusarium circinatum, is one of the most important pathogens of Pinus species. Sporadic outbreaks and epidemics caused by this fungus have been reported from numerous countries, including Central and South America, South Africa, Asia and, more recently, Europe. The disease is frequently associated with reduced yields and high levels of tree mortality in certain areas, resulting in significant economic losses. Nevertheless, there has been little research on *F. circinatum* in Europe to date and little information is available overall on host range in Europe, pathogen spread and disease control. The main aim of PINESTRENGTH is to establish a European-focused network to increase knowledge of the biology, ecology and pathways of spread of *F. circinatum,* to examine the potential for the development of effective and environmentally-friendly prevention and mitigation strategies and to deliver these outcomes to stakeholders and policy makers. To that end, a multi-disciplinary approach is being taken, including researchers, forest managers and policy makers from 35 countries to date. Furthermore, any interested party is encouraged to join this COST Action, participating in some of the six Working Groups (WG1 The pathogen-diagnosis, WG2 Interactions with other forest pests and pathogens, WG3 Pathway of disease spread, WG4 Pest risk analyses, WG5 Management of the disease in forest and nurseries and WG6 Coordination, identifying research gaps and dissemination). For further information, please check http://www.pinestrength.eu/.

112. FUNCTIONAL CHARACTERIZATION OF A Zn(II)2Cys6 TRANSCRIPTION FACTOR INVOLVED IN THE REGULATION OF THE TRICHOTHECENE GENE CLUSTER IN FIESC. A. Villani[1], D.W. Brown[2], R.H. Proctor[2], S. McCormick[2], A.F. Logrieco[1], A. Moretti[1], A. Susca[1]. *[1] Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche (ISPA-CNR), Via Amendola 122/O - 70126 Bari, Italy. [2] National Center for Agricultural Utilization Research, U.S. Department of Agriculture, 1815 N. University St.- 61604, Peoria, IL, USA. E-mail: alessandra.villani@ispa.cnr.it*

Members of *Fusarium incarnatum-equiseti* species complex (FIESC) have been commonly reported as contaminants of cereals, less often associated with major disease epidemics, although regularly identified with other pathogens in field surveys. In addition, the complex includes mycotoxigenic strains able to synthetize trichothecenes, both type A and type B. Due to the potential health hazards, trichothecenes (TRI) mycotoxins represent a particular concern, because they are potent inhibitors of protein synthesis in eukaryotes, and their consumption causes several symptoms in human and animals. The organization of trichothecene biosynthetic genes in *F. equiseti* is different than other TRI-producing species. In the FIESC, *TRI1* and *TRI101* are located in the trichothecene cluster, *TRI12* is absent, and the *TRI3-TRI7-TRI8* region of the cluster is in the opposite orientation and located at the opposite end of the cluster. Additionally, a gene named *OrfF*, encoding a *Zn(II)2Cys6* transcription factor, is exclusive for the FIESC, and it is present into all the TRI core clusters so far analyzed. In order to achieve more insight into the regulation of TRI biosynthetic genes in FIESC, we investigated the function of *OrfF* by deletion with a split marker recombination strategy. *OrfF* deletion mutants showed complete loss of TRI production, while complementation of the ΔOrfF mutants with the wild-type copy of the gene fully restored their ability to make TRI. Subsequent feeding experiments demonstrated that *OrfF* is required for expression of at least *TRI11* and possibly other late-pathway genes (i.e. *TRI13, TRI7, TRI3* and *TRI8*) required for synthesis of TRI.

113. CO-CULTURE OF BENEFICIAL MICROBES AS A NEW SOURCE OF BIOACTIVE METABOLITES. F. Vinale[1], R. Nicoletti[2], R. Marra[1], M. Pascale[3], G. d'Errico[3], F. Lacatena[3], M. Monetti[1], K. Abadi[4], M. Lorito[1,3]. *[1] Istituto per la Protezione Sostenibile delle Piante (IPSP-CNR), Via Università 133-80055 Portici (NA), Italy. [2] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria; [3] Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 133 - 80055 Portici (NA), Italy. [4] BTRC - Biotechnology Reserch Center, Tripoli, Libya. E-mail: francesco.vinale@ipsp.cnr.it*

Beneficial microbes biosynthesize secondary metabolites for competition, symbiosis, parasitism or pathogenesis with other organisms. Some of these compounds have been associated, directly and/or indirectly through the plant, to the biocontrol of phytopathogens, and to the activity of many beneficial microbes used as biopesticides or biofertilizers. Metabolomic analysis of the interactions between fungal antagonists, plants, and phytopathogens allows the identification of several microbial secondary metabolites that positively affect plant metabolism. Simulating naturally occurring interactions, the co-cultivation of two different fungi or a fungus with plant tissue represents an effective strategy to identify novel compounds that may not be produced under standard laboratory conditions. Co-culturing experiments of the biocontrol agent *Trichoderma harzianum* M10 and the endophyte *Talaromyces pinophilus* TP1, or the single fungus grown in the presence of tomato leaf tissue, resulted in increased biological activity. Metabolomic analysis revealed that the fungal co-culture induced the accumulation of the M10 siderophores ferricrocin, coprogen B and dimerumic acid, and the TP1 siderophore ferrirubin. The production of *Trichoderma* metabolites harzianic acid and iso-harzianic acid was not affected by *T. pinophilus*, but increased in the presence of plant tissue. Conversely, *Talaromyces* metabolites 3-O-methylfunicone and herquline B decreased when M10 was present, while no differences were observed with tomato tissue. Surprisingly, in the fungal co-culture, a novel metabolite was found, not previously observed in neither M10 nor TP1 culture filtrates. The complete chemical characterization of this compound, with the putative molecular formula $C_{11}H_{17}NO_5$, is in progress.

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114. FUNGAL DIEBACK DISEASE OF CYPRESS: AN INCREASING PROBLEM. S. Vitale, L. Luongo, L. Orzali, M. Galli, A. Belisario. *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero, 22 - 00156 Roma, Italy. E-mail: laura.luongo@crea.gov.it*

Fungal dieback disease seems to have increased in significance on garden conifers, mainly cypress, in recent years. During routine monitoring, twigs were collected from several cypress trees located in different Mediterranean areas. Symptoms were observed on twig tips progressing downwards, resulting in twig dieback and death. In some cases, dark acervula were present on the cankers. Isolations were made at the margins of twig lesions by removing small host tissues pieces and by placing them onto 2% malt extract agar (MEA). Species of *Pestalotiopsis* have constantly been isolated, among them *P. funerea* was the most common, but also *P. hollandica* and *P. maculiformis* were detected. Together with *Pestalotiopsis* spp. other fungal ubiquitous and polyphagous, occasional pathogenic and/or endophytic, were isolated, namely *Stemphylium* spp., and

Paraconiothyrium variabile. Species of the genus *Pestalotiopsis* are often found associated with leaf spots and diebacks, not just of conifers but of a very wide range of woody plants. They are usually regarded as weak pathogens, though once within the plant tissues they can cause quite extensive damage. However in recent years remarkable new reports of this pathogen have been published worldwide. This fungal genus has increased its occurrence and pathogenic attacks also in cold areas such as Norway and Scotland as a probable consequence of global warming.

115. *STEMPHYLIUM VESICARIUM* CAUSING GRAY LEAF SPOT ON HOT PEPPER IN ITALY. S. Vitale, L. Luongo, M. Galli, A. Belisario. *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: salvatore.vitale@crea.gov.it*

A mycological survey was carried out on hot pepper fields during 2014 growing season in two different Italian geographic areas of Italy. Gray leaf spot symptoms were observed on "Diavulicchio calabrese" (*Capsicum annum*) and "Fatalii white" (*C. chinense*) varieties. Leaf lesions were small circular spots, usually 3-5 mm in diameter, with a white center and a narrow dark edge. The disease incidence on these varieties were more than 40%. Colonies of *Stemphylium* sp. were consistently isolated from diseased leaf samples. Two isolates of *Stemphylium* sp. (ISPaVe2162 and ISPaVe2165) were collected from gray leaf spot lesions of *C. annum* and *C. chinense* of both geographic areas. Based on the morphological characteristics of colony colour and appearance, and morphology of conidia as well as sequences of internal transcribed spacer regions (ITS) and glyceraldehyde 3-phosphate (*gpd*) gene, the fungus was identified as *S. vesicarium*. The isolates showed 100% identity with either ITS region or *gpd* gene with *S. vesicarium* namely, JX424810 for ITS region and DQ000654 for *gpd* gene. Newly developed sequences were deposited in European Nucleotide Archive (ENA) with the accession Nos. LN896692 and LN896693 for ITS, and Nos. LN896694 and LN896695 for *gpd* gene, respectively. Pathogenicity tests confirmed *S. vesicarium* isolates as pathogenic onto artificially inoculated *C. annum* and *C. chinense* leaves. Leaf spots caused by *S. vesicarium* were reported on tomato in Italy, but not on other horticultural crops. To our knowledge this is the first report of *S. vesicarium* causing gray leaf spot on pepper in Italy or elsewhere.

116. A LAB-SCALE APPROACH TO SELECT YEAST STRAINS FOR BIOLOGICAL CONTROL OF POSTHARVEST PATHOGENS. D. Vitullo[1], A. Kheireddine[1,2], D. Palmieri[1], G. Ianiri[1], F. De Curtis[1], N. Sadfi-Zouaoiu[2], R. Castoria[1], G. Lima[1]. *[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis s.n.c. - 86100 Campobasso, Italy. [2]Department of Biology, Campus Academicals, University of Science of Tunis, 2090 Tunisia. E-mail: domenico.vitullo@unimol.it*

Biological control of postharvest decay of fruit and vegetables using antagonist yeasts has been explored as one of most promising alternatives to synthetic fungicides. For the biocontrol yeast activity several mechanisms have been proposed: competition for nutrients and space, secretion of antifungal compounds and lytic enzymes, induction of host resistance, yeast adaption to reactive oxygen species (ROS).

Several potential biocontrol yeasts were isolated from different fruit and vegetables and stored in an Italian and a Tunisian yeast collection. Based on our background, using as a model of study the host-pathogen system "Apple-*Penicillium expansum*", we set up a new lab-scale approach to evaluate candidate yeasts displaying the best biocontrol features by investigating: i) antagonistic activity, ii) influence of pH during antagonist-pathogen co-incubation, iii) temperature of growth, iv) biofilm production, v) reactive oxygen species (ROS) tolerance and vi) ability to produce siderophores. The most promising strains were identified by analysis of the ITS regions.

Our preliminary results showed that the most active biocontrol yeast strains were able to reduce the incidence of fruit decay with the involvement of different modes of action, which included mainly i) counteracting the tissues acidification exerted by *P. expansum*; ii) production of siderophores, and iii) ability to grow in the presence of ROS.

117. ASSESSING GENETIC DIVERSITY OF *PHYTOPHTHORA CAMBIVORA* ISOLATES. A. Zambounis[1], A. Xanthopoulou[2], P. Madesis[2], A. Tsaftaris[1,2], A. Vannini[3], N. Bruni[3], A. Tomassini[3], G. Chilosi[3], A.M. Vettraino[3,4]. *[1]Laboratory of Genetics and Plant Breeding, Faculty of Agriculture, Forestry & Natural Environment, Aristotle University of Thessaloniki, P.O. Box 261, Thessaloniki GR-54124, Greece. [2]Institute of Applied Biosciences, CERTH, Thermi, Thessaloniki, 570 01, Greece. [3]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Via S. Camillo de Lellis, I-01100 Viterbo, Italy. [4]National Research Council - Institute for Sustainable Plant Protection, (CNR-IPSP), Via Madonna del Piano 10, I-50019 - Sesto Fiorentino, Firenze, Italy. E-mail: vettrain@unitus.it*

Phytophthora cambivora (Petri) Buisman is a soil borne oomycete causing root rot on several woody species worldwide. Besides with *P. cinnamomi,* it is assigned as the most pathogenic species associated with chestnut ink disease in Europe. At the moment, no efficient management control strategies exist for ink disease. Resources regarding the population genetic variability of *P. cambivora* is almost limited, despite that might be useful for the development of integrated control programmes. According to our knowledge, no such studies have been performed in chestnut-growing regions across Europe. Thus in this study, we develop a reliable and accurate genotyping approach named HRM (High Resolution Melting) analysis in order to efficiently estimate the genetic variation among *P. cambivora* isolates collected in a chestnut growing area in Central Italy (region of Rieti). Degenerated primers were designed upon conserved regions of exons 3 and 4 of the gene locus *Ypt*1. The derived melting curves profiles accurately assigned all *P. cambivora* isolates in eight unique HRM genotypes based on their normalized curve profiles indicating rather a high intraspecific genetic variation among these isolates. Our data confirmed that the HRM approach might be on a wide scale a rapid, reliable and reproducible tool for efficient intraspecific genotyping of *P. cambivora* populations.

118. BIOCONTROL AGENTS INDUCE PATULIN BIOSYNTHESIS *IN VIVO* BUT DECREASE THE OVERALL PATULIN ACCUMULATION IN STORED APPLES. Q. Yang[2], X. Zheng[2], X. Zhang[2], M.T. Apaliya[2], G. Ianiri[1], H. Zhang[2], R. Castoria [1,2]. *[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, Via F. De Sanctis snc, Campobasso, Italy. [2]School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, Jiangsu, People's Republic of China. E-mail: castoria@unimol.it*

Synthetic fungicides are presently employed for the control of postharvest diseases. However, health concerns on the use of these chemicals require alternative control methods such as biocontrol based on biocontrol agents (BCAs). We have investigated the effect of two biocontrol yeasts, *Rhodotorula mucilaginosa* strain

3617 and *Rhodosporidium kratochvilovae* LS11, on blue mold and patulin contamination caused by strains PY and FS7 of *Penicillium expansum* in artificially inoculated Fuji apples stored at 20°C for 9 days. In general, strain LS11 proved to be more effective than strain 3617. In order to correlate the development of *P. expansum* in yeast-treated and untreated apples with patulin production, we quantified the pathogens' biomass in the infected fruits through a qRT-PCR method based on specific primers from patF, a gene involved in patulin biosynthesis in *P. expansum*. Both yeasts significantly reduced disease incidence by the two strains of *P. expansum* up to 5 days of incubation. Furthermore, the BCAs lowered the growth of pathogens' biomass and the progression of symptoms up to 9 days. Interestingly, both yeasts strains increased the rate of patulin production (ng of patulin/ng of fungal DNA) by the two pathogenic strains. Nevertheless, both BCAs reduced the overall level of patulin contamination, especially in the case of strain FS7, the higher patulin producer. To our knowledge, this is the first report of a quantitative assessment in stored fruits of the effect of BCAs both on the growth of mycotoxigenic fungal pathogens and on the specific rate of mycotoxin synthesis.