

ABILITY AND SYNERGISTIC EFFECTS OF ANTAGONISTIC *PSEUDOMONAS* AND *PANTOEA* spp. TO CAUSE VASCULAR DISCOLORATION AND PITH NECROSIS IN TOMATO PLANTS. D. Aiello¹, S. Abriano¹, A. Cinquerrui¹, G. Firrao², G. Polizzi¹ and G. Cirvilleri¹. ¹Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Sezione di Patologia Vegetale, Via S. Sofia 100, 95123 Catania, Italy. ²Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. E-mail: gcirvil@unict.it

The elimination of methyl bromide has had remarkable phytopathological consequences on tomato cultivation in greenhouses over the last decade, with an increased incidence of soil-borne diseases. Recently, vascular infections caused by bacteria have grown in importance in Sicily. In particular, infections caused by *Pseudomonas fluorescens* and *P. putida* induce leaf chlorosis, vascular and pith browning/discoloration of tomato plants grown in greenhouses and soilless cultures. During 2011-2012 pith necrosis, vascular discoloration and leaf chlorosis observed in soil-grown tomatoes were shown to be caused by *Xanthomonas perforans* strains, never reported previously as the cause of vascular diseases. In addition, surveys showed that numerous bacterial strains belonging to *P. fluorescens*, *P. putida*, *P. marginalis*, *P. citroneolis*, *P. straminea*, and *Pantoea agglomerans*, often associated in symptomatic tissues with *X. perforans* strains, were weakly virulent pathogens to tomato. *Pseudomonas* and *Pantoea* species are well-known antagonistic bacteria that often used for biological control. In further tests, our *Pseudomonas* spp. strains and *Pseudomonas* spp. strains from International Collections with well-established biocontrol properties were also shown to induce tomato vascular discoloration. Moreover, in some cases, co-inoculation with *X. perforans* resulted in an increased length of stem pith necrosis. The results of this work highlight the need for a careful evaluation of biocontrol agents during the screening procedures and before commercialization to avoid the risk of introducing strains that may be pathogenic to tomato plants.

RESISTANCE BEHAVIOUR TO ANTHRACNOSE DISEASE BY *GNOMONIA LEPTOSTYLA* IN VARIOUS SPECIES AND PROVENANCES OF *JUGLANS* AND *PTEROCARIA* FOR WOOD PRODUCTION. N. Anselmi¹, M. Gras², G. Mughini² and S. Zauli¹. ¹Department for Innovation in Biological, Agro-food and Forest Systems University of Tuscia, Viterbo, Italy. ²Agricultural Research Council, Research Centre for Intensive Wood Production, Rome, Italy. E-mail: anselmi@unitus.it

The aim of this work was to evaluate the behavior of various species and provenances of *Juglans* and *Pterocarya* for wood production with respect to anthracnose caused by *Gnomonia leptostyla*. The research was carried out in a 15-year-old experimental plantation near Tivoli, Rome (CRA, Cesurni farm). The material taken into consideration consisted of four *Juglans* species from the USA, *J. cinerea* (8 genotypes), *J. microcarpa* (18), *J. sieboldiana* (16) and *J. nigra* (166); different provenances of *J. regia* from France (27), Russia (16), Israel (28), Iran-Turkey (29), northern Italy (32), central Italy (96), southern Italy (122), as well as *Pterocarya fraxinifolia* (8) and *P. stenoptera* (6). During the 2002 vegetative season, the rainfall was abundant and therefore anthracnose incidence was very high. The mean number of necrotic spots and the percentage of necrotized leaf area were useful parameters to evaluate disease incidence. Although severely infected, *Pterocarya*, *J. cinerea* and *J. sieboldiana* showed only small (2-4 mm diameter) necrotic spots, while the other species exhibited necroses bigger than 1 cm², with a clear preference (in descending order) for *J. regia*, *J. nigra* and *J. microcarpa* and, among *J. regia*, for the provenances from France, Russia, Italy, Iran-Turkey, and Israel. *P. fraxinifolia* and *P. stenoptera* proved very resistant to anthracnose,

whereas *J. cinerea*, *J. microcarpa*, *J. nigra*, *J. sieboldiana* were basically resistant. Among *J. regia*, the genotypes from French origin were moderately resistant, those from USSR and Israel were tendentially susceptible, those from Turkey and Iran were very susceptible, whereas the genotypes from Italy ranged from very susceptible to moderately resistant. In the case of Italian *Juglans*, a significant inverse correlation between the incidence of the disease and diametric growth of the plants was found. These results represent an important point of reference for the selection and genetic improvement of walnut for wood production.

PYRENOCHAETA LYCOPERSICI GENOME ASSEMBLY SHOWED INTERESTING FEATURES RELATED TO ITS LIFESTYLE AND INTERACTION WITH THE HOST. M. Aragona¹, A. Minio², A. Ferrarini², M.T. Valente¹, P. Bagnaresi³, L. Orrù³, P. Tononi², G. Zamperin², A. Infantino¹, G. Valè^{3,4}, L. Cattivelli² and M. Delledonne². ¹Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy; ²Dipartimento di Biotecnologie, Università degli Studi, Strada le Grazie 15, 37134 Verona, Italy; ³Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per la Genomica e la Post Genomica Animale e Vegetale, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy; ⁴Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per la Riscicoltura, S.S. 11 per Torino Km 2.5, 13100 Vercelli, Italy. E-mail: maria.aragona@entecra.it

Pyrenochaeta lycopersici is a soil-borne pathogen that causes corky root rot disease in tomato (*Solanum lycopersicum*) and other *Solanaceae* crops, reducing fruit yields by up to 75%. *De novo* assembly of the *P. lycopersici* genome was based on Illumina sequencing and the functional characterization of the draft sequence by integrating RNA-Seq data was followed by an in-depth analysis of the virulence mechanisms and potential pathogenicity effectors encoded by this pathogen. We assembled a 54.9Mb *P. lycopersici* draft genome and annotated approximately 17,000 genes. The *P. lycopersici* genome is closely related to hemibiotrophs and necrotrophs, in agreement with the phenotypic characteristics of the fungus and its lifestyle. *P. lycopersici* genome reveals a significant expansion of specific genes families related both to pathogenesis and to reproduction mechanisms, including those responsible for plant cell wall degradation, nutrient absorption and fungicide detoxification. We also observed a significant expansion of the gene families associated with heterokaryon incompatibility (HI), which represents an important mechanism for this imperfect fungus for increasing genetic variability. The assembly constitutes an important resource to understand the molecular bases of corky root rot and more in general to enrich current knowledge of plant-pathogen interaction mechanisms.

CHARACTERIZATION OF THE E3-UBIQUITINE LIGASE ATL GENE FAMILY IN GRAPEVINE AND STABLE TRANSFORMATION OF *VITIS VINIFERA* WITH AN ATL GENE FROM *VITIS RIPARIA*. P. Ariani, A. Lovato, A. Giorgetti, A. Regaiolo and A. Polverari. Dipartimento di Biotecnologie, Università degli Studi, Strada Le Grazie 15, 37134 Verona, Italy. E-mail: annalisa.polverari@univr.it

Our research group is mainly focused on the molecular basis of grapevine resistance to *Plasmopara viticola*, which is being investigated through different approaches. In previous microarray analyses, we identified several hundred genes, specifically activated in the resistant species *Vitis riparia* and not in the cultivated susceptible

V. vinifera. Many of these genes are related to signal transduction or are homologous to known regulators of the defence response in other species. Among them, a group of genes encoding RING-Finger proteins (ATL subfamily) with E3-ubiquitine ligase activity are strongly upregulated only in the resistant species, very early after infection (12 h post inoculation). Based on published research, genes of this family could be interesting candidates for breeding or for biotechnological applications, to confer resistance against different stresses in crops. We have undertaken a project to characterize the ATL gene family in grapevine in terms of phylogenetic relationships, gene structure, chromosomal localization and expression in different biotic and abiotic stress conditions. In parallel, we started a stable grapevine transformation to express in *V. vinifera* under the 35S promoter an ATL gene from *V. riparia*, provisionally designed as VrATL, highly homologous to the ATL2 gene of *Arabidopsis thaliana*. Transformed plants have been obtained from cv. Shiraz embryogenic calli and are now grown for further molecular and phenotypic characterization. Moreover, *A. thaliana* plants are being transformed with the GUS reporter gene under the control of the VrATL promoter, to investigate the responsiveness of this promoter to different biotic and environmental stresses.

GENETIC DIVERSITY AND AGGRESSIVENESS OF COLLETOTRICHUM ACUTATUM SPECIES COMPLEX RELATED TO STRAWBERRY IN THE UK. R. Baroncelli^{1,2}, A. Zapparata², S. Sarrocco², G. Vannacci², E. Holub¹ and S. Sreenivasaprasad^{1#}. ¹School of Life Sciences and Warwick Crop Centre, University of Warwick, Wellesbourne Campus, Wellesbourne, Warwickshire, UK. ²Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. [#]Current address: Life Sciences, University of Bedfordshire, Luton, Bedfordshire, UK. E-mail: R.Baroncelli@warwick.ac.uk

Fragaria x ananassa (common name: strawberry) is a worldwide cultivated hybrid species belonging to the family Rosaceae. In 2010, strawberry total world production and cultivation were estimated as about 4,366,662 tons and 243,907 ha, respectively. *Colletotrichum acutatum* species complex has been considered economically the second worldwide most important pathogen after *Botrytis cinerea*. A set of 67 *C. acutatum sensu lato* isolates from strawberry in UK, obtained from FERA (Food and Environment Research Agency part of the Department for Environment, Food and Rural Affairs; authorities responsible for Plant Health) with a set of isolates globally representative of worldwide population has been used to genetically characterize the population structure of this pathogen in the UK. A subset of isolates was used to carry out pathogenicity assays on strawberry plants in order to investigate differences in aggressiveness across different species. This study has provided evidence of the occurrence of three distinct genetic groups on strawberry in the UK corresponding to three *Colletotrichum* species reported in the literature, namely *C. nymphaeae*, *C. fioriniae* and *C. godetiae*. Pathogenicity tests revealed a strong correlation between *C. acutatum sensu lato* species and aggressiveness. Isolates representative of the UK populations (*C. nymphaeae*, *C. fioriniae* and *C. godetiae*) with *C. acutatum sensu stricto* appeared to be the most aggressive. Strains isolated from strawberry were more aggressive compared to strains from the same taxa but isolated from other hosts clearly indicating a degree of host-preference. Isolates belonging to other groups were much less aggressive on strawberry fruits compared to the others and almost non-pathogenic on plants.

FIRST REPORT OF GRAPEVINE PINOT GRIS VIRUS IN EMILIA-ROMAGNA AND VENETO REGIONS. R. Beber¹,

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A recently discovered trichovirus, *Grapevine pinot gris virus* (GPGV), was suggested to be associated with a new grapevine disease in Trentino (northern Italy). The main symptoms are represented by leaf deformation with chlorotic mottling and various mosaic patterns. Shoots are also malformed, showing short internodes and abnormal branching. Diseased vines show a stunting, low vigour, reduced yields and low quality of the berries. Similar symptoms have been observed in some viticultural areas of Emilia-Romagna and Veneto. RT-PCR analysis was carried out to check the virological status of both symptomatic and symptomless grapevines. Results have revealed the presence of GPGV in all symptomatic vines but also in some apparently symptomless samples. PCR products were sequenced and all sequences showed a high identity with the published nucleotide sequence of GPGV (GenBank accession No. NC_015782). Further investigations are in progress to better define the distribution and aetiology of this grapevine disease in the Emilia-Romagna and Veneto vineyards.

TRANSMISSION TRIALS OF GRAPEVINE PINOT GRIS VIRUS BY THE ERIOPHYOID MITE COLOMERUS VITIS. R. Beber¹, E. de Lillo², V. Malagnini³, V. Gualandri³, C. Poggi Pollini¹, C. Ratti¹, P. Saldarelli⁴, D. Valenzano², P. Vernile² and F. Terlizzi¹. ¹Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, Bologna, Italy. ²Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. ³FEM-IASMA, Centre for Technology Transfer, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy. ⁴Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, Bari, Italy. E-mail: federica.terlizzi@unibo.it

Grapevine pinot gris virus (GPGV), a new trichovirus, was recently suggested to be associated with symptoms of leaf chlorotic mottling and deformations observed on grapevine plants of cv. Pinot gris in Trentino (northern Italy). GPGV has a genomic organization identical to that of *Grapevine berry inner necrosis virus* (GINV), which is known to be transmitted by the erineum or leaf blister grape mite, *Colomerus vitis* (Acari: Eriophyidae). This mite species is quite common in infected vineyards so that two experimental trials were carried out in order to investigate its possible involvement in GPGV transmission. *C. vitis* was transferred from galls of virus-free leaves to GPGV-infected grapevine leaves for the acquisition-access feeding. After various periods, some mites were analyzed by RT-PCR for the presence of GPGV and some were moved to virus-free grapevine seedlings for virus transmission. The second transmission experiment consisted in collecting *C. vitis* directly from galls of GPGV-infected leaves, then proceeding, as described above, with RT-PCR analysis and placing the mites on virus-free seedlings. Results demonstrated the presence of the virus in two pools of eriophyids analyzed, whereas no amplification was obtained from seedlings used for transmission trials. Parallel experiments were also conducted on pools or single individuals of *C. vitis* to characterize their ITS1 region, amplified using the specific primer pair 18S and 5.8Srev.

SEVERAL VIRAL AGENTS OF ACTINIDIA spp. DETECTED IN ITALY. R. Bicchieri¹, A.R. Babini², A. G. Blouin³, C. Poggi Pollini¹, D. Cohen³, A. Pisi¹, M.N. Pearson⁴ and C. Ratti¹. ¹Dipartimento di Scienze Agrarie, Università degli Studi, Viale G. Fanin 44,

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Plants of kiwifruit (*Actinidia chinensis*) cv. Hort16A, exhibiting viral symptoms, were observed in two orchards in the Faenza province (Emilia-Romagna, northern Italy). Symptoms include chlorotic and necrotic rings on the leaves and depressed areas on the fruits with consequent deformation of the berries. The causal agent was successfully transmitted to *Cenopodium quinoa*, *Nicotiana benthamiana*, *N. glutinosa* and *N. tabacum* by mechanical inoculation and viral particles with diameter of about 30 nm were present in purified preparations from infected *C. quinoa* plants. Random amplification and sequencing of nucleic acids isolated from purified preparations identified the virus as an isolate of *Pelargonium zonate spot virus* (PZSV). Cuttings obtained from symptomatic plants developed infected but symptomless leaves suggesting that a long incubation period, and therefore high viral titre, may be necessary for symptom expression. The new PZSV isolate has been characterized by sequencing and ultrastructural and immuno-transmission electron microscopy investigations. Infected fruit exhibit progressive decreased metabolic activity and significant reduction of cell wall water content, indicating early senescence of tissues in PZSV-infected fruit compared with uninfected samples. The recently described vitivirus *Actinidia virus B* (AcVB) was also detected in kiwifruit by RT-PCR and, in addition, preliminary evidence of a novel virus belonging to the family *Closteroviridae* has been obtained. Finally, kiwifruit plants showing symptoms likely determined by viral infection, such as leaf narrowing and curling and wood pitting, have recently been observed during surveys of commercial orchards, but none of the known kiwifruit-infecting viruses was identified. Unidentified filamentous virus particles were detected in all these symptomatic samples.

PATHOGENESIS OF *PHLYCTEMA VAGABUNDA* IN cv. CRISP PINK APPLES AND RELATIONSHIP WITH ORGANIC ACIDS CONTENT. I. Cameldi¹, M. Maucourt², D. Rolin² and M. Mari¹. ¹Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127, Bologna Italy. ²UMR 1332 Biologie du Fruit et Pathologie, Centre INRA de Bordeaux, 71 Avenue Edouard Bourlaux, B.P. 81, 33883 Villenave d'Ornon Cedex, France. E-mail: irene.cameldi2@unibo.it

Bull's eye rot (BER) of stored apples caused by *Pblyctema vagabunda* is an important and damaging disease in Europe, Australia, North and South America. Fruit infection occurs in the orchard, but the symptoms appear on fruits only 3-4 months after harvest, during cold storage. During this time the pathogen is present in apple lenticels and it remains in a quiescent state until complete fruit ripening. According to literature, young fruits are characterized by high content in antimicrobial compounds that decrease greatly during ripening. The main aim was to study the BER pathogenesis in naturally infected cv. Crisp Pink apples. Ten replicates of 70 fruits were harvested from our experimental orchard and stored for five months at 0°C. Every month apples were evaluated for BER incidence. In order to investigate the presence of preformed antifungal compounds in apple skin during field growth and storage, proton-NMR analysis was performed in skin apples. The first BER symptoms appeared after three months of storage (1%). The percentage of infected apples increased to 30% after five months (end of storage). Proton-NMR results showed that quinic acid, chlorogenic acids, malic acid, citric acid, citramalic acid, formic acid and succinic acid are among the major compounds in apple

skin. The changes in their content during fruit development in relation to BER will be presented.

RELATIONSHIP OF *CHALARA FRAXINEA* AND THE MEDITERRANEAN ENVIRONMENT. P. Capretti¹, E. Carrari¹, M. Feducci¹, N. Luchi² and A. Santini². ¹Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Patologia Vegetale ed Entomologia, Piazzale delle Cascine 28, 50144, Firenze, Italy. ²Istituto per la Protezione delle Piante del CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy. E-mail: paolo.capretti@unifi.it

Chalara fraxinea has threatened European ash trees from its first appearance in Poland in 1992. The fungus has been reported in northern Italy on *F. excelsior* since 2009. Because of the speed and efficiency of its spread, which is estimated of about 50 Km/year, the regional phytosanitary service of Tuscany (central Italy) promoted a systematic survey, in order to detect the possible presence of the pathogen in the Mediterranean environment, as a threat for biodiversity. Two main aspects were considered: (i) suitable climatic conditions for the pathogen and (ii) host susceptibility. As to climate, Tuscany is characterized by different environments including the Apennine mountains, where the climate is continental and comparable to that of central Europe (cold winters and mild summers). The region has also smaller mountains and hills where soil and air humidity persist for several months, making the environment suitable for the pathogen. The susceptibility of different hosts was evaluated through regular survey on sites where *F. excelsior* is commonly associated with *Tilia*, *Carpinus* and *Ulmus* trees and where *F. angustifolia* and *F. ornus* are present in both small forests and plantations. Evaluation of host susceptibility included the risk of disease spreading on other Mediterranean species of Oleaceae. To this aim, experimental inoculation were performed under controlled conditions on *Ligustrum*, *Phyllirea* and *Olea* seedlings. Results of these tests showed that the pathogen is potentially harmful also to species differing from *Fraxinus* sp.

SEVERE OUTBREAKS OF ZUCCHINI YELLOW MOSAIC VIRUS AND WATERMELON MOSAIC VIRUS 2 IN GREENHOUSES OF ZUCCHINI SQUASH IN CAMPANIA. G. Cennamo¹, A. De Blasio¹, M. del Vaglio¹, B. Greco² and G. Parrella². ¹Settore Sperimentazione, Informazione, Ricerca e Consulenza in Agricoltura, Laboratorio Fitopatologico della Regione Campania, Napoli, Italy. ²Istituto per la Protezione delle Piante del CNR, UOS Portici, Via Università 133, 80055 Portici (NA), Italy. E-mail: parrella@ipp.cnr.it

During spring 2013, very severe symptoms were observed in two adjacent greenhouse-grown zucchini squash crops of cv. Altea in Torre del Greco (Napoli province, southern Italy). About 70-80% of the plants were stunted and had a prominent yellow mosaic, distortion and narrowing of the leaves. Fruits were reduced in size and severely distorted. In some case slotted fruits or with scattered prominent knobs were also observed. In order to identify the virus(es) associated with the disease, infected plants were sampled from both greenhouses and screened for the presence of *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus 2* (WMV-2) and *Watermelon mosaic virus 1* (WMV-1) by DAS-ELISA using commercial kits. Results showed that ZYMV and WMV-2 were present in ca. 59% and 45% of symptomatic plants, respectively. Mixed infections of both viruses were found in about 27% of the samples. Nevertheless, in the 22% of the symptomatic plants, no

virus was detected. This could be explained by a relative low viral titer in these samples, probably below the ELISA threshold sensitivity provided by commercial kits or, less likely, by the presence of unknown virus(es). Although ZYMV and WMV-2 are very common in open field cucurbit crops, in protected cultivations it is very rare to observe severe outbreaks caused by these viruses, mainly because the crops are very efficiently protected against aphids. This suggests that other ways of spreading the virus would be involved, as recently demonstrated for ZYMV.

CORRELATION BETWEEN BUCKWHEAT ANTIOXIDANT PROFILE AND AB1-PRODUCER *ASPERGILLUS* GROWTH ON ACHENES: A PRELIMINARY STUDY. G. Chitarrini¹, C. Nobili², S. Procacci² and M. Reverberi³. ¹Dipartimento di Medicina Clinica, Sanità Pubblica, Scienze della Vita e dell'Ambiente, Università degli Studi dell'Aquila, Piazzale Salvatore Tommasi 1, Blocco 11, 67010 Frazione Coppito, L'Aquila, Italy. ²Laboratorio Innovazione Agroindustriale, Unità Tecnica Sviluppo Sostenibile ed Innovazione del Sistema Agro-industriale, ENEA C.R. Casaccia, Via Anguillarese 301, 00123 Roma, Italy. ³Dipartimento di Biologia Ambientale, Università degli Studi "Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. E-mail: chiara.nobili@enea.it

Buckwheat (*Fagopyrum* spp.) is a pseudo-cereal of great interest for the production of healthy foods as flours, derived from achenes, are rich in bioactive compounds. A radical innovation in food industry may be represented by the use of *Fagopyrum tataricum*, which is richer than common buckwheat (*F. esculentum*) in rutin, flavonoid with antioxidant activity. To ensure high quality of achenes it would be necessary to control fungal contamination both in the field and in post-harvest. Fungi can grow on many food commodities and can produce, under suitable conditions, mycotoxins, secondary metabolites dangerous for humans. In this study two buckwheat species, *F. tataricum* (Golden accession) and *F. esculentum* (Aelita accession) were inoculated with an AB1-producer fungus (*Aspergillus flavus*). Correlation between antioxidant activity of some healthy buckwheat compounds and *A. flavus* growth was investigated on achenes. Results suggest that Golden seeds, which have a higher content of antioxidant compounds, are less susceptible to infection and consequently to mycotoxin contamination.

INVESTIGATIONS ON ENATIONS, A VIRUS-LIKE GRAPEVINE DISEASE. M. Chiumentì¹, A. Giampetruzzi², C. Pirolò¹, P. Saldarelli², A. Minafra², G. Bottalico¹, A. De Stradis², V. Roseti¹, A. Campanale², V. Savino¹ and G.P. Martelli^{1,2}. ¹Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. ²Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: p.saldarelli@ba.iva.cnr.it

Enation disease of the grapevine is an erratic disorder, whose symptoms recall a teratological condition possibly deriving from hormonal unbalance. Even though graft transmissibility of enations supports a viral aetiology of the disease, its putative agent has not yet been identified. We investigated the "virome" of enation-infected grapevines of seven plants of cv. Panse precoce from a 10-year-old commercial vineyard. Two different libraries of pooled double-stranded (dsRNAs) and small (sRNAs) RNAs from leaf tissues were deep-sequenced using Illumina technology. Analysis of the data revealed the presence of 10 different viruses: *Grapevine leafroll-associated virus* 1, 2, 3, 4 (strain 5) and 9 (GLRaV-1,-2,-3,-4,-9), *Grapevine virus A* and *B* (GVA, GVB) *Grapevine rupestris stem-pitting*

associated virus (GRSPaV), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV) and two viroids *Grapevine yellow speckle viroid-1* (GYSVd-1) and *Hop stunt viroid* (HSVd), whose genome coverage was different in the two sequenced libraries. A group of pot-grown vines originated from partially sanitized explants from vines of the same vineyard did not show enation symptoms for over six years. These vines, however, were still infected by GFLV, GRSPaV, GFkV and GVA. Paraffin-embedded thin sections of leaf tissues at the enation level confirmed the reversal of the palisade with the spongy tissue. Expression profile of known *Vitis vinifera* micro RNAs (vvi-miRNAs) in enation-showing leaf tissues showed an increase of miR166 which controls leaf morphogenesis by targeting transcription factors of the Class III HD-Zip gene family members in *Arabidopsis thaliana*. Preliminary hybridization results confirmed the increased expression of vvi-miR166 in enation-showing tissues as compared with enation-free tissues from the same leaves. These data do not solve the problem of the putative viral origin of enation disease, but indicate that symptomatic plants contain an undetermined factor that affects leaf morphogenesis.

APPROACHING MLVA TO INVESTIGATE INTRAPATHOVAR VARIABILITY OF *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE*. S. Ciarroni, M.C. Taratufolo, L. Gallipoli, G.M. Balestra and A. Mazzaglia. Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: angmazza@unitus.it

Kiwifruit bacterial canker is a severe disease caused by the Gram-negative bacterium *Pseudomonas syringae* pv. *actinidiae* (*Psa*), which produces a series of typical symptoms, as leaf spots with chlorotic haloes, whitish to reddish exudates on woody tissues, sudden death of young vines, collapse of fruits and browning of buds and flowers. Nowadays, the pathogen threatens kiwifruit orchards in almost every country of the world where this plant is cultivated. Currently, multi locus sequence typing (MLST) is the prevailing technique for molecular typing of bacteria based on the comparison of a few house-keeping gene sequences. In the last years, more detailed information on differences among strains of some human bacterial pathogens was achieved by means of a molecular technique named MLVA (multiple locus VNTR analysis). This technique relies on the detection of variations in length of particular DNA sequences referable to the differences in number of copies of defined repeated units: these sequences are known as variable number of tandem repeats (VNTR). Several VNTR loci were found *in silico* within *Psa* genomes available in NCBI database by means of specific tools; some of these sequences have shown noticeable variability in the number of repetitions. Therefore, these differentiating loci were used to explore intrapathovar variability among *Psa* populations. Preliminary results obtained testing 12 VNTRs on an extensive and worldwide *Psa* strains collection indicate a surprising capability to discern clonal complex according to the geographical origin of the strains. Further development of the MLVA approach could allow to get high resolution information on the route and mode of transmission of this dire phytopathogenic bacterium.

SET UP OF THE BEST DNA EXTRACTION METHOD AND THE BEST SAMPLING STRATEGY FOR STANDING VINES IN ORDER TO DETECT THE PRESENCE OF TRUNK DISEASES ASSOCIATED FUNGI BY MEANS OF A MICROARRAY TOOL (MYCORRAY). T. Cinelli, G. Marchi, B. Ginetti, D. Bossio and L. Mugnai. Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Patologia Vegetale ed

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Grapevine trunk diseases (GTD) including the whole Esca complex of diseases, Eutypa dieback, Botryosphaeria cankers and Black foot are widespread in all grapevine-growing areas causing losses in quality and quantity of the crop. Since different fungal species associated with GTD can be found in the woody tissues of the same vine, the possibility of their simultaneous detection would be an important goal both for vine growers and nurseries. With this purpose a microarray tool (named MYCORRAY) has been developed. In order to set up an efficient detection assay, the best DNA extraction method from woody tissues and the best sampling strategy are the starting points to be determined. Seven DNA extraction methods were compared to ensure maximum fungal DNA extraction and purification coupled with the highest sensitivity and minimum testing costs. Moreover to establish the best sample type, woody tissues surrounding the wounds were collected by drilling, i.e. using a non lethal method for standing vines. The wood sawdust obtained was analyzed for fungal detection by means of the nested-PCR protocols available for *Phaeoemoniella chlamydospora*, Botryosphaeriaceae, *Neofusicoccum parvum*, *Fomitiporia/Phellinus*, *Ilyonectria* spp. and *Eutypa lata*. After the set up of the best DNA extraction protocol, to reliably assess the sanitary status of individual plants, single and pooled samples were tested and compared with traditional isolation techniques. Pooled samples included sampling from four points surrounding pruning wounds and samples from the rootstock and roots. This approach was shown to minimize the number of DNA extractions required being as efficient and reliable as many single samples.

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EFFECTS OF A NEW DISINFECTANT IN CONTROLLING BACTERIAL SPOT AND PITH NECROSIS CAUSED BY XANTHOMONAS PERFORANS ON TOMATO PLANTS. A. Cinquerrui¹, S. Abriano¹, A. Vitale¹, M.A. Dimartino², A. Myrta³, G. Cirvilleri¹ and G. Polizzi¹. ¹Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Sezione di Patologia Vegetale, Via S. Sofia 100, 95123 Catania, Italy. ²Certis Europe B.V., Via J.M.E. de Balaguer 6, Saronno, Italy. ³Certis Europe B.V., Boulevard de la Woluwe 60, 1200 Brussels, Belgium. E-mail: gpolizzi@unict.it

Xanthomonas perforans is a widespread pathogens known to cause bacterial spot of tomato and pepper. Very recently, it was reported for the first time as causal agent of pith necrosis in greenhouse tomato plants in southern Italy (Sicily). The use of copper compounds is a common method for the control of this pathogen. However, as reported for other *Xanthomonas* species, the exclusive use of copper compounds should be avoided for limiting the risk of development of copper-resistant strains. The efficacy of a new disinfectant (JetFive, Certis Europe) containing 5% peracetic acid and 20% hydrogen peroxide applied at two rates (200 and 400 ml/hl) in pre and post-inoculation was evaluated for the control of bacterial leaf spot and pith necrosis on tomato plants cv. Sir Elyan. The effect of JetFive in reducing diseases incidence and severity was compared with the activity of Cu hydroxide (Funguran-OH 250 SC, Certis Europe, 280 ml/hl) and *Bacillus subtilis* (Serenade Max, BASF, 250 g/hl). *X. perforans* strain 4P1S2 was spray-inoculated before and after treatments on wounded leaves and stems. All treatments were effective in reducing disease infection on leaves and stems. *B. subtilis* was the least effective in reducing bacterial spot and pith

necrosis. In contrast, JetFive (400 ml/hl) proved more effective in reducing disease symptoms caused by the pathogen. A significant reduction of incidence and severity was also observed when JetFive was used as curative treatment 24 h after pathogen inoculation. The treatments did not cause any adverse effects on tomato plants. The results encourage the use of JetFive as a sustainable strategy for achieving good control of *Xanthomonas* infections.

INVESTIGATION OF MYCOTOXIN CONVERSION TO THEIR MASKED FORMS IN WHEAT. M. Cirilini¹, S. Generotti¹, C. Dall'Asta¹, A. Massi² and G. Galaverna¹. ¹Department of Food Science, University of Parma, Parco Area delle Scienze 95/a, 43124 Parma, Italy. ²Società Produttori Sementi, Via Macero 1, 40050 Argelato, Italy. E-mail: silvia.generotti@nemo.unipr.it

Fusarium head blight (FHB), caused by *Fusarium* species, *F. graminearum* and *F. culmorum* in particular, is one of the major fungal disease of wheat, barley, oats, rye and maize. *Fusarium* fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions of America, Europe, and Asia; these mycotoxins have been shown to cause a variety of toxic effects in both animals and humans. Among them, deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins are just some of the most representative and in response to their production, glucoside derivatives, known as masked mycotoxins, can be formed by *Fusarium*-infected plants.

In this study, results about the ability of different wheat lines to convert native mycotoxins to glucoside conjugates upon toxin contamination under greenhouse controlled conditions are presented. In particular, five groups have been considered: plants treated with DON, NIV, T-2 toxin, HT-2 toxin and control plants. Contaminations were made on the flowering ears at selected levels, inducing typical FHB symptoms to the plant. Plants ears were sampled at different times from the contamination and analyzed for native and derivative mycotoxins occurrence by UPLC/MS/MS analysis. Data were statistically elaborated and compared to those obtained for control plants. Findings demonstrated that the ability to convert mycotoxins to their masked forms is genotype-related also in wheat.

EMERGING FUSARIUM MYCOTOXINS AND THEIR PRODUCING SPECIES: OCCURRENCE IN SOFT AND DURUM WHEAT IN AN AREA OF CENTRAL ITALY. L. Covarelli¹, G. Beccari¹, S. Generotti^{1,2}, F. Etruschi^{1,2}, A. Prodi³, G. Meca² and J. Mañes Vinuesa². ¹Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. ²Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Avenue Vicent Andres Estelles, 46100 Burjassot, Valencia, Spain. ³Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. E-mail: lorenzo.covarelli@unipg.it

Samples (162) of durum and soft wheat harvested in 2009 and 2010 were collected in an area of Central Italy (Umbria) and investigated for the presence of the most important "emerging" mycotoxins such as enniatins (ENs: ENA, ENA1, ENB, ENB1), beauvericin (BEA) and fusaproliferin (FUS). ENs and FUS were quantified by liquid chromatography (LC) with a diode array detector and BEA by LC coupled with a mass spectrometer. A set of strains of *F. avenaceum* (FA) and *F. poae* (FP), two potential "emerging" mycotoxin producers isolated from the same grain samples, was screened for the presence of the *esn1* gene, encoding the multifunctional enzyme enniatin synthetase for the detection of potential enniatin-producing isolates. In addition, their mycotoxin production *in vitro* was determined. All the analysed samples resulted to be significantly

contaminated by ENs, both in terms of frequency and quantity. BEA was frequent but contamination levels appeared to be low. Conversely, FUS was present with a low incidence but showed considerable levels of contamination. In general, a remarkable presence of “emerging” mycotoxins was found, particularly ENs. Co-contamination of different mycotoxins also occurred. About 80% of FA and 100% of FP strains resulted to be positive for the presence of the *esyn1* gene. All FA strains showed the ability to biosynthesize ENs *in vitro* but not BEA. Conversely, all FP strains resulted to be BEA producers and some of them co-biosynthesized ENs.

DETECTION OF MYCOTOXIGENIC *FUSARIUM* SPECIES AND MYCOTOXINS IN DIFFERENT MALTING BARLEY VARIETIES. L. Covarelli¹, G. Beccari¹, M. Giannini², F. Tini¹, U. Bonciarelli¹ and A. Prodi². ¹Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. ²Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. E-mail: lorenzo.covarelli@unipg.it

A study was carried out in order to evaluate the presence of mycotoxigenic *Fusarium* spp. associated to *Fusarium* head blight (FHB) and mycotoxin contamination in 15 malting barley varieties harvested at the Experimental Station of the Faculty of Agriculture of Perugia (Papiano, Umbria, central Italy) in 2012. Grain samples were subjected to fungal isolation and to the determination of the infecting fungal species. The isolates belonging to the *Fusarium* genus were identified by species-specific PCR or by sequencing the *TEF1α* gene. Fungal genotype characterization by *tri5* (trichothecene producers), *tri12* (trichothecene chemotypes NIV, 3ADON or 15ADON producers) and *esyn1* (enniatin producers) gene amplification, was realized to determine the mycotoxigenic potential of the isolated strains. Quantification of the mycotoxins DON and T-2 present in the kernels was also performed by ELISA. Anthesis time and weather data were collected to detect the possible presence of favourable conditions to FHB. In the surveyed year, unfavourable for the development of FHB, the incidence of fungal infections was 21% for *Fusarium* spp., 30% for *Aspergillus* spp., 28% for *Alternaria* spp. and 17% for *Penicillium* spp.. The most frequently detected species were *F. tricinctum* followed by *F. proliferatum*, *F. graminearum* and *F. avenaceum*. *F. graminearum* strains were potential trichothecene producers and belonged to the NIV chemotype. *F. tricinctum* and *F. avenaceum* were all potential enniatin producers. The average DON concentration did not exceed the EU legal limits. The average T-2 toxin contamination was critical if compared to the level recently recommended by the EU (2013/165).

MONITORING KIWI FRUIT BACTERIAL CANKER IN CAMPANIA. L. Cozzolino¹, P. Spigno² and A. Zoina¹. ¹Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. ²Servizio Fitosanitario della Regione Campania. E-mail: lucia.cozzolino@unina.it

Pseudomonas syringae pv. *actinidiae* (*Psa*), the causal agent of kiwifruit bacterial canker, was detected for the first time in Campania (southern Italy) in 2011 in one Jin Tao kiwifruit orchard in the province of Caserta. The Regional Phytosanitary Services were alerted and an intensive survey program was initiated in March 2012 to identify bacterial canker outbreaks and to prevent the spread of the disease in the region. *Psa* presence was determined in twig and leaf samples by isolation on current bacteriological media, direct-PCR and Biolog test (Biolog MicroStation (TM) System/Microlog) on bacterial colonies and DNA extraction from plant tissues followed by PCR analysis. PCR was carried out with specific primers on

bacterial colonies and DNA extracted from plant tissue. The Biolog analysis constantly identified *Psa* as *P. syringae* pv. *anthirrhini* since the pathovar “*actinidiae*” is not yet present in the GenIII Database of the Biolog system. In 2012, from March to November, more than thirty orchards and four nurseries were inspected and over 200 samples were analyzed. The disease was found on a single plant in the same orchard already affected in 2011. In other areas of the region other infections were found caused by *P. syringae* pv. *syringae* and *Pseudomonas viridiflava*. These bacteria cause symptoms very similar to those of *Psa* but determine much less severe damages. Monitoring for the year 2013 is currently underway to contain the spread of kiwifruit bacterial canker.

CHARACTERIZATION OF BETAFLEXIVIRIDAE VIRUSES INFECTING SWEET CHERRY ORCHARDS IN EMILIA-ROMAGNA. M. Dall'Ara¹, A.E. Cornejo Valdivia¹, A.R. Babini², D. Dradi³, C. Lanzoni¹, C. Rubies Autonell¹, C. Poggi Pollini¹ and C. Ratti¹. ¹Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. ²Servizio Fitosanitario Regionale, Via di Corticella 133, 40129 Bologna, Italy. ³Astra Innovazione e Sviluppo, Via Tebano 45, 4018 Faenza (RA), Italy. E-mail: claudio.ratti@unibo.it

Several members of the virus family *Betaflexiviridae* such as *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry virus A* (CVA), *Cherry mottle leaf virus* (CMLV) and *Cherry green ring mottle virus* (CGRMV) are associated with symptomatic cherry plants in France or USA. Cherry plants (*Prunus avium*) with a generalized decline and narrow, rolled or rusty leaves have been observed, in the last years, in experimental and commercial orchards in Emilia-Romagna (northern Italy). Some sweet cherry cultivars show different levels of symptom severity when grafted on vigorous or not vigorous and dwarfing rootstocks. Furthermore, conditions inducing water stress seem to worsen the disease suggesting a graft-incompatibility between scion and rootstock intensified by viral infections. Total RNA was extracted from leaf samples collected from symptomatic and symptomless sweet cherry trees. Degenerate deoxyinosine-substituted primers were used to amplify, by nRT-PCR, 198 or 362 nucleotide long regions from a polymerase domain portion highly conserved within members of *Trichovirus*, *Capillovirus*, *Foveavirus* and *Vitivirus* genera (*Betaflexiviridae*). Results showed a high incidence (50%) of betaflexiviridae in the tested plants. Moreover, sequence analyses showed that amplicons obtained by nRT-PCR share high levels of nucleotide identity with several members of the family such as *Apple stem pitting virus* (86%), CNRMV (96%), *Apple chlorotic leaf spot virus* (91%), *Citrus leaf blotch virus* (69%) and CVA (80%). This study was focused on the molecular characterization of viral strains in order to confirm and define the viral etiology of diseases affecting sweet cherry in Emilia-Romagna.

PRELIMINARY INVESTIGATIONS ON THE OCCURRENCE OF CITRUS TRISTEZA VIRUS IN CENTRAL-WESTERN SICILIAN GROVES AND ON THE MYCORRHIZAL STATUS OF CITRANGES. S. Davino, V. Mondello, L. Torta, G. Conigliaro and S. Burrano. Dipartimento di Scienze Agrarie e Forestali, Viale delle Scienze, 90100 Palermo, Italy. E-mail: salvatore.davino@unipa.it

In the last decade, since the first outbreak of *Citrus tristeza virus* (CTV) in the province of Catania (2002), several new CTV foci were found in Sicily. In order to determine CTV occurrence in central-western Sicily, identify and characterize viral strains and verify the mycorrhizal status of citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.], one of the most utilized tolerant rootstock, an

epidemiological survey was carried out in 2011. Symptomless samples from citrus groves from different Sicilian provinces (Agrigento, Palermo, Trapani, Caltanissetta) were collected and submitted to serological assay. Only samples from Agrigento and Palermo provinces were ELISA-positive. Moreover, RT-PCRs of p18, p20 and p23 genes and single strand conformation polymorphism (SSCP) assays, showed two CTV strain variants in the infected samples from Palermo. Cloning and sequencing of the detected CTV variants for phylogenetic study are in progress. Citrange root samples taken from rootstocks of field-grown plants and seedlings were collected and submitted to an optimized staining technique aimed at visualizing the endomycorrhizal fungal structures. Seedling and micro-propagated plants, kept under laboratory conditions, were also inoculated with both endemic and commercial endomycorrhizal inoculum. First results indicate a limited root colonization. The lack of the mycorrhizal advantage could play a role on the well-known susceptibility of citrange to common soil pathogens.

FUSARIUM OXYSPORUM f.sp. PISI CAUSING DECLINE OF CHICKPEA PLANTS IN SOUTHERN ITALY. F. De Curtis, D. Palmieri, D. Vitullo and G. Lima. *Dipartimento Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, Via F. De Sanctis, 86100 Campobasso, Italy. E-mail: decurtis@unimol.it*

Epidemiological investigation in chickpea fields of southern Italy disclosed severe cases of decline. Affected plants showed browning and necrosis of the crown and main roots while the vegetation was less vigorous and chlorotic. From symptomatic plant tissues three fungi were isolated and preliminarily identified as *Fusarium* spp. Pathogenicity tests performed on healthy chickpea plants showed the high virulence of one of the isolates, which reproduced the field symptoms. The pathogen was re-isolated and showed the same morphological features of the inoculum, thus fulfilling Koch's postulates. The fungal isolate was identified by PCR using specific primer pairs (ITS1F/ITS4) designed to amplify a stretch of the rDNA intergenic spacer 563-573 bp in size, which was sequenced. The ITS sequence of the new fungal isolate was analyzed by overlaying it with those of the «International Mycological Association» through the website (www.mycobank.org). The overlay revealed 99% similarity of the new fungal isolate with *F. oxysporum* f.sp. *pisi*. Additional molecular studies using specific primers (Foc0-12/Foc0-12rF), have excluded the possible belonging of the new fungal isolate to *F. oxysporum* f.sp. *ciceris*. According to these results, additional pathogenicity tests were carried out also on pea and faba bean plants. The results confirmed the high virulence of the new isolate of *F. oxysporum* f.sp. *pisi* to chickpea and pea, whereas no symptoms were observed on faba beans. This is the first report of *F. oxysporum* f.sp. *pisi* as a new pathogen of chickpea.

STUDY OF MORPHOLOGICAL AND SEXUAL DIFFERENTIATION IN THE ASCOMYCETE *BOTRYTIS CINEREA* THROUGH WHOLE TRANSCRIPTOME ANALYSIS. R.M. De Miccolis Angelini, C. Rotolo, S. Pollastro and F. Faretra. *Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: ritamilvia.demiccolisangelini@uniba.it*

Sexual compatibility in the heterothallic bipolar fungus *Botrytis cinerea* (*Botryotinia fuckeliana*) is controlled by a single *MAT1* locus with two alternative idiomorphic sequences conferring sexual identity. The Illumina NGS technology was used to gain insight into gene expression profiles associated with different developmental stages and identify the key fungal genes involved in regulation of

morphogenesis and sexual process. RNA-Seq analyses were carried out on two near-isogenic reference strains (SAS56 and SAR11004) of opposite mating type at the following stages: (i) actively growing mycelium; (ii) mature sclerotia; (iii) carpogenesis-induced sclerotia; (iv) spermatized sclerotia; (v) apothecial primordia; (vi) fully developed apothecia. About 16 million short sequence reads per sample were aligned to the reference *B. cinerea* genome and analyzed to measure transcript levels. A wide range of variations in the number of regulated genes and their patterns of regulation was recorded. Genes showing great differences in transcription levels ($FC > 20$) in the two strains of opposite mating types included: mating-type pheromone precursors and pheromone receptors (GPCRs) as well as other membrane proteins, *bet* domain-containing proteins, transcription factors and signal transducers, and several proteins of unknown function. Tag density of sequence reads resulted adequate for quantitative analysis even for genes weakly transcribed, and led to ascertain constitutive transcription of the idiomorph-specific genes at the *MAT1* locus (*MAT1-1-1* or *MAT1-2-1*, encoding transcription factors, and *MAT1-1-5* and *MAT1-2-4*, encoding proteins of unknown function), in all development stages. RT-PCR experiments revealed, for the *MAT1-2-1* gene, two transcript isoforms resulting from antisense gene transcription and alternative splicing.

IDENTIFICATION OF *PHYTOPHTHORA* SPECIES INFECTING CITRUS IN VIET NAM. A. De Patrizio^{1,2}, S.O. Cacciola², C. Olsson³, R. Faedda², M. Ramstedt⁴, S. Wright⁵, N.M. Chau⁶, N.M. Hoa⁶, A. Pane², M.I. Prigigallo¹, L. Schena¹ and G. Maggano di San Lio¹. ¹Dipartimento di Agraria, Università Mediterranea, 89122 Reggio Calabria, Italy. ²Department of Agri-Food and Environmental Systems Management, University of Catania, 95123 Catania, Italy. ³Department of Biological and Environmental Sciences, Gothenburg University, 40530 Gothenburg, Sweden. ⁴Department of Forest Mycology and Plant Pathology, Swedish Agricultural University, 75007 Uppsala, Sweden. ⁵Department of Electronics, Mathematics and Natural Sciences, University of Gävle, 80176 Gävle, Sweden. ⁶Southern Horticultural Research Institute, Mytho, Vietnam. E-mail: olgacacciola@unict.it

In a survey aimed at identifying *Phytophthora* species infecting fruit crops in Viet Nam, *P. citrophthora* and *P. nicotianae* were the species most commonly recovered from soil and trees in citrus plantations. *P. nicotianae* was prevalent in citrus groves in the Ben Tre and Tien Giang provinces (southern Viet Nam). Interestingly, both A1 and A2 mating types of the latter species were found, while in other citrus-growing areas of the world, such as the Mediterranean region, South Africa, Australia and the Americas, A1 is the only mating type occurring in citrus groves, suggesting southern-east Asia could be a centre of origin of this *Phytophthora* species. Mixed infections of three *Phytophthora* species were detected on the fruits of pomelo (*Citrus grandis*) with symptoms of brown rot collected in the Mekong Delta region (southern Viet Nam). The three species were identified as *P. colocasiae*, *P. insolita* and *P. meadii* on the basis of morphological characters and DNA analysis. The ITS-rDNA sequence of three representative isolates obtained from pomelo fruits showed 99, 99 and 100% similarity with reference ITS-rDNA sequences of *P. colocasiae*, *P. insolita* and *P. meadii* from GenBank, respectively. Koch's postulates were fulfilled by pathogenicity tests on fruits of pomelo and other citrus species. To our knowledge, this is the first report of natural infections of these three *Phytophthora* species on citrus fruits worldwide and the first report of *P. insolita* in Viet Nam.

MONITORING OF *MONILIA FRUCTICOLA* ON STONE FRUIT MUMMIES IN NORTHERN ITALY FROM 2008 TO 2012. M. Del Pilar Bustos Lopez¹, D. Spadaro¹, L. Nari², A. Garibaldi³ and M.L. Gullino³. ¹Dipartimento Scienze Agrarie, Forestali ed Alimentari, Università degli Studi di Torino, Grugliasco (TO), Italy. ²Centro di Ricerca e Sperimentazione per l'Ortofrutticoltura Piemontese, Cuneo, Italy. ³Centro di Competenza per l'Innovazione in campo agro-ambientale (AGROINNOVA), Università degli Studi di Torino, Grugliasco (TO), Italy. E-mail: marialodovica.gullino@unito.it

Brown rot, the most important disease of stone fruits, is mainly caused by *Monilinia* spp., in particular for peaches and nectarines where the disease can affect over 50% of the crop. In Europe the endemic species are *Monilinia laxa* and *Monilinia fructigena* plus *M. fructicola*, which is more aggressive than the others and was recorded for the first time in 2009 in Piedmont. The three species are very similar and sometimes they can be found simultaneously on the fruits. *M. fructicola* can infect unripe fruits and it can overwinter in cankers on branches and in mummified fruits, originating primary infections in spring. During the winter seasons of 2008 to 2012 mummified peaches were analysed. In 2008-09 *M. laxa* was the major species (91.8%), followed by *M. fructicola* (6.2%) and *M. fructigena* (2.0%). The incidence of *M. fructigena* was constantly low during the three following years. On the contrary the percentage of *M. fructicola* increased from 10.2% in 2009-10, to 33.9% in 2010-11, reaching 49.6% in 2011-12. The increase in *M. fructicola* was accompanied by a consequential decrease of the incidence of *M. laxa*. In 2011-12, the incidence of *M. fructicola* was, for the first time, higher than that of *M. laxa*. Most of the mummies attacked by *M. fructicola* were found in nectarines, while *M. laxa* was prevalent in peaches.

BENYVIRUS CHIMERAS INDICATE A SPECIFIC INTERACTION BETWEEN SILENCING SUPPRESSION PROTEINS AND VIRAL RNAs. A. Delbianco¹, M. Dall'Ara¹, A. Flobinus², K. Hleibieh², E. Klein², C. Rubies Autonell¹, D. Gilmer² and C. Ratti¹. ¹Dipartimento di Scienze Agrarie, Sezione di Patologia Vegetale, Università degli Studi, Viale G. Fanin 44, 40127 Bologna, Italy. ²Institut de Biologie Moléculaire des Plantes du CNRS, Université de Strasbourg, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France. E-mail: claudio.ratti@unibo.it

Beet necrotic yellow vein virus (BNYVV) and *Beet soil-borne mosaic virus* (BSBMV), genus *Benyvirus*, possess a multipartite genome comprising four ssRNAs(+) and are both transmitted by the plasmodiophorid *Polymyxa betae*. BSBMV and BNYVV are closely related since they possess the same host range, vector and genome organization. Recent studies demonstrated a possible amplification and transmission of BSBMV RNAs by BNYVV helper strain. In the USA, both benyviruses are frequently present in the same cultivated field, infecting the same plant but no chimeric forms have been described from field isolates so far. The possibility that BNYVV/BSBMV chimeras may be generated has been investigated. *Chenopodium quinoa* was locally infected using *in vitro* infectious transcripts of BNYVV and BSBMV RNA-1 and -2 and the behavior of BSBMV/BNYVV chimeras and wild type isolates was compared. The chimera BoStras12 (BSBMV RNA-1+BNYVV RNA-2) induced severe necrotic lesions on the leaves, probably due to a hypersensitive response of the plant, in contrast to the typical chlorotic lesions of wild type combinations. The necrosis disappeared when the plant was co-inoculated with BoStras12 together with a viral replicon expressing BSBMV p14, a cysteine-rich protein acting as a suppressor of post-transcriptional gene silencing. Thus, the properties of *Benyvirus* p14s were investigated. Experiments were carried out to investigate the relationships between BSBMV/BNYVV p14s and VSR activity, their link with the "coremin" sequence necessary

for long distance movement and the absence of natural chimeras of *Benyviruses*.

POSTHARVEST DISEASES CONTROL BY YEAST VOLATILE ORGANIC COMPOUNDS. A. Di Francesco and M. Mari. CRIOF, Università degli Studi di Bologna, Via Gandolfi 19, 40057 Cadriano (BO), Italy. E-mail: marta.mari@unibo.it; alessand.difrancesc3@unibo.it

The volatile organic compounds (VOCs) produced by biocontrol agents (BCAs) were evaluated for their activity against some fungal pathogens. Two strains of *Aureobasidium pullulans* (L1 and L8), were previously assayed on a wide range of fungi (*Monilia laxa*, *Monilia fructicola*, *Monilia fructigena*, *Penicillium expansum*, *Penicillium italicum*, *Penicillium digitatum*, *Botrytis cinerea*, *Rhizopus* spp). The aim of this study was to evaluate the production of VOCs as part of BCAs mechanisms of action. To this purpose the production of VOCs was assayed in *in vitro* and *in vivo* trials against the pathogens listed above. For *in vitro* trial a double Petri dish test was carried out to evaluate the conidial germination of the above pathogens. The results showed that the germination of *M. laxa* and *M. fructicola* was completely inhibited by strains of *A. pullulans*, while the germination of the other pathogens was reduced by over 74%. *B. cinerea* conidia germination was reduced by 69% and 53% by VOCs produced by L1 and L8 respectively. Both *A. pullulans* strains showed a fungistatic activity. An *in vivo* test was performed on cv. Golden Delicious apples wound-inoculated with *P. expansum* and placed in glass boxes saturated, 48 h before, with L1 or L8 VOCs. After one week exposure to L1 and L8 VOCs, blue mould incidence was reduced by 72 and 77% by L1 and L8, respectively. This study show that VOCs production by BCAs may be a mechanism of action explaining the efficacy of BCAs against fungal fruit pathogens.

ENZYMATIC ACTIVITIES OF *AUREOBASIDIUM PULLULANS* STRAINS CORRELATED WITH PARASITIC ACTION ON THE PATHOGEN'S CELL WALL. R. Di Francesco, R. Roberti and M. Mari. Dipartimento di Scienze Agrarie, Università degli Studi, Viale G. Fanin 44, 40127 Bologna, Italy. E-mail: marta.mari@unibo.it

Aureobasidium pullulans strains L1 and L8 isolated from peach cv. Red Heaven have shown an efficient control of postharvest diseases of fruits caused by pathogens like *Monilia laxa*, *Monilia fructicola* and *Monilia fructigena* (Mari *et al.*, 2011; *Biological Control* 60,132-140). *A. pullulans* strains were grown in nutrient yeast liquid broth (NYDB) for 96 h and checked for the production of chitinase (endo- and esochitinases) and β ,1-3,glucanase esoenzymes. Chitinase and β ,1-3,glucanase activities were assayed in NYDB using specific substrates and measured at 405 nm and 660 nm absorbance, respectively. L1 showed an increased chitinase activity in the culture filtrate from 24 h of growth onwards, L8 from 48 h onwards. Among chitinase activities, endochitinase was less expressed than esochitinases by both L1 and L8. β ,1-3,glucanase activity picked at 96 h in L1 and at 48 h in L8. The enzymatic activity of L1 and L8 reflects the growth of both strains, measured spectrophotometrically at 660 nm absorbance, exhibiting an upward trend from 24 h onwards and a decrease trend after 96 h.

MOVEMENT OF BLUE STAIN CANKER ELICITED BY *CERATOCYSTIS PLATANI* IN FLORENCE DURING THE

LAST 20 YEARS. M. Feducci, M. Fabbri and P. Capretti. *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: deviltora@alice.it*

Ceratocystis platani has been reported in Italy in 1954 and since then it has spread in every urban area where *Platanus* is commonly planted in public parks or boulevards. The present study focused on the manner and speed of pathogen spread during the last 20 years in Florence. Based on the urban map, the city was divided into four sectors, all plane trees present in parks and boulevards were inspected and the occurrence of the disease registered. In total sixteen sites, representing 6.4 km of trees in line, were considered. Eleven sites out of sixteen were infected with 25 trees colonized by *C. platani* and 67 recently cut because of the disease. Using historical data, collected in the 1980s and '90s, it was estimated that the area covered by the disease included a length of 8.3 km and an area of 2.5 km². In those years, the pathogen was present only the eastern part of Florence. Updating the data to 2012, the above figures increase to 13 km in length and area of 4.0 km², while the disease had hit the western part of the city. Despite of containment operations, the blue stain canker has spread in Florence overstepping also the natural barrier represented by the Arno river.

CLIMATE CHANGE EFFECTS ON *FUSARIUM OXYSPORUM* f. sp. *LACTUCAE* ON LETTUCE PLANTS GROWN UNDER SIMULATED ENVIRONMENTAL CONDITIONS. I. Ferrocino^{1,2}, W. Chitarra^{1,2}, M. Pugliese^{1,2}, D. Spadaro^{1,2}, G. Gilardi¹, M.L. Gullino^{1,2} and A. Garibaldi¹. *¹Centro AGROINNOVA, Università degli Studi di Torino, Via L. da Vinci, 44, 10095 Grugliasco (TO), Italy. ²Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, 10095 Grugliasco (TO), Italy. E-mail: massimo.pugliese@unito.it*

A peat growing substrate was infested with *Fusarium oxysporum* f. sp. *lactucae* to reach a final concentration of 1×10⁴ CFU g⁻¹ of substrate. Lettuce plants were then transplanted into the 2 litre pots and grown in phytotrons under four simulated environmental conditions: (i) 800 ppm CO₂, 22-26°C; (ii) 800 ppm CO₂, 18-22°C; (iii) 400 ppm CO₂, 22-26°C and (iv) 400 ppm CO₂, 18-22°C. Non infested pots were used as control. Disease severity was evaluated every seven days after transplanting, accompanied by physiological analysis like chlorophyll content. Substrate samples were collected from each phytotron at 7, 14, 21 and 28 days after transplanting. Plate counts, enzymatic assays, and polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analyses were carried out on substrate samples to evaluate the effect of climate change on the microbial population. The severity of *Fusarium* wilt of lettuce and *Fusarium* spp. population were significantly influenced by temperature (22-26°C). Disease severity, enzymatic activities and PCR-DGGE fingerprints of the ascomycota community were not affected by CO₂ concentration. In conclusion, only higher temperatures are expected to increase *Fusarium* wilt of lettuce, while high carbon dioxide levels (800 ppm) are not relevant for the disease.

PSEUDOMONAS MEDITERRANEA ASSOCIATED WITH PSEUDOMONAS CORRUGATA ON CHRYSANTHEMUM PLANTS IN SARDINIA. M. Fiori¹, R. Marongiu¹, V.A. Prota¹, G.A. Bravo Jimenez¹, M. Losada Rodriguez¹ and G. Marchi². *¹Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola, 07100 Sassari, Italy.*

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In this study we have characterized 14 bacterial strains (six *Pseudomonas mediterranea* and eight *Pseudomonas corrugata*) isolated from chrysanthemum plants grown in greenhouses located in central-southern Sardinia (insular Italy). Infected chrysanthemum plantlets showed symptoms of rot and blight and, at blooming, chlorosis and necrosis of leaves. In pathogenicity tests all the strains tested induced similar symptoms on chrysanthemum, tomato and pepper plants. Initially, the presence of two different species was ascertained by using different media. Among them, TZCAgar proved to be particularly suitable. The identification and the differentiation of the two species was confirmed by genomic analysis. According to multiplex PCR, performed with specific primers PC1/1, PC1/2 and PC5/1, PC5/2, the six strains of *P. mediterranea* yielded the typical 600 bp band, while the eight strains of *P. corrugata* showed the typical 1100 bp band. Cluster analysis of results of rep-PCR performed by using BOXA1R, and ERIC1R and ERIC2 primers, clustered the two species in two distinct groups. On the contrary, Biolog phenotypic fingerprinting analysis identified the 14 strains as *P. corrugata*. However, *P. mediterranea* strains could be distinguished from *P. corrugata* strains on the basis of the metabolic profile determined by Biolog GEN III microplates. *P. mediterranea* is sensitive to lincomycin antibiotic and oxidize some sugars and organic acids including D- mannose, D-mannitol, L-glutamic acid, L-galactonic acid lactone. To determine their role in the disease, the two bacterial species are currently being tested with experimental inoculations, alone or in combination, onto chrysanthemum, geranium, pepper and tomato plants.

BIOCONTROL ACTIVITY OF FOUR NON-FERMENTING YEASTS AGAINST THE PATULIN PRODUCER *PENICILLIUM EXPANSUM*. S. Fiori^{1*}, A. Marcello¹, W. Hammami^{3*}, S. Jaoua³ and Q. Migheli^{1,2}. *¹Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia ed Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. ²Centro Interdisciplinare per lo Sviluppo della Ricerca Biotecnologica e per lo Studio della Biodiversità della Sardegna e dell'Area Mediterranea, Viale Italia 39, 07100 Sassari, Italy. ³Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, P.O. Box: 2713, Doha, Qatar. E-mail: qmigheli@uniss.it*

Penicillium expansum causes severe rots on apple fruits during storage and shelf life. Aiming at the development of new antagonistic yeast active in controlling different postharvest fruit pathogens, the efficacy of four non-fermenting yeast strains (*Candida intermedia*, *Candida friedrichii*, *Cyberlindnera jadinii* and *Lachancea thermotolerans*) was tested on apple fruits against a patulin producer strain of *P. expansum*. All strains were inoculated in artificial wounds (10 µl of 10⁸ CFU/ml) and showed significant biocontrol activity against the pathogen inoculated on fruits with 10 µl of a spore suspension (1×10⁴ conidia/ml). However, they were less efficient against more concentrated pathogen spore suspensions (10⁷ and 10⁶ conidia/ml). Preliminary *in vitro* experiments were performed to confirm the *in fructo* results: living yeast cells, filtered and autoclaved supernatants were tested against *P. expansum* on YES medium. Only living cells were able to completely inhibit the pathogen growth, suggesting that their biocontrol activity is mainly due to competition for space and nutrients and not because of the release of some antifungal compounds. The four antagonistic yeast are now being tested against a wider array of filamentous fungi, such as *Aspergillus ochraceus*, *A. westerdijkiae*, and *A. carbonarius*.

The availability of non-fermenting yeast displaying antagonistic capability may be valuable in the biocontrol of mycotoxin-producing moulds affecting fruit and fruit-based beverages commercialised in countries where the presence of ethanol residues may represent a religious concern.

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HORIZONTAL TRANSFER OF THE CERATO-ULMIN GENE IS WIDESPREAD BETWEEN *OPHIOSTOMA NOVO-ULMI* AND *GEOSMITHIA* spp.

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Preliminary work had shown the presence of a gene encoding class II hydrophobin cerato-ulmin (*cu*) from the phytopathogenic fungus *Ophiostoma novo-ulmi* in the unrelated species *Geosmithia pallida*. As the two species coexist inside elm trees occupying the same habitat but different ecological niches, a horizontal gene transfer event was proposed. We used PCR amplification to search for the *cu* gene in 70 *Geosmithia* spp. strains from different European locations (Czech Republic, Hungary, Spain, Italy) and host plants: 46 strains were derived from insect vectors infesting elm trees or from decaying elm trees and 24 strains were from insect vectors on plants other than elms. The gene was present in 52.1% of the strains from elm trees, while none of those isolated from non-elms possessed it. The presence of *cu* mRNA was determined by real time PCR in the reference strain *G. pallida* IVV7 grown in liquid shaken culture for 4, 8, 12, 16 and 20 days. *cu* mRNA was present in *G. pallida*, even if in very low amount, reaching its maximum after 8 days of growth. The same time-point was used to test for *cu* expression in seven isolates of different species, where *cu* mRNA was found in amounts comparable to IVV7. Finally, the induction of *cu* gene expression was tested in IVV7 grown on elm sawdust and in dual culture with *O. novo-ulmi*. In both conditions the expression level was increased, however the amount of *cu* mRNA remained extremely low thus raising the question of its functional significance.

INTERSPECIFIC VARIABILITY OF THE CLASS II HYDROPHOBIN GEO1 IN THE GENUS *GEOSMITHIA*.

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Members of the genus *Geosmithia* (Ascomycota:Hypocreales) are mitosporic filamentous fungi distributed worldwide and living in the galleries drilled by phloeophagous insects in broad-leaved trees. Previous work had shown that the species *Geosmithia pallida* harboured a gene encoding the class II hydrophobin GEO1. In order to analyze the variability of the *geo1* gene in the genus *Geosmithia*, we investigated the presence of its sequence in 35 species representing the whole genetic diversity of the genus and derived from different host plants and geographic locations. The *geo1* gene was detected in 26 species where it maintained the general organization shown in *G. pallida*, with three exons and two introns. Size variations were found in both introns and in the first exon, the latter being due to the presence of a microsatellite which corresponded to a stretch of glycine residues in the deduced proteins. At the amino acid level the deduced proteins had 44.6% homology and no major differences were found in the biochemical parameters (pI, GRAVY index, hydrophobicity plots). Finally, GEO1 production in the fungal culture filtrate was tested by turbidimetric assay and Western blotting. Phylogenetic analysis based on the *geo1* sequences did not correspond to the multigene tree generated from neutral markers, thus suggesting that sequence similarities could be influenced by factors other than phylogenetic relatedness.

PRELIMINARY SURVEY ON PATHOGENIC FUNGAL SPECIES ASSOCIATED TO *LARIX DECIDUA-PINUS CEMBRA* DECLINE.

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Since 2004, increasing decline symptoms were observed in natural *Larix decidua-Pinus cembra* stands growing next to the lake Antorno (eastern Dolomites, Belluno, northern Italy). In summer 2012 a preliminary survey was carried out to determine the possible role played by pathogenic fungal species isolated from declining trees. When compared to trees growing far from the lake, a higher incidence of crown diseases was observed along the lake shore, likely associated to air humidity stagnation. In *P. cembra*, *Lophodermium* spp. was frequently isolated from casted needles and wilting shots. *Hypodermella laricis* and *Mycosphaerella laricina* were isolated from larch needles, while *Lachnellula willkommii* was widely detected in branches and stems. *Armillaria* spp. was also observed in both tree species, but its role in decline is still unclear.

BENEFICIAL RHIZOSPHERIC MICROORGANISMS AGAINST FLAVESCENCE DOREÈ PHYTOPLASMA.

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Bacteria living on and inside the root system and arbuscular mycorrhizal (AM) fungi form strict associations with most plants and may influence growth and health by improving plant nutrition and its ability to overcome biotic and abiotic stresses. Plant inoculation with beneficial microorganisms may represent a valid tool for controlling phytoplasma diseases in the frame of an IPM strategy. The effects of AM fungi, bacterial endophytes and rhizobacteria against flavescence dorée phytoplasma (FDP) infection were assessed in periwinkle (*Catharanthus roseus*) used as model plant. In nature, FDP is associated with grapevine yellows, a serious threat for grape production in Europe and especially in north-western Italy. Plant protection from the disease, FDP multiplication, pattern and severity of symptom expression were evaluated in plant inoculated with the microorganisms in different combinations as well as in the non inoculated controls. Physiological parameters, such as wet and dry weight of entire treated plants, roots, shoots and leaves, were also evaluated. In a preliminary experiment, 12 different bacterial strains, belonging to *Pseudomonas*, *Bacillus* and *Streptomyces* genera, as well as two mixed mycorrhizal inocula were assayed. We then focused on two bacterial endophytes, *P. fluorescens* YsS6 and *Pseudomonas* spp. 8R6, producing aminocyclopropane-carboxylic acid (ACC) deaminase and their mutants lacking of this enzymatic activity. Interestingly, strain 8R6 induced some level of resistance to phytoplasma infection in terms of symptom severity and FDP multiplication rate. The evaluation of the activity of rhizospheric microorganisms in mitigating phytoplasma damage under field conditions on grapevine is currently under investigation.

BIOCONTROL AGENTS OF *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* FROM PHYLLOSPHERE OF *ACTINIDIA* spp. L. Gallipoli¹, A. Mazzaglia¹, M. Marinelli¹, E. Mariotti, F. Mastrogiovanni², S. Bernardini², E. Ovidi², A. Tiezzi² and G.M. Balestra¹. ¹Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. ²Dipartimento per l'Innovazione dei Sistemi Biologici, Agroalimentari e Forestali, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: balestra@unitus.it

Pseudomonas syringae pv. *actinidiae* (Psa) is the causal agent of kiwifruit bacterial canker. It causes cankers on branches, angular necrosis surrounded by a yellow halo on the leaves, browning on flowers and buds, wilting of fruits, thus proving the most destructive pathogen of kiwifruit plants in most of the world's cultivation areas of *Actinidia* spp. To reduce Psa damages, its control is mainly centered on the use of copper compounds associated with appropriate cultural practices. The search and use of natural antagonists as biological control agents (BCA) is considered one of the most promising methods for more rational and safe crop-management practices. In this study, the screening of a huge number of bacteria isolated from the phyllosphere of *Actinidia deliciosa* (cv. Hayward) and *Actinidia chinensis* (cv. Hort16A) affected by Psa, has led to the selection of six potential BCA (bacteria). These isolates have shown a remarkable aptitude at inhibiting the growth of known Psa isolates *in vitro*. In addition, the selected bacterial antagonists have shown to be strong producers of biofilm. This suggests that they are able to produce specific substances belonging to different chemical classes and that their production could be regulated by quorum sensing system. The same isolates were also able to colonize readily the surface of kiwifruit plantlets, preventing or drastically reducing the damages caused by artificially inoculated known Psa strains (*in planta*). Detailed analyses of biofilm production as BCA identification and characterization are in progress.

INVESTIGATIONS ON THE BIOSYNTHESIS OF OCHRATOXIN A IN *ASPERGILLUS CARBONARIUS* THROUGH WHOLE TRANSCRIPTOME ANALYSIS. D. Gerin, R.M. De Miccolis Angelini, S. Pollastro and F. Faretra. Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: stefania.pollastro@uniba.it

Ochratoxin A (OTA) is a mycotoxin produced by several fungi including *Aspergillus carbonarius* (Bainier) Thom., the main responsible of OTA contamination of grapes and derived products. Although the biosynthetic pathway of OTA has not yet been clarified, the involvement of multi-enzyme complexes, such as the polyketide synthase and non-ribosomal peptide synthase has been proposed in some fungal species. Next generation sequencing (Illumina technology) was applied to study the transcriptional responses of two OTA producer strains of *A. carbonarius* grown under inductive and non-inductive conditions. Mycelium collected after 4, 6 and 8 days under the two growth conditions were submitted to cDNA library construction using standard protocols for RNA-Seq experiments. About 10 million of short-sequence reads (50 bases) generated from each of twelve libraries were aligned to the re-annotated reference genome of the fungus (*A. carbonarius* ITEM 5010, assembly v3 <http://genome.jgi.doe.gov/Aspca3/Aspca3.home.html>) and analyzed to measure transcript levels. Comparing the OTA-inductive and non-inductive conditions, differentially expressed genes were identified, assigned to functional categories, and examined for their potential involvement in OTA biosynthetic pathway. Transcripts showing the greatest induction (fold change \leq 8) included sequences encoding enzymes involved in the biosynthesis of secondary metabolites (16%) having the biological function of polyketide synthase (7), non-ribosomal peptide synthase (6), methyltransferase (7), p450 monooxygenase (8), chloroperoxidase (1), dehydrogenase (5) and hydrolase (3). Genes involved in the biosynthesis of melanin, responsible for conidia pigmentation, and hydrophobins, secreted on the surface of conidia and other aerial structures, already described for other species of the genus *Aspergillus*, were also identified.

EVALUATION SCALE OF FUSARIUM HEAD BLIGHT SYMPTOMS IN BARLEY. M. Giannini, S. Tonti, A. Prodi, G. Innocenti, P. Nipoti and A. Pisi. Dipartimento di Scienze Agrarie, Università degli Studi, Viale G. Fanin 44, 40127 Bologna, Italy. E-mail: marta.giannini2@unibo.it

Fusarium head blight (FHB), or scab, is a cereal disease spread throughout the world and studied mainly in wheat. FHB can cause significant yield losses and quality reduction, especially if during fungal infection dangerous mycotoxins for human and animal health are produced. In the last years, in Italy, barley production for malting industry has undergone a positive trend that has generated a higher attention for barley diseases. FHB symptoms in barley differ in part from those in wheat. In wheat, premature death or bleaching of spikelets is a typical symptom, particularly clear on emerged immature heads. Pink to salmon-orange spore masses of the fungus are often formed on the infected spikelets and glumes during prolonged wet weather. In barley, symptoms of infection show as bleaching but also as a browning or water-soaked appearance of spikelets and the presence of small brown spots on the glumes. Salmon-orange spore masses of the fungus are rarely observed. In wheat the severity index of the FHB is calculated with the rating scale proposed by Parry *et al.* (1984, *Journal of the National Institute of Agricultural Botany* 16: 465-468) that evaluates the percentage area infected on individual ears with values from 0% to 90%. The aim of this work was to set up a FHB scale for barley,

similar to that used by Parry and coworkers taking as reference a tow-row barley cultivar artificially inoculated in an experimental field. This rating scale can be useful also to the breeders to evaluate the different susceptibility of barley cultivars.

OCCURRENCE OF *PHYTOPHTHORA RAMORUM* IN TUSCAN NURSERIES: RESEARCH EFFORTS TO FACE THIS SERIOUS THREAT. B. Ginetti, S. Carmignani, A. Ragazzi and S. Moricca. *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Patologia Vegetale ed Entomologia, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: beatrice.ginetti@unifi.it*

Phytophthora ramorum, the quarantine pathogen agent of "sudden oak death", is responsible for the extensive mortality of a number of plant species in the northern hemisphere. This oomycete induces bleeding cankers on trees and leaf necrosis and aerial twig dieback on ornamental shrubs. The microorganism was recently found on *Viburnum tinus* plants in a nursery of ornamentals in Pistoia (Tuscany, central Italy). Symptoms on *V. tinus* include shoot folding and blight, brown-bronze lesions on petioles and leaves. On the leaf lamina, lesions extend from the petiole towards the midrib or develop from the tip and the edge. Difficulties inherent to a proper identification of the microorganism could cause its uncontrolled spread in the area, both in nurseries and on the local flora. If this would happen, the damage would be incalculable for the nursery industry and the environment. We have developed conventional and molecular methods for the accurate and reliable identification of the pathogen. These assays are helping the regional Plant Protection Services with the identification of the oomycete in suspected areas and nurseries. Our diagnostic protocols have greatly enhanced pathogen monitoring efforts and enable prompt action of the authorities for its eradication. Such research achievement could help safeguarding forests as well as the economy of nursery activities in the Pistoia district. It is a known fact that many countries have decided of no longer importing a wide range of species and varieties of ornamentals from areas where *P. ramorum* is known to occur epidemically.

EFFECT OF VOLATILES PRODUCED BY ANTAGONISTIC RHIZOBACTERIA ON *ARABIDOPSIS THALIANA* GROWTH. A. Giorgio¹, P.A.H.M. Bakker² and N.S. Iacobellis¹. ¹*Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy.* ²*Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. E-mail: iacobellis@unibas.it*

Recent studies have shown that rhizobacteria may employ volatiles as signals in their interactions with plants and rhizosphere microbial communities. In this study we investigated the *in vitro* effect of volatiles from selected antagonistic bacteria isolated from bean rhizosphere and identified as *Pseudomonas* and *Bacillus* spp., on the growth of *Arabidopsis thaliana* Col-0. The effects of bacterial volatiles on *A. thaliana* seed germination and plant growth depended on the bacterial isolate used, but also on the growth conditions for the bacteria and on the plant growth stage at which they are exposed to volatiles. Contrasting effects ranging from inhibition of seed germination to a moderate growth inhibition as well as to an augmented plant biomass were observed. The former effect was observed for some *Pseudomonas* spp. isolates which produced, beside other volatiles, hydrogen cyanide which may be responsible for the toxic effect on seed germination. Other *Pseudomonas* spp. isolates, which did not produce hydrogen cyanide, showed slight inhibition

of *A. thaliana* growth. In contrast, volatiles from a *Bacillus* spp. isolate caused a huge biomass increase. Plant growth and development is endogenously regulated by hormones such as SA, JA and ET that are also involved in plant responses to abiotic and biotic stresses. To study the contribution of SA, JA and ET signaling pathways on the growth of *A. thaliana* mediated by bacterial volatiles, a set of plant hormone mutants, including NahG, jar1-1 and ein2-1, defective in mentioned hormones signaling, were evaluated for their responses to inoculation with these bacteria.

DEFINE: A MULTIDISCIPLINARY RESEARCH PROJECT TO INVESTIGATE THE EFFECTS OF EXOTIC PLANT PATHOGENIC FUNGI AND INSECTS ON NATIVE ECOSYSTEMS. P. Gonthier¹, N. Luchi², E. Petrucco Toffolo³, R. Balestrini⁴, S. Colazza⁵, M. Faccoli³, M. Garbelotto⁶, A. Giorcelli⁷, L. Giordano¹, S. Guarino⁵, G. Leone¹, F. Loreto², A. Mello⁴, M. Michelozzi², A.L. Pepori², A. Santini², F. Sillo¹, A. Vizzini⁸ and E. Zampieri^{1,8}. ¹*Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy.* ²*Istituto per la Protezione delle Piante del CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy.* ³*Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente Agripolis, Università degli Studi di Padova Viale dell'Università 16, 35020 Legnaro (PD), Italy.* ⁴*Istituto per la Protezione delle Piante del CNR, UOS Torino, Viale Mattioli 25, 10125 Torino, Italy.* ⁵*Dipartimento di Scienze Agrarie e Forestali, Università degli Studi, Viale delle Scienze, 90128 Palermo, Italy.* ⁶*Department of Environmental Science, Policy and Management, University of California, 54 Mulford Hall, 94720 Berkeley, California, USA.* ⁷*CRA, Unità di Ricerca per le Produzioni Legnose Fuori Foresta, Strada Frassineto 35, 15033 Casale Monferrato (AL), Italy.* ⁸*Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi, Viale Mattioli 25, 10125 Torino, Italy. E-mail: paolo.gonthier@unito.it*

The effects of biological invasions have been mostly studied in terms of financial losses and ecological impact on native species. However, there is a lack of information on the extent to which invasive organisms may determine physiological and genetic changes in native components of the ecosystems. A research project named DEFINE (Deciphering the Effects of invasive Fungi and Insects on Native Ecosystems) was recently granted by the Italian Ministry of University and Research (MIUR), within the FIRB program. The project aims at investigating the potential impact of invasions by fungal plant pathogens and phytophagous insects on the main components of the native ecosystems: plants, their pathogens, pests and symbionts. Three model systems are studied, each including either an exotic pathogen or an exotic insect in Europe (the tree pathogen *Heterobasidion irregulare* and the phytophagous insects *Hyphantria cunea* and *Bagrada hilaris*) and its main host in the invasion area (*Pinus pinea*, *Populus nigra*, *Brassica oleracea*). Effects of invasive organisms on a range of native components, including infected/infested hosts and neighbouring healthy plants (inter-plant signalling), are determined by conducting comparative inoculation/infestation experiments with native host-associated pathogens and insects. Host responses are assessed by analysing volatile organic compounds (VOCs) and gene transcripts in plant tissues. Effects are also investigated on the occurrence and gene expression of native ecto- and endo-mycorrhizal fungi, and on genomics of native species that may have experienced allelic introgression from the invasive organism. For this purpose, whole-genome genotyping of 15 *Heterobasidion* isolates is in progress in order to clarify allelic introgression between invasive and native species.

DEVELOPMENT OF MARKER-ASSISTED BACKCROSS BREEDING FOR SIMULTANEOUS INTROGRESSION OF TOMATO MOSAIC VIRUS AND TOMATO SPOTTED WILT VIRUS RESISTANCE INTO TOMATO ELITE GERMPLASM.

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The Sorrentino tomato ecotype is a large fresh market tomato, round or semi-round shaped. Its pulp is very meaty and with a sweet and delicate taste. It is mainly grown in a limited area of Sorrento peninsula (Campania, southern Italy) that coincides with its land of origin. As many other tomato ecotypes, it is highly susceptible to tomato pathogens and it is usually grafted on resistant rootstocks to avoid some common soil-borne tomato diseases. Nevertheless, this ecotype still remains susceptible to viruses. Recently (2011-2012), several protected cultivations of this ecotype have been affected by multiple virus infections caused by ToMV (*Tomato mosaic virus*), TSWV (*Tomato spotted wilt virus*) and PMoV (*Parietaria mottle virus*) in particular. Depending on the greenhouse inspected, the percentage of affected plants ranged from 20% to 80%. The use of resistant genotypes is probably the best manner to control viral diseases and for this reason we have undertaken a breeding programme to introgress ToMV and TSWV resistances into the Sorrentino ecotype by developing a marker-assisted backcross (MAB) method. A resistant fresh market tomato line was crossed with a selected Sorrentino line. The resulting F₁ generation were screened through PCR-based markers using specific primers for the simultaneous detection of both resistant genes (*Sw5* and *Tm2²*) in a single plant/PCR reaction. Effectiveness of the MAB method developed, was confirmed in the first two segregating backcross populations, so that it will be possible, within the next three backcross generations, to recover the entire elite Sorrentino germplasm with the addition of the two resistance genes.

“CURRENT AND EMERGING PHYTOPHTHORA spp” A PROJECT FOR HARMONIZATION OF DETECTION AND IDENTIFICATION METHODS AT THE EUROPEAN LEVEL.

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Euphresco is an EU-funded ERA-NET project with the aim of creating a network of funders to coordinate the research on plant health aspects. One of the research projects born in the mainframe of Euphresco is the project “Current and emerging Phytophthoras” (CEP), that involves different European countries and deals with *Phytophthora* species such as *P. ramorum*, *P. kernoviae* and *P. lateralis*, which are quarantine pathogens for UE member countries, and other current *Phytophthorae*. The topics are risk assessment, disease management, genotyping, but it focuses especially on the harmonization of diagnostic methods. Participants share procedures regarding both traditional methods, based on direct isolation and baiting methods, and molecular techniques. Within the latter, participants collaborate to improve techniques on DNA extraction, sample preparation and methodologies of detection, including field approaches such as LFD and LAMP. Our main work is addressed to: (i) improve DNA extraction from soil by testing different methodologies, including commercial kits, on different soils, and (ii) set up a microarray detection platform which allows the simultaneous detection of several *Phytophthora* pathogens. This characteristic is very appreciable for complex diseases and/or for a faster and reliable diagnosis of several pathogens on the same matrix. We used the microarray for the detection and identification

of different *Phytophthora* species including *P. ramorum*, *P. kernoviae*, *P. lateralis*, but also other widespread species that are a serious threat in Italy such as *P. cactorum* and *P. cinnamomi*.

TESTA-SEED HEALTH: DEVELOPMENT OF SEED TREATMENT METHODS, EVIDENCE FOR SEED TRANSMISSION AND ASSESSMENT OF SEED HEALTH. C. Henry¹, T. Aveling², P. Bonants³, J.M. Carstensen⁴, V. Cockerell⁵, M. Ebskamp⁶, V. Grimault⁷, M.A. Jacques⁸, S.L. Nielsen⁹, F. Petter¹⁰, D. Spadaro¹¹, E. Stefani¹² and J.E. Thomas¹³. ¹FERA, Sand Hutton, York, UK. ²University of Pretoria, Pretoria, South Africa. ³Plant Research International, Wageningen, Netherlands. ⁴Videometer A/S, Hørsholm, Denmark. ⁵SASA, Roddinglaw Road, Edinburgh, UK. ⁶NAKT, Roelofarendsveen, Netherlands. ⁷GEVES, Beaucouzé, France. ⁸INRA, Beaucouzé, France. ⁹Aarhus University, Slagelse, Denmark. ¹⁰EPPO, Paris, France. ¹¹Agrinova, University of Torino, Grugliasco, Italy. ¹²Department of Life Sciences, Reggio Emilia, Italy. ¹³NIAB, Cambridge, UK. E-mail: emilio.stefani@unimore.it

A wide range of diseases and pests are carried by seeds. Seeds may carry pest already present in the European Community, but also introduce new ones via this route. Under the EU 7th Framework Programme a collaborative project was granted to a research Consortium, in order to face open questions related to seed pathology, such as pathogen transmission by seed, development, implementation and validation of sampling and detection methods, development of effective disinfection protocols for contaminated seeds. The TESTA project will develop a range of novel methods to underpin the control of these diseases, including faster, more accurate methods to assess the mode of seed transmission, economic and practical sampling approaches for the detection of pathogens present in low levels in large seed lots, novel and efficient generic detection methodologies, non-destructive testing methods and improved, effective and sustainable disinfection procedures. Several target crops and disease combinations have been identified in consultation with EPPO, ISHI-ISF and ISTA. Among them, most relevant are following: Tomato/pepper and their bacterial and viral pathogens, such as *Clavibacter michiganensis* subsp. *michiganensis*, Xanthomonads, and a number of pospiviroids; cucurbits and their most important bacterial pathogen, *Acidovorax citrulli*; brassicas and other crucifers with *Xanthomonas campestris* pv. *campestris* and *Phoma lingam*; wheat and its fungal pathogens *Tilletia* spp. and Fusaria, parasitic nematodes of legumes. Important aims of TESTA are: (i) enhance knowledge of the biology of seed transmission, both by developing novel methods based on labelled microorganisms and through extensive field trials; (ii) establish a comprehensive web-based database as a global resource, detailing all known pests and diseases of crop plants transmitted by seed; (iii) develop novel methods for assessing levels of seed transmission and their relevance to disease levels in crops; (iv) improve sampling strategies and methodologies for seed lots, in order to generate representative samples for lab testing; (v) establish generic platforms for seed testing methods, together with the assessment of innovative protocols, possibly using non-destructive methodologies; (vi) develop novel disinfection/sanitation procedures for seed, and assessing their proficiency; (vii) disseminate results to national policy stakeholders, testing laboratories and inspection services. Complete information is available on the website: www.seedtesta.eu.

ROLE OF VOLATILE ORGANIC COMPOUNDS IN THE INTERACTION OF RHIZOBACTERIA AND PLANT PATHOGENIC FUNGI. N.S. Iacobellis, A. Giorgio, D. Lamorte and P. Lo Cantore. *Scuola di Scienze Agrarie, Forestali, Alimentari ed*

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Among 162 bacteria isolates obtained from bean plants rhizosphere 60 inhibited in dual plate assays the growth of bean fungal and bacterial pathogens. When the 60 antagonistic bacteria were applied to bean seeds and the relative plants were challenged with a highly virulent strain of *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* six isolates highly protected bacterized bean plants. The protection by the six bacterial strains, though at different level, was observed either *in vitro* or in greenhouse cotyledon and trifoliolate pathogenicity leaf assays, respectively. Induced systemic resistance (ISR) is apparently involved and, in fact, ISR has been recently ascertained in parallel studies using the pathosystem *Arapidopsis thaliana* and *X. campestris* pv. *amoriaceae* for three of the above rhizobacteria. The six antagonistic bacterial strains produced an array of hydrolytic enzymes, diffusible antimicrobial substances and volatile organic compounds (VOCs) which inhibited the growth of several plant pathogenic fungi as assessed either in dual plate VOCs assays. In particular, strains of *Sclerotinia sclerotiorum* proved highly sensitive to both diffusible and volatiles, hence this fungus was selected for further studies. Pure synthetic VOCs such as 2-nonanone, 2-undecanone, m-cymene, limonene, dimethyl disulfite, dimethyl trisulfide highly inhibited, though with different MIC, the growth of *S. sclerotiorum* strains and showed haemolytic activity suggesting that membrane systems are the target of the above VOCs. Preliminary observation with optical and electron microscopes of *S. sclerotiorum* mycelia appear to confirm that membranes are the target of the above VOCs.

CHARACTERIZATION OF PSEUDOMONAS "REACTANS" ISOLATES ASSOCIATED WITH CULTIVATED MUSHROOMS. D. Lamorte, P. Lo Cantore and N.S. Iacobellis. *Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: nicola.iacobellis@unibas.it*

Pseudomonas "reactans", a saprophyte associated to cultivated mushrooms and used for the identification of *P. tolaasii* in the white line assay, has been established to be the causal agent of *P. eryngii* yellowing and to be involved with *P. tolaasii* and other fluorescent pseudomonads in the development of the brown blotch on *A. bisporus* and the yellowing of *P. ostreatus*. Isolates of this pathogen share the common feature to form white precipitates when grown near *P. tolaasii* in the "white line" assay and although they appear to belong to *P. fluorescens* (biovar III and V), ultimate identification at the species level is yet to be achieved. The purposes of this study was to characterise 20 isolates of *P. "reactans"* for phenotypic and genotypic traits with the final aim to contribute to its characterization and classification. Isolates were evaluated for biochemical, physiological and nutritional features, for the productions of antimicrobial and hemolytic substances, siderophores and volatile organic compounds (VOCs), and for their pathogenicity to *A. bisporus*. *Pseudomonas*-selective PCR primer amplification of the 16S rDNA gene and amplicon sequences, deposited at EMBL-EBI, indicate the bacterial isolates as belonging to *P. fluorescens* though some differences have been observed among them. Since additional REP-PCR analysis showed the genetic heterogeneity of *P. "reactans"*, further amplification and sequencing of other housekeeping genes is in progress with the final aim to assess the genetic relatedness of this bacterium.

BIOACTIVE SECONDARY METABOLITES AND HYDROLYTIC ENZYMES PRODUCED BY STRAINS OF BURKHOLDERIA GLADIOLI pv. GLADIOLI. D. Lamorte, P. Lo Cantore

and N.S. Iacobellis. *Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: nicola.iacobellis@unibas.it*

The genus *Burkholderia* includes more than 40 validly described species associated to plants and animal with beneficial or detrimental features in the occupied niches. Some beneficial *Burkholderia* species are potentially exploitable in the biotechnological and agricultural industry or for bioremediation of recalcitrant xenobiotic but, unfortunately, the fact that some members of the genus are involved in human infections is hampering their exploitation. *Burkholderia gladioli*, initially described as a phytopathogenic species, contains the pathovars *gladioli*, *allicola* and *agaricicola*, which cause gladiolus and onion bulb rot, and soft rot of cultivated mushrooms, respectively. Recently, the new pathovar *cocovenenans* has been described, which is associated primarily with toxicity of food processed from plants commodities, apparently due to the highly toxic compounds toxoflavin and bongkrekeic acid. Unlike *B. gladioli* pv. *agaricicola* whose ability has recently been determined to produce antimicrobial secondary metabolites and hydrolytic enzymes as well as the QS regulation of their production and potential role in the pathogen virulence, no comparable information is available for *B. gladioli* pv. *gladioli* (*Bgg*). In this study the production of antimicrobial metabolites in dual plate assays as well as hydrolytic enzymes by representative strains of *Bgg* has been ascertained. Liquid culture extracts of virulent strains of *Bgg* showed antimicrobial and haemolytic activities. HPLC analyses of bioactive culture extracts produced individual fractions whose chemical and biological characterization is in progress.

FUMONISIN B, A AND C AND HIDDEN FUMONISINS IN FUSARIUM VERTICILLIOIDES AND FUSARIUM. PROLIFERATUM COLONIES. I. Lazzaro¹, C. Falavigna², C. Dall'Asta², G. Galaverna² and P. Battilani¹. ¹*Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy.* ²*Dipartimento di Scienze Alimentari, Università degli Studi, Viale delle Scienze 17/A, 43124 Parma, Italy. E-mail: paola.battilani@unicatt.it*

Maize is the second most important crop on a worldwide basis. Concerns for human and animal health arise from fungal infection, by *F. verticillioides* and *F. proliferatum* in particular, the two main fumonisin producers on maize. Fumonisins B are the most abundant in nature; while fumonisin C (FC) and A occur at lower concentrations (< 5%); the P series has never been detected. Hidden fumonisin (HFUM) were also recovered on maize. Our study aimed at defining the production profile of fumonisin and HFUM by *F. verticillioides* and *F. proliferatum* in relation to water activity (a_w), pH and growing medium changes (malt extract, cornmeal and corn-starch). Fumonisin B, C and A were produced by both fungi, were enhanced when $a_w=0.990$, with an increase in production from 21 to 45 days of incubation with a peak at 30 days. Among the growing media, cornmeal allowed the highest fumonisin production in both species, followed by corn-starch, suggesting an important role of amylopectin as fumonisin inducer. As to pH, it was confirmed that acidic conditions induce fumonisin biosynthesis, irrespective of the growing medium. The production of HFUM on malt extract was negligible, while their occurrence in corn-based medium was proved, although at low levels. Moreover, we showed, for the first time, the production of fatty-acid esters of fumonisins in cornmeal medium cultures and naturally contaminated maize, and their absence in malt extract cultures, confirming the hypothesis that esters are formed by the fungus only on a complex substrate rich in lipids, such as maize.

GENOME SEQUENCING OF TWO PREVALENT ITALIAN ISOLATES OF *CITRUS TRISTEZA VIRUS*. G. Licciardello, A. Lombardo, M. Russo, G. Scuderi and A. Catara. *Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Blocco Palma I, Str.le Lancia 57, 95121 Catania, Italy. E-mail: glicciardello@pstsicilia.it*

Analyses carried out over the past 5 years in a Sicilian area heavily infected by *Citrus tristeza virus* (CTV) revealed that viral population is mainly composed by virulent isolates inducing severe seedling yellow and mild symptomless isolates. Two representative isolates (SG29 and Bau282) were selected for further analysis by genome sequencing after biological and molecular characterization. Biological analysis showed that SG29 induces seedling yellow on sour orange and rarely stem pitting on Duncan grapefruit but not on sweet orange, whereas Bau282 induced no symptoms in sour orange and Duncan grapefruit. Molecular analysis by CE-SSCP and Multiple Molecular Marker showed distinct genomic profiles. Full genomes were obtained by sequencing small RNAs and assembly of overlapping sequences by reference alignment from libraries using Illumina GAIx platforms. The complete SG29 and Bau282 genomes are 19,259 and 19,250 nucleotides in length, respectively, with 12 open reading frames (ORFs). Phylogenetic analysis based on 31 full CTV genomes showed that SG29 clustered with the "Asian" VT-lineage comprising isolates T318A (Spain), AT-1 (China), Nuaga (Japan) and CT11A (China) and has the highest homology identity with T318A (98.4%) and AT-1 (97.4%). Divergences in the 5' end of the genome, which contains the replication-associated proteins ORF1a and b, showed that SG29 can be distinguished from T318A but is quite similar to AT-1, as revealed by multiple alignment. Bau282 clustered within the mild isolates T30 (Florida) and T385 (Spain) and blast analysis showed a very high (99%) identity level.

IN SILICO EVALUATION OF PRIMERS AND PROBES FOR *CANDIDATUS LIBERIBACTER* spp. DETECTION AND CHARACTERIZATION. G. Licciardello¹, P. Bella², A. Sicilia², R. La Rosa², A. Catara¹ and V. Catara². ¹Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Blocco Palma I, Str.le Lancia 57, 95121 Catania, Italy. ²Dipartimento di Scienze delle Produzioni Agricole e Alimentari, Università degli Studi, Via Santa Sofia 100, 95131 Catania, Italy. E-mail: vcatara@unict.it

'*Candidatus Liberibacter*' is a genus of phloem-limited Gram-negative bacteria in the Rhizobiaceae family vectored by psyllids. Most of the member of the genus are included in the EPPO A1 list of quarantine bacteria and are associated with severe plant diseases, i.e. '*Ca. L. asiaticus*', '*Ca. L. africanus*' and '*Ca. L. americanus*' with Citrus huanglongbing (HLB), '*Ca. L. solanacearum*' with diseases of potato and tomato in the Americas and New Zealand and carrot and celery in Europe. A new species, '*Ca. L. europaeus*', has been found in symptomless pear trees in Italy and Hungary and in scotch broom showing stunting and leaf dwarfing in New Zealand. Over the years a number of diagnostic protocols were developed aiming at the detection and characterization of '*Ca. Liberibacter* spp.' To investigate the potential of a one-step discrimination of different members of the genus by designing protocols for multiplex PCR assays from plant DNA extracts we have carried out an *in silico* evaluation of available primers and probes and sequences deposited in GenBank. The 16S rRNA gene sequences of '*Ca. L. asiaticus*', '*Ca. L. americanus*', '*Ca. L. africanus*', '*Ca. L. solanacearum*', '*Ca. L. europaeus*' and *Liberibacter crescens* were aligned together with those of four plant-associated α -Proteobacteria, included as outgroups. The phylogenetic tree originated by the 16S rDNA alignment helped to check the conformity of the source organism with its taxon. New primer sets and candidate probes based on

conserved and variable regions have been selected.

ROLE OF ANTAGONIST MICROFLORA AND SOLID MATRICES IN THE CONTROL OF FUNGAL PATHOGENS OF ROSE IN HYDROPONIC CROP. G. Lima¹, M. Fierro², P. Ferrara², I. Carulli² and S. Frisullo². ¹Dipartimento Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, Via F. De Sanctis, 86100 Campobasso, Italy. ²Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università degli Studi, Via Napoli 25, 70100 Foggia, Italy. E-mail: lima@unimol.it

Soil-less cultivations are good alternatives to open field crops in terms of productivity, lower energy consumption, control of plant growth, independence from soil quality, as well as reduced environmental impact. However, the higher risk of crown and root pathogens spreading through the nutrient solution, is one of the main limiting factor for a widespread of closed-loop systems. The present study was aimed at monitoring the state of rose plant health during the crop cycle in closed hydroponic systems and to characterize bacterial and fungal species present in flower greenhouse farms of Apulia. To this aim experiments were performed to: (i) monitor, quantify and identify the main pathogens and non pathogenic microorganisms of rose; (ii) evaluate *in vitro* and *in vivo* and on different hydroponic matrices, the antagonist activity of selected biocontrol agents against rose fungal pathogens. The main pathogens of rose recovered from hydroponic growth matrices were fungi belonging to *Fusarium*, *Acremonium*, *Phaeoacremonium* and *Cylindrocarpon* spp. A higher population density of nonpathogenic microorganisms (fungi and bacteria) was recovered where coconut fiber was used as a solid matrix. In biocontrol tests carried out in a hydroponic greenhouse farm, isolates of *Trichoderma viridae* and *T. barzianum*, were more effective antagonists of rose fungal pathogens than some potential antagonist bacteria. The results are discussed in relation to the potential combined application of growth media and biocontrol agents to control fungal pathogens of flower plants in hydroponic cultivation systems.

DIPLODIA QUERCIVORA, A NEW OAK PATHOGEN. B.T. Linaldeddu, B. Scanu, A. Deidda, L. Maddau and A. Franceschini. *Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola 1, 07100 Sassari, Italy. E-mail: ben@uniss.it*

Several species of Botryosphaeriaceae are known as pathogens involved in the aetiology of oak decline worldwide. In recent years these species have caused serious concern because of their negative impact on oak ecosystems in the Mediterranean countries, where they limit both the vitality and productivity of trees. During a study of the species of Botryosphaeriaceae associated with oak decline in Tunisia, a large collection of *Diplodia* strains were isolated from *Quercus canariensis* trees showing a progressive dieback of shoots and branches, trunk canker and exudates and collar rot. In particular, a new *Diplodia* species, recently described as *Diplodia quercivora* Linaldeddu et al. Phillips, was obtained from sunken cankers on branches. In this study the relative susceptibility of three Mediterranean oak species (*Quercus ilex*, *Q. pubescens* and *Q. suber*) to *D. quercivora* infection was tested with log inoculation trials. The results obtained suggest that this fungus is an aggressive pathogen on all three oak species tested. The fungal infection developed from bark tissues towards the wood where it penetrated by a few millimeters. Lesion size ranged from 67.8 cm² on holm oak to 377.8 cm² on pubescent oak. Given its virulence, *D. quercivora* currently poses a threat to Mediterranean oak ecosystems. In order to prevent its

spread in other Mediterranean countries, it is important to improve oak disease monitoring and to understand the pathways by which this pathogen can be spread.

NOVEL HIGH-THROUGHPUT DIAGNOSTIC TOOLS FOR THE SIMULTANEOUS DETECTION OF INVASIVE CITRUS PATHOGENS. G. Loconsole¹, V. Savino¹, R.K. Yokomi² and M. Saponari³. ¹Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. ²United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Parlier, CA 93648, USA. ³Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: giuliana.loconsole@uniba.it

A number of important citrus pathogens are spread by graft propagation, arthropod vector transmission and inadvertent import and dissemination of infected plants. For these reasons, citrus disease management and clean stock programs require economical and sensitive pathogen detection systems apt to maintaining a healthy industry. To this end, multiplex quantitative real-time PCR (qPCR) assays were developed allowing high-throughput and simultaneous detection of major invasive citrus pathogens. Automated high-throughput extraction comparing several bead-based commercial extraction kits were tested and compared with tissue print and manual extraction to obtain nucleic acids. Then two one-step TaqMan-based multiplex RT-qPCR assays were developed. The first assay included primers and probes for broad spectrum detection and genotype differentiation of 'Candidatus Liberibacter asiaticus' (CLAs) and *Citrus tristeza virus* (CTV). In the second assay primers and probes were used for *Hop stunt viroid* (HSVd), *Citrus exocortis viroid* (CEVd) and the mitochondrial NADH dehydrogenase (nad5) mRNA as an internal citrus host control. The assays were validated using infected tissues from our pathogen collection (HSVd, CEVd and CTV) or non-infectious CLAs infected tissues obtained from China. Based on quantitation cycle values, automated high-throughput extraction of samples proved to be as suitable as manual extraction. The multiplex RT-qPCR assays detected both RNA and DNA pathogens in the same dilution series as singleplex assays and yielded similar quantitation cycle values. Taken together, high throughput extraction and multiplex RT-qPCR assays reported in this study provided a rapid and standardized method for routine and simultaneous diagnosis of different RNA and DNA invasive citrus pathogens.

PLANTS CRY FOR HELP: BIOTIC AND ABIOTIC STRESSES ATTRACT BENEFICIAL MICROBES TO THE ROOTS. N. Lombardi¹, A. Pascale¹, M. Ruocco², S. Woo^{1,2}, G. Manganiello¹, F. Vinale², R. Marra¹, S. Lanzuise¹, V. Matteoli¹ and M. Lorito^{1,2}. ¹Dipartimento di Agraria, Università di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. ²Istituto di Protezione delle Piante del CNR, UOS Napoli, Via Università 133, 80055 Portici (NA), Italy. E mail: lorito@unina.it

Trichoderma spp. are ubiquitous saprophytic fungi, known for their ability to colonize a variety of niches, antagonize and control plant pathogenic microorganisms and establish a direct beneficial interaction with plants, resulting in the enhancement of growth, nutrient uptake and systemic resistance to diseases. It has been recently demonstrated that the root colonizing fungus *Trichoderma longibrachiatum* strain MK1 positively affects the production of volatile compounds that attract both predators and antagonists of the aphid *Macrosiphum euphorbiae* (Battaglia *et al.*, 2013. *Molecular Plant-Microbe Interaction* DOI: 10.1094/MPMI-02-13-0059-R).

Therefore, we hypothesize that a similar mechanism is used by the plant to promote the growth and the activity of beneficial root-colonizing microbes (RCM) upon the exposure to pathogen attack or certain abiotic stresses. This may occur by the release of compounds that, more or less specifically, modify the root microbiome in favour of species that help the plant to overcome the incoming stresses. We observed in a split root experiment that the contact with *Pythium ultimum* on one side stimulates the development of *Trichoderma* colonies located in a separated compartment, also increasing the chemiotactic growth of the mycelium toward the plant. This suggests the release of specific compounds through the root system that attract and stimulate beneficial root-colonizing microbes, which helps the plant to overcome biotic and/or abiotic stresses.

DEVELOPMENT OF A NEW VIRUS-INDUCED GENE SILENCING VECTOR FOR GRAPEVINE, BASED ON GRAPEVINE ALGERIAN LATENT VIRUS. A. Lovato¹, L. Santi², C. Malvezzi¹ and A. Polverari¹. ¹Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy. ²Department of Science and Technology for Agriculture, Forestry, Nature and Energy, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: annalisa.polverari@univr.it

Establishment of functional genomics in *Vitis* sp. is still challenging, due to the lack of reliable high-throughput tools for grapevine transformation. Virus-induced gene silencing (VIGS) makes use of a plant virus-based vector carrying a sequence of an endogenous plant gene. By delivery of the vector and replication of the recombinant virus within the plant, the plant defence mechanism known as post-transcriptional gene silencing (PTGS) is activated against the virus resulting in silencing of the plant gene. This system has been recently applied on *Vitis vinifera* plants with vectors based on the *Grapevine Virus A* (GVA) and *Grapevine leafroll-associated virus 2* (GLRaV-2). We developed a new VIGS system using a vector based on *Grapevine Algerian latent virus* (GALV), a member of the genus *Tombusvirus*. The whole GALV genomic sequence was cloned into the binary vector pK7WG2 and modified to carry a polylinker site in which different sequences could be easily inserted to induce silencing against any gene of interest. Preliminary results of *Agrobacterium*-mediated GALV infections in grapevine have been obtained. GALV could replicate and spread systemically in several grapevine varieties. The presence of a ribozyme sequence immediately downstream of the viral sequence was crucial for viral replication. The silencing-inducing ability of the GALV-based vector was confirmed by downregulation of the phytoene desaturase gene in *N. benthamiana*, associated to the expected bleaching phenotype. Availability of an efficient GALV-based VIGS vector for grapevine could be precious for functional genomics as it is a systemic and latent grapevine virus.

WIDESPREAD OCCURRENCE OF APPLE PROLIFERATION DISEASE IN LOW-INTENSITY ORCHARDS OF BASILICATA. C. Marcone¹ and E. Seemüller². ¹Dipartimento di Farmacia, Università degli Studi di Salerno, Via Giovanni Paolo II, 134, 84084 Fisciano (SA), Italy. ²Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, 69221 Dossenheim, Germany. E-mail: cmarcone@unisa.it

Visual symptom assessment and PCR amplification were used to survey the occurrence of apple proliferation (AP) disease in low-intensity orchards in the Agri valley, a major cultivation area of

Basilicata (southern Italy). The apple trees examined, whose cultivars were not determined as they consisted mostly of local types, were more than 20-year-old. Therefore, these plants had been exposed to insect vectors for a long time. The survey revealed that a high percentage of trees were infected reaching more than 50% in some locations. The symptoms of diseased trees were generally mild and consisted of enlarged stipules, rosettes, witches'-brooms as well as subterranean witches'-broom-like growth arising from large roots. However, the incidence and severity of symptoms in the aerial parts of affected trees were more pronounced in trees which had been heavily pruned in the previous dormant season. Specificity of the primers used and RFLP analysis of PCR-amplified 16S rDNA sequences employing *SspI* and *BsaAI* restriction endonucleases showed that the trees testing positive by PCR were infected by the AP agent '*Candidatus* Phytoplasma mali'. The high incidence of AP infections in low-intensity orchards of the Agri valley is likely due to inappropriate vector control. The trees examined were not or rarely treated with insecticides. Although a few AP-affected apple trees grown in a low-intensity orchard in the Agri valley had previously been observed, our survey shows that the distribution of AP disease in Europe extends further south than previously thought and that the climatic conditions of southern Italy are not unsuitable for this quarantine disease.

BIOACTIVE METABOLITES PRODUCED BY *TRICHODERMA*: NOVEL EFFECTORS FOR PLANT BIOSTIMULATION.

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Fungi of the genus *Trichoderma* are successfully applied worldwide in the form of live inocula as biofungicides and biofertilizers due to their ability to protect crops from various diseases, as well as to increase plant growth, development and yield under different conditions. These microbes are capable of producing a plethora of chemically different compounds, i.e. lytic enzymes, antibiotics, resistance inducers, etc. that have beneficial effects on the plant and a negative activity on pathogens. Treatment with *Trichoderma* metabolites produces significant modifications of the plant expressome, proteome and metabolome, acting on specific pathways involved in the defence response to biotic/abiotic stresses and nutrient uptake. The direct use of bioactive metabolites may provide various advantages over the application of the living microbes. For instance, the semi-purified compounds are less susceptible to losses of activity due to storage or environmental changes, thus allowing to overcome some of the performance inconsistencies often observed in the field. We have conducted a large *in vivo* study by testing isolated metabolites from different *Trichoderma* species, both singly and in a variety of combinations also with the living microbes, on many different crops, pathogens and applications. Selection of the compounds to be used was based on the actual knowledge of the mechanisms of action, in order to complementing different beneficial effects. New biopesticides based on secreted proteins and/or secondary metabolites related to the biocontrol/plant-growth-promoting-activity of *Trichoderma* spp. are being patented, registered and included in the pipeline for commercialization.

PREFORMED ANTIFUNGAL COMPOUNDS IN PEACH PEEL FRUIT AT DIFFERENT DEVELOPMENTAL STAGES AGAINST *MONILINIA* spp. C. Martini¹, L. Ugolini², C.

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The aim of this study was to relate peach fruit susceptibility to brown rot, the main disease on stone fruits caused by *Monilinia* spp., to the presence of secondary metabolites with antifungal properties, that are mostly concentrated in the peel at the immature stage. The susceptibility to pathogens was evaluated weekly as the percentage of infected fruits after artificial inoculation in wounded and unwounded fruits incubated for seven days at 20°C. The susceptibility presented the same seasonal pattern for the three *Monilinia* species and a high resistance to *Monilinia* rots was found 6-8 weeks after full bloom. In this phase, the pit hardening occurred (S2). The presence of antifungal compounds ethanol extracts from peach peels of cvs Maycrest, Red Heaven, and Tardibelle at different developmental stages was evaluated by 1D TLC bioassays. Using a solvent system 60:40:30 (hexane:ethylacetate:methanol), inhibition spots with R_f values of 73.3, 53.3 were found in S1 (4 weeks after full bloom), S2 (pit hardening) and S4 (harvest) stages. Spots with R_f values of 26.6, 13.3 were also observed in S2. No inhibition spots were detected on cvs Maycrest and Tardibelle in S4, while very feeble inhibition spots were found in cv. Red Heaven with R_f 73.3 and 53.3. These results suggest that the presence of preformed compounds in peach peel could be considered partially responsible for the resistance to brown rot observed in fruit 6-8 weeks after full bloom. However, further investigations are required to identify these compounds and understand their role in brown rot susceptibility.

TEMPERATURE INFLUENCE ON THE RESISTANCE AND SENSITIVITY OF *MONILINIA LAXA*, *MONILINIA FRUCTICOLA* AND *MONILINIA FRUCTIGENA* ISOLATES TO TEBUCONAZOLE AND THIOPHANATE METHYL FUNGICIDES: *IN VITRO* AND *IN VIVO* TRIALS. C. Martini, A. Di Francesco and M. Marta. ¹CRIOF, Università degli Studi di Bologna, Via Gandolfi, 19, 40057 Cadriano (BO), Italy. E-mail: camilla.martini2@unibo.it

The effect of environmental factors such as temperature should be studied to better assess the resistance of *Monilinia* spp. to chemical fungicides used in the orchards. For *in vitro* assay, PDA plates amended with tebuconazole or thiophanate methyl at a concentration corresponding to EC₅₀ value for each isolate were inoculated with both sensitive (S) and resistant (R) isolates. Thirteen *M. laxa*, seven *M. fructicola*, and three *M. fructigena* isolates were tested. For *in vivo* assay peaches were wounded and inoculated with a spore suspension (10³ conidia ml⁻¹) of *M. laxa* or *M. fructicola* six hour after treating the fruits with the fungicides at a concentration of half the label rate. To determine the effect of the temperature, the plates and the fruits were incubated at 15, 20, 25, 30°C for seven days in the dark. The percentage of infected fruits were calculated in the *in vivo* assay. *In vitro* results showed that the growth of *M. laxa* on tebuconazole-amended substrates incubated at 20°C was significantly reduced (-78%) while in the case of *M. fructigena* isolates the treatment was more effective at 15°C (-90%). *In vitro* assays with thiophanate methyl showed that the largest pathogen reduction was obtained when *M. laxa* and *M. fructicola* isolates were incubated at 30°C (with a reduction of -62% and -73%, respectively). Similar results were observed in *in vivo* tests with both fungicides, demonstrating that the temperature affects the efficacy of the fungicide treatments against *Monilinia* isolates.

OCCURRENCE OF EGGPLANT MOTTLED DWARF VIRUS ON POTATO IN ITALY. R. Miglino¹, R. Sorrentino², A. De Stradis³, A. Zoiana² and D. Alioto². ¹Dutch Flower Bulb Inspection Service, PO Box 300, 2160 AH Lisse, The Netherlands. ²Dipartimento di Agraria, Università di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. ³Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: alioto@unina.it

Since 2004, a potato disease consisting of a severe necrosis of vascular rings and adjacent flesh of tubers was observed in several potato-growing areas of Italy. In 2012, tuber extracts from five affected plants collected in Emilia Romagna (northern Italy) were used for mechanical inoculations of herbaceous test plants. Twenty-three days post inoculation *Nicotiana benthamiana*, *N. glutinosa*, *N. rustica*, *N. tabacum* cvs Samsun and White Burley plants reacted with yellow spots on the inoculated leaves followed by systemic vein clearing. No local or systemic infection occurred on *Chenopodium amaranticolor*, *C. quinoa* and *C. murale*. Electron microscopy (EM) and immuno-electron microscopy (IEM) showed the presence of rhabdovirus-like particles in extracts of potato tuber necrotic tissues and symptomatic leaves of *N. glutinosa*. Particles were recognized by a polyclonal antiserum to an Iranian potato isolate of *Eggplant mottled dwarf virus* (EMDV). A DAS-ELISA using a commercial kit (Loewe Biochemica, Germany) confirmed the presence of EMDV. For molecular characterization, total RNA extracted from potato tubers and *N. glutinosa* leaves using the RNeasy Plant Mini Kit (Qiagen Germany) was used in a RT-PCR with a specific primer pair, covering the polymerase gene. RT-PCR yielded, both in potato and *N. glutinosa*, a distinct DNA fragment of about 600 bp in size. Direct sequence of the amplicons (GenBank accession No KC760150B) showed 86% nucleotide sequence identity with EMDV (AM922322.1). To our knowledge, this is the first report of EMDV on potato plants in Italy, although this virus is endemic and infects several other crops in the country.

PLURIANNUAL LATENCY OF A GFP-MARKED PSEUDOMONAS SYRINGAE pv. ACTINIDIAE IN ACTINIDIA CHINENSIS ADULT PLANTS. P. Minardi¹, S. Ardizzi² and C. Lucchese². ¹Dipartimento di Scienze Mediche Veterinarie Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. ²Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 424, 40127 Bologna, Italy. E-mail: paola.minardi@unibo.it

In Italy, a leading kiwifruit producer in the world (excluding China), the recent outbreaks caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*) in both green (*Actinidia deliciosa*) and yellow-fleshed (*Actinidia chinensis*) cultivars and the pollinators of these crops gave rise to heavy yield losses. To overcome the current phytosanitary emergency it is now required to further investigate some critical epidemiological aspects. More specifically, the information presently available on the life-cycle of *Psa* is still inadequate with regard to its "latency" within the susceptible host plant. Indeed, a feature of the species to which *Psa* belongs is to survive endophytically in adult plants without inducing symptoms. Recent epidemiological studies showed that *Psa* can be isolated from trunks and branches of symptomless kiwifruit plants. Similarly to *P. syringae* pv. *syringae*, the causal agent of the bacterial canker of stone fruits, *Psa* may cause systemic infections which are completely symptomless or have a delayed expression. To shed light on this dangerous "latent phase", the duration of which is still unknown, the survival of *Psa* at low inoculum dose was studied in susceptible plants over three years. In 2010, shoots of two-year-old plants of *A. chinensis* cv. Hort16A grown outdoors were inoculated with a virulent *Psa* *gfp*-expressing/Rif-resistant-strain. The microbiological (re-isolation on selective media), biological (pathogenicity/HR assay) and molecular (PCR)

analyses of the whole plants were carried out from May 2011 at the appearance of the cankers in the inoculated shoots. The results obtained have highlighted the ability of *Psa* to survive within asymptomatic plants.

LONG-TERM SURVIVAL OF PSEUDOMONAS SYRINGAE pv. ACTINIDIAE IN SYMPTOMLESS MICROPROPAGATED PLANTS OF ACTINIDIA DELICIOSA. P. Minardi¹, S. Ardizzi², C. Lucchese² and A. Bertaccini². ¹Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. ²Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. E-mail: paola.minardi@unibo.it

In recent years the spread of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is creating serious problems in Italy, especially for the early detection of the pathogen in micropropagated plant material from which most of the plants used for the establishment of new stands originate. Indeed, the use of micropropagation techniques overcomes the seasonal limitations encountered with cuttings or grafting and it is favoured when a large number of plants is required in a short time. The possibility to assess innovative nursery techniques allowing a reliable and fast *Psa* detection in asymptomatic micropropagation material is essential to prevent the further spread of the pathogen, which can remain latent for a long time at very low concentrations. To evaluate *Psa* survival in micropropagated material, over three years ago the apical part of micropropagated shoots of *Actinidia deliciosa* cv. Hayward were artificially inoculated with a virulent *Psa* *gfp*-expressing/Rif-resistant strain (*Psa::gfp*) and used as explants for the production of kiwifruit plantlets. In the following 10 months, seven generations of shoots were obtained in which the presence of *Psa::gfp* was confirmed. After the multiplication and rooting phase, the plantlets were transferred to the greenhouse for re-establishment. The microbiological and molecular analyses of the plants three years post inoculation of the shoot tips showed that *Psa::gfp* was able to survive endophytically without inducing disease symptoms. A method for *Psa* detection at low inoculum concentration in symptomless nursery material has been developed.

ARTICHOKE ITALIAN LATENT VIRUS ENTERS THE SHOOT APICAL MERISTEM OF TOBACCO BEFORE THE PLANT RECOVERS FROM DISEASE SYMPTOMS. S.A. Minutillo¹, T. Mascia^{1,2}, A. De Stradis² and D. Gallitelli^{1,2}. ¹Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. ²Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: serenaminutillo@hotmail.it

We have shown that in tobacco plants *Artichoke Italian latent virus* (AILV) is unable to interfere with plant defence response based on RNA silencing (RNAi). As a result, plants recover from disease symptoms between 21 and 28 days post inoculation but symptomless leaves still contain infectious virus particles. Here we show also that unlike the nepovirus *Tomato black ring virus* AILV was present in the tobacco topmost leaf at very early stage of infection and before initiation of the recovery phase while the reduction of virus titre observed in shoot apical meristem at 40 days post inoculation was concomitant with the steady-state level of viral RNA loads during the recovery phase in lower leaves. Therefore, at least with AILV, invasion of the shoot apical meristem and initiation of recovery seem to be distinct processes. This is consistent also with the observation that AILV invaded the first leaf after the site of

inoculation not earlier than 7 days post inoculation i.e. two-days after the colonization of the topmost leaf. In this first leaf, viral RNA load reaches a threshold level high enough to prime a silencing mechanism that impairs virus spread into successive leaves moving at or ahead the infection front.

OCCURRENCE OF IRIS YELLOW SPOT VIRUS ON ONION IN CAMPANIA. M. Minutolo¹, R. Sorrentino¹, R. Griffo², F. Scala¹ and D. Alioto¹. ¹Department of Agricultural Sciences, Section of Biology and Protection of Agriculture and Forest Systems. University of Naples "Federico II", Via Università 100, 80055 Portici (NA), Italy. ²Plant Protection Service of Campania Region, Napoli, Italy. E-mail: alioto@unina.it

In April-June 2013, during a surveillance program (URCOFI) in collaboration with the Regione Campania (southern Italy), symptoms resembling those caused by *Iris yellow spot virus* (IYSV), genus *Tospovirus* family *Bunyaviridae* were observed in onion (*Allium cepa*) commercial and seed crops in different locations of Salerno and Avellino provinces with 5-20% incidence. The most common symptoms were chlorotic/necrotic diamond-shaped lesions on the scapes and leaves. Symptomatic samples were tested by ELISA using commercial polyclonal antisera (Loewe Biochemica, Germany) against IYSV, *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). All samples had a positive reaction with IYSV whereas none of them tested positive for TSWV or INSV. To biologically characterize IYSV isolate, two ELISA-positive samples (one from Avellino and one from Salerno province) were mechanically inoculated onto *Chenopodium murale*, *Nicotiana glutinosa*, *N. benthamiana*, *Datura metel*, *D. stramonium*, *Vigna unguiculata*. All inoculated plants showed local chlorotic and/or necrotic lesions. Systemic symptoms were observed only on *N. glutinosa* and *N. benthamiana*. For molecular characterization, total RNA was extracted and RT-PCR carried out using the primer pair IYSV-NF/IYSV-NR, designed on the nucleocapsid (N) gene. A 775 bp amplicon was amplified from each of the two assayed plants and sequenced. Blast analyses showed 99% sequence identity with the IYSV N gene sequence (GenBank accession No. EU586203.1). The analysis of weeds collected in the affected fields gave negative results. To our knowledge, this is the first report of IYSV in Campania. The presence of the virus in two distant areas of this region suggests that IYSV constitutes a serious threat to onion production in this country.

PERMSELECTIVE PROPERTIES OF NATURAL POLYMERS APPLIED TO THE DEVELOPMENT OF BIOSENSORS FOR MYCOTOXIN DETECTION. P. Monti^{1,2*}, G. Calia^{1,4*}, S. Marceddu³, G. Delogu², P.A. Serra⁴ and Q. Migheli¹. ¹Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia Agraria ed Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. ²Istituto di Chimica Biomolecolare del CNR, Traversa La Crucca 3, 07100, Sassari, Italy. ³Istituto di Scienze delle Produzioni Alimentari, Traversa La Crucca 3, 07100, Sassari, Italy. ⁴Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi, Viale San Pietro 43/b, 07100 Sassari, Italy. E-mail: qmigheli@uniss.it

A biosensor combines the specificity of a biological component with the sensitivity of an electrochemical transducer. The variable inhibition showed by aflatoxin and other mycotoxins towards acetyl choline esterase (AChE) can be exploited in a multi-enzyme biosensor design: the more AChE is inhibited by mycotoxins, the less choline is oxidized by choline oxidase (ChO) to betaine aldehyde

and H₂O₂. Hence, the H₂O₂ oxidation signal is influenced by the presence of mycotoxin. In the present study, AChE and ChO were co-immobilized onto a Pt/Ir electrode surface coated with different electrosynthesized polymers. In order to prevent signal of interferents, *ortho*-phenyldiamine (*o*PD) is currently utilized. Regrettably, this compound is highly carcinogenic and alternative, non toxic, polymerizing compounds would be highly desirable. We have tested different permselective films generated by non-toxic natural monomers belonging to phenylpropanoids and C₂-symmetric dimers. The compounds were electropolymerized by constant potential amperometry (CPA) and by cyclic voltammetry (CV) and characterized by scanning electron microscopy (SEM) and permselectivity analysis. Differences in permselectivity towards H₂O₂ over ascorbic acid and dopamine were detected in poly-monomers and poly-C₂-dimers. The presence of a 2-propenyl chain in the phenol ring seems to enhance permselectivity and electrocoating quality. A bi-enzyme sensor with AChE/ChO coated with these natural compounds may therefore represent a promising analytical device for mycotoxin detection in agricultural and food matrices.

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THE NEW SPECIES PECTOBACTERIUM AROIDEARUM DOES NOT PREFERENTIALLY ATTACK MONOCOTYLEDONOUS PLANTS. C. Moretti¹, R. Fakhr², I. Cleenwerck³, C. Cortese¹ and R. Buonauro¹. ¹Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Via Borgo XX Giugno 74, 06121 Perugia, Italy. ²Faculty of Agricultural and Food Sciences, Holy Spirit University of Kaslik, Lebanon. ³Laboratorium voor Microbiologie, Universiteit Gent, B-9000 Gent, Belgium. E-mail: chiara.uce.moretti@unipg.it

A new phytopathogenic species *Pectobacterium aroidearum* sp. nov. preferentially attacking monocotyledonous plants (family Airodeae) was recently described (Nabhan *et al.*, 2012, *International Journal of Systematic and Evolutionary Microbiology* 63:2520-2525). The same species causes severe soft rot on potato plants in Lebanon. During surveys, carried out in 2009-2012 on several potato fields located in the Bekaa valley and the Akkar region, many bacteria belonging to the *Pectobacterium* genus were isolated from diseased tubers and stems. Among them, we focused our attention onto five highly virulent isolates that were phylogenetically separated from the validated *Pectobacterium* spp. (before *P. aroidearum* sp. nov. description) when subjected to MLST analysis carried out on *acnA*, *atpD*, *gyrB*, *infB*, *mdh* and *rpoB* genes. These results were confirmed by fingerprinting analyses performed by digestion (*Rsa*I and *Cfo*I) of the 16S-23S intergenic transcribed spacer region and rep-PCR, as well as by phenotypic characterization obtained with API50CHE. Further analyses are in progress to verify whether our isolates and those reported by Nabhan *et al.* (2012) belong to two distinct subspecies.

REALTIME PCR ASSAY BASED ON RPB2 GENE FOR IDENTIFICATION OF BOTRYTIS CINEREA FROM PELARGONIUM ZONALE. C. Moretti, M. Quaglia, D.E. Nicosia and R. Buonauro. Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Via Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: chiara.uce.moretti@unipg.it

The disease caused by *Botrytis cinerea* Pers. ex Fr., also known as grey mold, is arguably the most significant disease problem for *Pelargonium zonale* cultivation in greenhouse. Often present as a latent infection, this fungus has the potential to cause damaging symptoms on pelargonium following a period of quiescence of unpredictable duration. In the present study, a real time PCR Eva Green assay to detect and quantify *B. cinerea* in *P. zonale* leaves was developed. Based on DNA-dependent RNA polymerase subunit II (RPB2) gene sequence data from *B. cinerea*, other related *Botrytis* species and two additional species belonging to the family Sclerotiniaceae (*Monilia fructigena* and *Sclerotinia sclerotiorum*), a pair of specific primers was designed. A standard curve was established to quantify the fungus in question. The system had high efficiency (95%) and proved to be specific and sensitive, enabling quantification of as little as 5.7 pg of *B. cinerea* DNA. Fungal specific primers were tested for quantifying *B. cinerea* in artificially inoculated leaves and to monitor disease progress. This real time PCR assay proved to be rapid and sensitive and may be used to monitor *B. cinerea* infection/presence in greenhouses.

MOLECULAR ANALYSIS OF COLLETOTRICHUM SPECIES ASSOCIATED WITH OLIVE IN CALABRIA. S. Mosca¹, M.G. Li Destri Nicosia¹, M.I. Prigigallo¹, G.E. Agosteo¹, R. Faedda², S.O. Cacciola², G. Magnano di San Lio¹ and L. Schena¹. ¹Dipartimento di Agraria, Università Mediterranea, 89122 Reggio Calabria, Italy. ²Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: lschena@unirc.it

A new molecular approach based on the use of genus-specific primers targeting the internal transcribed spacer (ITS) regions of rDNA, was developed and used to study the diversity of *Colletotrichum* species associated with the olive canopy in the Gioia Tauro plain (Calabria, southern Italy). Representative symptomatic and symptomless samples of leaves, flowers and fruits were collected during 2012 and analyzed by extracting total DNA and amplifying the target region with the genus-specific primers. Amplicons were cloned and sequenced in order to use the ITS as a barcode gene. No *Colletotrichum* species were detected in the first sampling period (May 28, 2012), whereas around 15% of the analyzed samples including leaves, dead floral parts and symptomless fruits proved to be colonized in the second (June 29, 2012) and third sampling (October 17, 2012). A significantly higher colonization rate was found in the fourth sampling (December 12, 2012) with *Colletotrichum* species detected in 74% of the analyzed samples, including many asymptomatic fruits and leaves. On the whole *C. clavatum*, *C. acutatum sensu stricto* and *C. gloeosporioides sensu stricto* were the most common species accounting for 54, 22 and 21% of the sequenced clones, respectively. Few sequences belonged to *C. karstii* and to a *Colletotrichum* sp., closely related to *C. coccodes*. Most samples were colonized by two or three different species. The new method proved very effective for discriminating multiple *Colletotrichum* species colonizing olive tissues and could be also applied to detect *Colletotrichum* spp. in other plant species.

DELAYED FUSARIUM HEAD BLIGHT SYMPTOMS IN DURUM WHEAT TRANSGENIC PLANTS EXPRESSING THE XYLANASE INHIBITOR TAXI-III. I. Moschetti¹, S. Tundo¹, M. Janni^{1,4}, L. Sella², K. Gazzetti², A. Tauzin³, T. Giardina³, S. Masci¹, F. Favaron² and R. D'Ovidio¹. ¹Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia, Università della Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy. ²Dipartimento Territorio e Sistemi Agro Forestali, Agripolis, Università

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Fungal pathogens secrete cell wall degrading enzymes (CWDEs) to breach the cell wall barrier and colonize host tissues. Endo- β -1,4-xylanases are key enzymes in the degradation of xylans, one of the major hemicelluloses in secondary cell walls of dicots and grasses. The primary role of these enzymes in pathogenesis was demonstrated for the necrotrophic fungal pathogen *Botrytis cinerea* infecting tomato leaves and grape berries. The activity of microbial xylanases is inhibited *in vitro* by specific protein inhibitors (xylanase inhibitors, XIs). Because of this inhibition capacity, and additional features including their induction following pathogen infection, XIs are considered part of the defence mechanisms counteracting microbial pathogens. Nevertheless, as yet, no evidence for this role has been *in planta* has been obtained. Therefore, we have produced transgenic plants constitutively over-expressing TAXI-III, a member of the TAXI type Xis, which is induced by pathogen infection. Results showed that TAXI-III endows the transgenic wheat with new inhibition capacities. We showed that TAXI-III is correctly secreted into the apoplast and possesses the expected inhibition parameters against microbial xylanases. The new inhibition properties of transgenic plants correlate with a significant delay in the appearance of fusarium head blight (FHB) disease symptoms caused by *Fusarium graminearum*, but do not influence significantly leaf spot symptoms caused by *Bipolaris sorokiniana*. We have shown that this contrasting result may be due to the ability of TAXI-III to inhibit the xylanase activity of *F. graminearum* whereas no such effect was exerted on the xylanase activity of *B. sorokiniana*. These results provide for the first time a straightforward evidence *in planta* that XIs are involved in plant defence against fungal pathogens and show that, by manipulating TAXI-III accumulation, wheat resistance to *F. graminearum* can be enhanced.

SPREAD OF BOIS NOIR IN A cv. CHARDONNAY VINEYARD ACCORDING TO VMP1 GENE CHARACTERISATION. S. Murolo, V. Mancini, A. Servili and G. Romanazzi. Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy. E-mail: g.romanazzi@uniupm.it

Bois noir (BN), a grapevine disease caused by stolbur phytoplasma, is widespread in all European and Mediterranean viticultural areas, and can lower the quality and quantity of the yield. The recent BN recrudescence has encouraged the investigations on the use of molecular markers to assess the genetic diversity of stolbur phytoplasma strains. The aim of this study was to record BN incidence and severity in the field, and to monitor the genetic structure of stolbur population based on the molecular characterization of variable membrane protein (*vmp1*) genotypes. Three visual inspections were carried out in a vineyard in July, August and September 2011. The number of symptomatic vines drastically increased from July (100 vines) to September (more than 700 vines). The analysis of dispersal indices showed that the spatial distribution of BN-infected vines was uniform in the vineyard. However, BN incidence was higher in the vines at the border than in those located in the centre of the stand. This disease pattern agrees with the presence of a natural source of inoculum to which vectors have access for acquiring and spreading BN in the vineyard. Stolbur population, analyzed by PCR-RFLP carried out by *RsaI* digestion of TYPH10F/R amplicons, was composed of two prevalent *vmp* genotypes (V14, V12) across both years, along with other minor haplotypes (V3, V4, V9,

V11, V15, V18). Our data indicate that the *vmp1* gene is an efficient marker to study the population structure of stolbur phytoplasma, to track the movement of the pathogen, and to identify the inoculum source, which will all serve in the planning of control strategies.

STRAWBERRY VOLATILE COMPOUNDS COULD INFLUENCE *BOTRYTIS CINEREA* AND *COLLETOTRICHUM ACUTATUM* DEVELOPMENT. F. Neri¹, M. Mari¹, A. Spadoni¹, I. Cameldi¹, D. Mazzoni¹, S. Grandi² and P. Bertolini¹. ¹CRIOF-Dipartimento di Scienze Agrarie, Università degli Studi di Bologna, Via Gandolfi 19, Cadriano (BO), Italy. ²La-Co-Ve-Dipartimento di Scienze Agrarie, Università degli Studi di Bologna, Via Gandolfi, 19 Cadriano (BO), Italy. E-mail: fiorella.neri@unibo.it

Recent studies have shown that plant volatile organic compounds may have important roles as infochemicals for the recognition of typical host and assessment of host status for fungal pathogens. To examine the influence of strawberry volatiles on *Botrytis cinerea* and *Colletotrichum acutatum* development, 34 strawberry volatiles were tested for their activity on the pathogen's conidial germination and mycelial growth. Changes in susceptibility to *B. cinerea* and *C. acutatum* infections and in volatile constituents were then examined in strawberry cvs Alba and Monterey harvested at four ripening stages. Some compounds influenced the development of the pathogens either with stimulatory and/or inhibitory effects, in relation to their concentration. In particular, ethyl butyrate and furaneol promoted *B. cinerea* conidial germination in a wide range of concentrations (from 0.062 to 12.30 µl/litre) and *C. acutatum* mycelial growth at 0.062 µl/litre. In addition, β-ionone (0.062 µl/litre) stimulated *B. cinerea* conidial germination and the mycelial growth of both pathogens. *Trans*-2-Hexenal completely inhibited *B. cinerea* and *C. acutatum* conidial germination at 12.3 µl/litre, while the same concentration of nonanal showed the best inhibition of *B. cinerea* mycelial growth (-66.8%). An increase of most volatiles having stimulant activity on the pathogens (methyl butyrate, ethyl butyrate, 2-methylbutyl acetate, hexyl acetate and hexyl butyrate) and a decrease of compounds that inhibited them (*trans*-2-hexenal and nonanal) was observed as ripening progressed in two strawberry cultivars with different aroma profiles. Results of our study suggest that fruit volatiles may influence the complex process of inhibition and/or stimulation of *B. cinerea* and *C. acutatum* latent infections.

SYSTEMATIC LITERATURE REVIEW FOR MODELLING BLACK ROT DISEASE OF GRAPEVINE. G. Onesti, S.E. Legler, T. Caffi and V. Rossi. Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy. E-mail: giovanni.onesti@unicatt.it

Systematic literature review (SLR) is a method to retrieve, analyse and summarize research studies, conducted through an explicit and reproducible methodology. SLR includes meta-synthesis and meta-analysis to: (i) develop new conceptualizations and interpretations; (ii) combine the results of different studies in order to identify the overall trend of the results, and (iii) transform the findings of existing studies in raw data for further analysis and interpretation. SLRs have been carried out in many disciplines, but none was performed in plant pathology with the aim of developing mechanistic, dynamic models of specific patho-systems. In this work, the available knowledge on *Guignardia bidwellii*, the causal agent of black-rot of grapevine, was retrieved through a SLR and analysed to conceptualize the life cycle of the pathogen. Following the 'systems analysis' approach the life cycle was divided in three compartments: (i) production and maturation of ascospores from

pseudothecia and conidia from pycnidia in berry mummies and cane lesions (i.e., the primary inoculum of the disease); (ii) infection caused by ascospores and conidia; (iii) disease onset and production of secondary inoculum. A mechanistic model, driven by weather and vine phenology data was developed from a meta-analysis of the quantitative information available in the literature. In particular, new equations were developed for ascospore and conidial maturation in overwintered fruiting bodies, spore release and survival, infection occurrence and severity, incubation and latency periods, onset of the lesions, production of pycnidia and infectious period. Finally, the model was evaluated to demonstrate its capability to represent the real system and in helping understanding black-rot epidemics.

THE ROLE OF IRON IN THE DEVELOPMENT OF NECROSIS BY *PHAEOACREMONIUM ALEOPHILUM* IN KIWI-FRUIT YOUNG VINES. F. Osti¹, R. Roberti², G. Innocenti², A. Rombolà² and S. Di Marco¹. ¹Istituto di Biometereologia del CNR, Via Piero Gobetti 101, 40129 Bologna, Italy. ²Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. E-mail: s.dimarco@ibimet.cnr.it

Wood decay of kiwifruit is a complex disease widespread in all kiwifruit-growing areas. Foliar symptoms usually appears on 10-year-old vines, but pathogens can degrade wood also in young plants. Among the microorganisms associated with this disease, tracheomycotic fungi (mainly *Phaeoacremonium aleophilum*), seem to play an important role, especially in young plant infections. Different kind of soil treatments have been tested with the aim to produce conditions potentially unfavourable to the necrosis caused by *P. aleophilum* on 2-year-old kiwifruit potted vines artificially inoculated with the pathogen. Iron chelate (Fe-EDDHMA) or a mixture of *Festuca rubra*, *Poa pratensis* and *Lolium perenne* proved to be the most effective in significantly reducing *P. aleophilum* necrosis. Such treatments improved the availability of iron in the soil. *In vitro* tests were conducted to evaluate the effect of ferrous iron on *P. aleophilum* mycelial growth, and on the fungal non-enzymatic and enzymatic activities associated with wood degradation. Polygalacturonase and 1-4-β-endoglucanase activities were significantly reduced by the presence of iron also at concentrations that did not reduce mycelial growth. A certain reduction of pectin-lyase activity was also observed. In some cases, the production of hydrogen peroxide and hydroxyl radical seemed to increase. Furthermore, iron chelate and the weed mixture favoured the natural mycorrhization of the roots. These results support a mechanistic explanation of the iron effect on the reduction of wood necrosis by *P. aleophilum*. Further studies are underway to assess whether iron can reduce the impact of the disease in the vines.

EVALUATING ANTIFUNGAL ACTIVITY OF SELECTED WILD *CAPSICUM* MEALS AGAINST PHYTOPATHOGENIC FUNGI. C. Pane, M. Caputo, M. Parisi and M. Zaccardelli, Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Orticoltura, Via dei Cavalleggeri 25, 84098 Pontecagnano (SA), Italy. E-mail: catello.pane@entecra.it

Toxicological concerns associated with the use of fungicides in agriculture and increased awareness about safe foods, have sparked the interest on the use of natural substances in pathogens eradication. The exploitation of phytochemical compounds, either as crude or purified, is a promising approach for identifying new tools for sustainable pest management. In this study, thirteen different wild *Capsicum* genotypes, including *Capsicum annuum*, *C. annuum*

var. *glabrumsculum*, *C. baccatum* var. *baccatum*, *C. baccatum* var. *pendulum*, *C. chacoense*, *C. chinense*, *C. eximium*, *C. frutescens*, *C. praetermissum*, *C. pubescens* and *C. tovarii*, were examined for their potential in providing phytochemicals for antifungal applications. Leaves dried at 70°C were ground and added, at a dose of 500 mg ml⁻¹, to autoclaved potato dextrose agar medium, then assayed for supporting the growth of phytopathogenic fungi. Wild pepper meals exhibited varying degrees of inhibitory activity against *Alternaria alternata*, *Rhizoctonia solani*, *Sclerotinia minor* and *Verticillium dahliae*. The clear-cut mycelial growth suppression indicates the presence, in the media, of unidentified molecules carried by raw plant materials that exhibited direct fungal toxicity. Among the studied plant materials, almost all the meals showed antifungal activity. Meals showing the maximum inhibition activity were those from *C. eximium* against *R. solani*, *C. chinense* against *A. alternata*, *C. annuum* var. *glabrumsculum* against *V. dahliae* and *C. baccatum* var. *baccatum* and *C. chacoense* against *S. minor*. Due to their antimicrobial properties, leaf meals from certain *Capsicum* plants may be used as natural protective substances in low-impact fungicidal formulations.

CHANGES IN SUPPRESSIVE PROPERTIES OF AERATED COMPOST TEA INDUCED BY DIFFERENT ORGANIC ADDITIVES. C. Pane¹, A.M. Palese², D. Villecco¹, G. Celano² and M. Zaccardelli¹, ¹Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Orticoltura, Via dei Cavalleggeri 25, 84098 Pontecagnano (SA), Italy. ²Dipartimento delle Culture Europee e del Mediterraneo: Architettura, Ambiente, Patrimoni Culturali, Università degli Studi della Basilicata, Via S. Rocco 3, 75100 Matera, Italy. E-mail: catello.pane@entecra.it

Aerated compost teas (ACT) are fermented extracts of composted materials used in plant disease control. The regulation of some productive parameters, such as compost aqueous extractant additives, to generate conditions leading to the development of beneficial organisms can be essential for suppression induction. Six compost teas were produced at the laboratory scale by a 48 h-aerated extraction of a commercial municipal waste compost suspended in water (ratio 1:5) with different additives, including sheet dairy byproducts, at two concentrations (150 and 75 ml l⁻¹), and sugar beet marc at three concentrations (10, 5 and 3 g l⁻¹). Teas were chemically and biologically characterized and the influence of the various additives was evaluated based on their antibiosis ability against a set of phytopathogenic fungi. *In vitro* assays were carried out against *Fusarium solani*, *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium sambucinum*, *Fusarium semitectum*, *Alternaria alternata*, *Botrytis cinerea*, *Verticillium dahliae*, *Colletotrichum lindemutianum*, *Pyrenochaeta lycopersici* and *Rhizoctonia solani*. An *in vivo* trial was carried out on the savoy cabbage-*R. solani* pathosystem. All ACT significantly inhibited the mycelial growth of the tested pathogens, showing an antibiosis-like mechanism at levels unrelated to the type of additive. In *in planta* assays ACT produced with the two additives at lowest concentration, consistently provided an efficient control of diseases. Sheet-containing teas were more efficient against *Rhizoctonia* disease of savoy cabbage, while the addition of marc was depressive.

A PROTEOMIC STUDY INVESTIGATING THE EFFECT OF NATURAL AND NATURAL-LIKE COMPOUNDS ON *FUSARIUM GRAMINEARUM*. G. Pani¹, T. Serchi², G. Delogu³, B. Scherm¹, V. Balmas¹, J. Renaut², L. Hoffmann², Q. Migheli¹ and M. Pasquali². ¹Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia and Unità di Ricerca Istituto Nazionale di

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Fusarium graminearum causes significant crop losses to wheat and other cereals and strongly affects the quality of the products due to the accumulation of mycotoxins. Several studies have investigated the action of compounds alternative to conventional fungicides able to interfere with growth and mycotoxins biosynthesis. In our work, we studied the effects of natural and natural-like compounds on *F. graminearum* protein profile. Six compounds with the ability to either induce or repress mycotoxin production (15-acetylated deoxynivalenol and deoxynivalenol) were used. Total protein extracts of the *F. graminearum* sequenced strain PH-1 were obtained at two time points during growth in liquid cultures corresponding to two key stages in toxin production (maximum trichothecene gene cluster (tri) expression levels and maximum concentration of toxin). Four biological replicates for each condition, six compounds at two different time points, were analysed by 2-D difference in gel electrophoresis (DiGE) using a pH gradient 3 to 10. A total of 763 differentially abundant (abundance of variation, 1.3-fold, P < 0.01) spots were picked from gels and analysed by MALDI-Tof/Tof. Of the 526 identified spots, 319 were single protein species. All the identified protein species were classified for their putative localization within the cell and their biological function. Most of the identified proteins were localized in the cytoplasm (26%) and in mitochondria (23%), followed by those present in the nucleus (10%) and in peroxisomes (9%). The identified proteins were specifically overrepresented in some functional categories, namely: "protein with binding function or cofactor" (p = 1.05E-39); "energy" (p = 1.75E-27); "metabolism" (p = 1.70E-16); and "protein involved in the responses to oxidative stress" (p = 2.47E-06). Moreover, the conditions used in this study lead to the detection of 48 proteins (mainly involved in metabolism), identified for the first time in *Fusarium graminearum* when compared with all the previously described proteomic studies so far. The comparison of the proteomic profiles shed light on some metabolic changes occurring in the interaction of the fungus with each compound as well as in the two stages of toxin production monitored.

NEW OUTBREAKS OF GEMINIVIRUSES IN TOMATO CROPS ASSOCIATED WITH THE SPREAD OF A NEW INVASIVE GENOTYPE OF *BEMISIA TABACI* IN CAMPANIA. G. Parrella and M. Giorgini. Istituto per la Protezione delle Piante del CNR, UOS Portici, Via Università 133, 80055 Portici (NA), Italy. E-mail: parrella@ipp.cnr.it

The geminiviruses *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV) were detected for the first time in Campania during 2003, in tomato plants showing symptoms of tomato yellow leaf curl disease (TYLCD). Most recent surveys of *Bemisia tabaci* populations associated with TYLCD outbreaks, revealed a constant rising of the frequency of biotype Q1 populations and displacement of the biotype B. Even so, the distribution of TYLCSV and TYLCV was detected at the same ratios and the TYLCD presence remained limited to a precise tomato greenhouse area. Recently (2012-2013), we sampled additional *B. tabaci* populations on insecticide-sprayed tomato crops and wild plants from the same greenhouse area and characterized their diversity by restriction analysis of the partial COI gene and bacterial endosymbiont composition. In almost all the populations tested, we detected again the Q1 biotype plus biotype Q2, which had never been previously recorded in Italy. The invasive biotype Q2 was prevalent (almost 90% of the whiteflies sampled) compared

to Q1. Moreover, we found a high TYLCD incidence in tomato plants infested by *B. tabaci*, with TYLCV detected in 90% of the symptomatic samples. Outbreaks of TYLCD were found in new areas of tomato cultivation in Campania (southern Italy). This has led to the hypothesis that the two whitefly biotypes have different fitness and may not be equally competent vectors for TYLCV and TYLCSV. The role of endosymbionts in increasing the fitness of biotype Q2 and its specificity in TYLCV transmission is hypothesized and discussed.

DESCRIPTION OF A PUTATIVE NOVEL PHYTOPLASMA TAXON ISOLATED FROM PERIWINKLE IN SPAIN.

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Phytoplasmas are wall-less bacteria of the class Mollicutes that inhabit phloem sieve cells in plants and colonize different tissues in insect vectors. Their evolutionary genome reduction resulted in loss of genes encoding various metabolic pathways making it difficult their isolation in axenic culture and classification based on phenotypic character observation. A phytoplasma classification has been established on the basis of 16S rRNA gene sequences, and a provisional 'Candidatus Phytoplasma' species taxonomy has been adopted. In this study, we assessed the taxonomic position and group classification of a phytoplasma responsible for virescence and phyllody symptoms in naturally diseased Madagascar periwinkle plants in southern Spain. Unique regions in the 16S rRNA gene of this phytoplasma distinguished the isolate from all previously described 'Candidatus Phytoplasma' species. In the alignment with full-length 16S rRNA gene sequences the Spanish isolate shared less than 97.5% sequence similarity with that of previously described 'Ca. Phytoplasma' species, justifying the recognition of a putative novel taxon. The 16S rRNA gene F2nR2 fragment from the Spanish isolate exhibited a distinct restriction fragment length polymorphism (RFLP) profile, different from representative phytoplasmas classified in any of the 31 previously delineated 16Sr groups. Phylogenetic analyses of full length sequences of three other genes, encoding the Elongation Factor Tu, ribosomal proteins L22 and S3 and protein translocase subunit SecY, shared less than 92% sequence similarity with other 'Ca. Phytoplasma' species sequences, supporting the identification of a novel taxon.

A NEW METHOD TO EVALUATE THE OCCURRENCE OF FIVE ALTERNARIA TOXINS IN DIFFERENT FOOD MATRICES.

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Different species of *Alternaria* are able to produce several toxic secondary metabolites, called alternaria mycotoxins (ATs), which include alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), tentoxin (TEN), and tenuazonic acid (TeA). A new method for the detection of AOH, AME, ALT, TEN, and TeA was developed by liquid chromatography-triple quadrupole

mass spectrometry equipped with atmospheric pressure chemical ionisation (APCI). A single extraction was used to recover the five ATs from apple juices, beers, tomato sauces, olives and dried basil. Different solid phase extractions (SPE) and clean-up were selected to optimize the purification step for each food matrix. Limits of detection and quantification were in the range of 0.16-12.31 ng g⁻¹ and 0.54-41.04 ng g⁻¹, respectively. Recovery rates were generally above 70%, except for dried basil and olives. Thirty of 70 samples analysed (seven apple juices, 14 beers and nine tomato sauces) resulted positive to at least one alternaria toxin investigated. AOH was the most common AT (14 samples), followed by ALT (10 samples). The highest concentration of ATs was found in commercial apple juices (35.33 ng g⁻¹). The presence of ATs in some food samples analysed, suggests the need of a wider monitoring of food products present on the market, and indicates the importance of determining maximum thresholds for AFs in Europe and internationally, to secure a high level of food safety.

MOLECULAR ANALYSIS OF PHYTOPHTHORA DIVERSITY IN ORNAMENTAL NURSERIES.

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A molecular approach based on the use of genus-specific nested-PCR primers (Scibetta *et al.*, 2012. *Journal of Microbiological Methods* 88: 356-368) was utilized to detect *Phytophthora* species in soil and root samples of potted ornamentals, collected across Apulia and Calabria, (southern Italy). Analyzed samples comprised many plant species with different levels of decline symptoms of the canopy and root rots. Extraction protocols were optimized to obtain DNA samples of appropriate quality from soil and roots. Sequence analysis of cloned nested-PCR amplicons enabled the identification of different *Phytophthora* species including *P. nicotianae*, *P. cinnamomi*, *P. cryptogea*, *P. palmivora* and *P. niederhauserii*. Interestingly, a higher level of intraspecific variability was detected within each *Phytophthora* species as compared with the results of previous investigations using the same method in natural ecosystems. Although, the existence of PCR artifacts due to Taq DNA polymerase errors cannot be completely excluded, the detection of the same genotypes (commonly differentiated by one or few polymorphic nucleotides) in different soil and/or root samples, confirmed the reliability of the results. As a consequence, the higher intraspecific variability detected in ornamental nurseries seem to be the results of a more intensive sexual recombination favoured by the concurrent cultivation of many plant species, which increases the meeting of different genetically distant isolates of the pathogens.

USE OF TRICHODERMA ASPERELLUM 2046 IN NEW "TAILOR MADE" SUBSTRATES FOR ORNAMENTAL PLANTS.

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Peat, the most important component of substrates for ornamental plants, has been defined a "slow renewable resource", which calls for a decrease of its use. The MIPAAF funded project "Messaggio a punto di substrati artificiali innovativi per il florovivaismo" for

reducing the use of peat for the cultivation of *Limonium sinuatum*, *Cupressus sempervirens* and *Camellia japonica*, aimed at developing an innovative, economical and suitable “tailor-made” substrate which was improved with the addition of beneficial fungi belonging to *Trichoderma* genus. At the end of more than three years of research activity, starting from a collection of almost 250 *Trichoderma* sp. isolates, *T. asperellum* 2046 showed beneficial effects in terms of growth promotion, on all the three ornamental species and colonized endophytically their roots. In addition, the antagonistic activity against the *Limonium* pathogens *Sclerotinia sclerotiorum*, *S. minor* and *Rhizoctonia solani*, was assessed by both *in vitro* and *in vivo* tests. With the aim to reduce the amount of peat in these substrates, different media containing composts and other components were formulated by a chemometric approach, and preliminarily evaluated on *Cupressus* and *Camellia*. Substrates selected by this screening were inoculated with *T. asperellum* 2046 to assess its beneficial effects. Plants growth data confirmed the biostimulating effects of the fungus and showed that it is possible to reduce by up to 30% the amount of peat in “tailor made” growing substrates by the combined addition of different composts and the selected *Trichoderma* isolate.

THE ARABIDOPSIS SEPAL FUNCTIONS AS DEFENSE BARRIER OF THE DEVELOPING FLOWER AGAINST THE BIOTROPHIC PATHOGEN *GOLOVINOMYCES CICHORACEARUM*. M. Quaglia¹, S. Pasqualini², C. Gehring³, A. Dawe⁴ and L. Ederli². ¹Dipartimento di Scienze Agrarie e Ambientali, Borgo XX Giugno 74, 06121 Perugia, Italy. ²Dipartimento di Biologia Applicata, Borgo XX Giugno 74, 06121 Perugia, Italy. ³Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal 23955–6900, Saudi Arabia ⁴South African National Bioinformatics Institute, University of the Western Cape, Bellville 7535, South Africa. E-mail: mara.quaglia@unipg.it

Arabidopsis sepals are modified green leaf-like structures containing polyploid giant pavement cells. It has been postulated, but not demonstrated, that these cells may play a role in the defense of developing flowers against biotic and abiotic stresses. In the present work, comparative analysis of sepal, petal and rosette leaf transcriptomes showed that several stress-responsive genes, including those involved in salicylic acid (SA) defence pathway, are differentially expressed in sepals as compared with petals and leaves. We investigated, in particular: (i) the constitutive levels of free and total SA in Arabidopsis Col-0 sepals and leaves; (ii) the level of infection in Col-0 and *nabG* Arabidopsis sepals and leaves inoculated with *Golovinomyces cichoracearum* or *Botrytis cinerea*. It was shown that sepals act as defense barrier for the developing reproductive organs against the biotrophic pathogen *G. cichoracearum* but not against the necrotrophic pathogen *B. cinerea*, thus emphasizing the prominent role of SA in defence pathway against biotroph.

EXTRACTS OF VEGETATION WASTE WATER FROM OLIVE MILLING AGAINST POMEGRANATE POSTHARVEST PATHOGENS. M. Quaglia¹, G. Linoci¹, S. Urbani² and A. Taticchi². ¹Dipartimento di Scienze Agrarie e Ambientali, Borgo XX Giugno 74, 06121 Perugia, Italy. ²Dipartimento di Scienze Economico-Estimative e degli Alimenti, Via San Costanzo, 06121 Perugia, Italy. E-mail: mara.quaglia@unipg.it

Pomegranate (*Punica granatum* L.) is a deciduous shrub widely grown in many tropical and subtropical regions, including Mediterranean countries. Its cultivation is rapidly expanding also in southern Italy. The nonclimatic fruits are harvested at full ripening

when the organoleptic and nutritional values, but also the susceptibility to pathogen attacks, are the highest. *Botrytis cinerea* and *Penicillium* spp. are the most important postharvest pathogens. These pathogens can be contained by a widely used fludioxonil-based postharvest treatment the use of which is not allowed in Italy. In the present investigation, a phenolic concentrate obtained from olive mill wastewater (COMW) through a membrane filtration process and a related purificate (PCOMW) deprived of the sugar were produced and *in-vitro* tested against an isolate of *B. cinerea* and one of *Penicillium* spp. obtained from decayed pomegranates. At the dose of 4 and 8 mg ml⁻¹ phenols both COMW and PCOMW significantly reduced the growth of *Penicillium* sp. while only PCOMW at the dose of 8 mg ml⁻¹ phenols significantly reduced the growth of *B. cinerea*, suggesting a different effect of both phenolic and sugar component on the pathogen growth. Treatments significantly reduced also conidia germination and germ tube elongation of both pathogens. These preliminary results suggest the possible use of COMW and PCOMW for postharvest treatment of pomegranate. These products could represent an health- and environmental-safe alternative to pesticides and an alternative way of utilization of olive mill wastewater, whose high content of phenols determines serious disposal problems.

FIRST REPORT OF GRAPEVINE PINOT GRIS VIRUS INFECTING cv. GLERA IN THE CONEGLIANO-VALDOBBIADENE AREA. A. Raiola¹, C. Scopel¹, D. Ferrigo¹, F. Taglietti², C. Duso³ and R. Causin¹. ¹Dipartimento Territorio e Sistemi Agro-forestali, Università degli Studi di Padova, Legnaro (PD), Italy. ²Consorzio Conegliano Valdobbiadene D.O.C.G., Legnaro (PD), Italy. ³Dipartimento Agronomia, Animali, Alimenti, Risorse Naturali e Ambiente, Università degli Studi di Padova, Legnaro (PD), Italy. E-mail: alessandro.raiola@unipd.it

Grapevine pinot gris virus (GPGV) has recently been associated with a new grapevine disease in Trentino (northern Italy). Phylogenetic trees constructed with viral RNA-dependent RNA polymerase and coat protein grouped this virus with a closely related thricovirus, *Grapevine berry inner necrosis virus* (GBNV) occurring only in Japan. RT-PCR analysis carried out to check the presence of GPGV in symptomatic vines of cv. Glera from Valdobbiadene locations (Veneto, northern Italy) gave positive reactions with specific GPGV primers. Sequenced PCR products were identical at the nucleotide level with comparable GPGV sequences (GenBank accession No. NC_015782). Chlorotic mottling, mosaic patterns, puckering, shoot malformation and abnormal branching were the main symptoms observed in cv. Prosecco. Symptom recovery of the new shoots of infected vines was observed in the warmer summer months. The yield and quality of the bunches of diseased vines are reduced, so that infected plants are removed, thus causing important economic losses in one of the most important grape-growing areas of northern Italy. Our analysis shows for the first time the presence of this new grapevine viral disease in cv. Glera in the Conegliano Valdobbiadene D.O.C.G. area, which is indicative of its wider distribution. Further investigations on GPGV spreading and epidemiology on cv. Glera are in progress.

CONSTITUTIVE EXPRESSION OF PECTIN METHYLESTERASE INHIBITORS LIMITS TOBAMOVIRUS SPREADING IN TOBACCO AND ARABIDOPSIS. A. Raiola¹, V. Lionetti², E. Fabri², F. Cervone² and D. Bellincampi². ¹Dipartimento Territorio e Sistemi Agro-forestali, Università di Padova, Legnaro (PD), Italy. ²Dipartimento di Biologia e Biotecnologie “Charles Darwin”, Sapienza Università di Roma, Italy. E-mail: alessandro.raiola@unipd.it

Plant viral infection is a complex process influenced by the interaction of virus-encoded proteins and host factors that support virus replication and movement. Successful infection requires viral movement proteins (MPs) that modify the plasmodesmata (PD) size exclusion limit during cell-to-cell movement. Pectin methylesterase (PME) was shown to interact *in vitro* with the MP of different viruses and the MP-PME interaction was proposed to be necessary for *Tobacco mosaic virus* (TMV) cell-to-cell spreading. PME is also required for the systemic movement of TMV through the host vascular system. Pectin demethylation directed by PME is likely to be a source of methanol that has been recently found to facilitate TMV spreading by triggering PD dilation. We here report that the ectopic expression of a PME inhibitor from *Actinidia chinensis* (AcPMEI) in *Nicotiana tabacum* significantly delays the TMV cell-to-cell and systemic spreading. A reduced susceptibility against *Turnip vein clearing virus* (TVCV) was also observed in Arabidopsis plants over-expressing a PME inhibitor from Arabidopsis (AtPMEI-2). Overall, our results indicate PMEIs as efficient tools to limit Tobamovirus infection.

GENOTYPIC AND PHENOTYPIC VERSATILITY OF *ASPERGILLUS FLAVUS* DURING MAIZE EXPLOITATION. M. Reverberi¹, M. Punelli¹, S. La Starza¹, V. Scala¹, M. Scarpari¹, P. Uva², W.I. Mentzen², A.L. Dolezal³, C. Woloshuk⁴, F. Pinzari², A.A. Fabbri¹, G.A. Payne³ and C. Fanelli¹. ¹Dipartimento di Biologia Ambientale, Università degli Studi "La Sapienza", Roma, Italy. ²CRS4 Bioinformatica, Parco Scientifico e Tecnologico Polaris, Pula (CA), Italy. ³Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA. ⁴Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA. ⁵Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo, Roma, Italy. E-mail: massimo.reverberi@uniroma1.it

Aspergillus flavus is a cosmopolitan fungus able to respond to external stimuli and to shift both its trophic behaviour and the production of secondary metabolites, including that of the carcinogen aflatoxin (AF). To better understand the adaptability of this fungus, we examined its genetic and phenotypic responses when it was grown under four conditions that mimic different ecological niches ranging from saprophytic growth to parasitism. Global transcription changes were observed in both primary and secondary metabolism in response to these conditions, particularly in secondary metabolism, where transcription of nearly half of the predicted secondary metabolite clusters changed in response to the trophic states of the fungus. The greatest transcriptional change was found between saprophytic and parasitic growth, particularly related to the secondary metabolite cluster 32. This cluster contains two fungal effectors: the necrosis and ethylene-inducing peptide and a salicylate hydroxylase. This cluster is apparently controlled by two ethylene responding transcription factors. We inactivate one of this for checking change in *A. flavus* virulence phenotype on maize. We also examined tolerance of *A. flavus* to oxidative stress and found that growth and secondary metabolism were altered in a superoxide dismutase (*sod*) mutant and an alkyl-hydroperoxide reductase (*abp*) mutant of *A. flavus*. Data presented in this study show a multifaceted response of *A. flavus* to its environment and suggest that oxidative stress and secondary metabolism are important in the ecology of this fungus, notably in its interaction with host plant and in relation to changes in its lifestyle (i.e. saprobic to pathogenic).

GENETIC VARIABILITY OF THE ITALIAN POPULATIONS OF *GUIGNARDIA BIDWELLII*, THE AGENT OF "BLACK

ROT" OF GRAPEVINE. P.A. Rinaldi¹, D. Paffetti², C. Comparini¹, G.A.L. Brogini³, C. Gessler³ and L. Mugnai¹. ¹Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Patologia Vegetale ed Entomologia, Piazzale delle Cascine 28, 50144 Firenze, Italy. ²Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Genetica Agraria, Via S. Bonaventura 13, 50145 Firenze, Italy. ³Plant Pathology Integrative Biology Zurich, ETH Zurich LFW-C16, Universitaetstrasse 2, 8092 Zurich, Switzerland. E-mail: pieroantonello.rinaldi@unifi.it

Guignardia bidwellii is the causal agent of black rot of grapevine, an ascomycetous fungus that has a homothallic production of perithecia. The pathogen was first recorded in Tuscany (central Italy) in 1891, but since then heavy damages were not caused in this country until the end of the 20th century, when they occurred in Liguria in the 1970s, in Friuli in the 1980s, and in Sardinia in 2010, while in Tuscany heavy damage was recorded only since 2010. This fungus now occurs in all regions of northern Italy, but not in the south, Sicily included. To explain this sudden increase in black rot incidence in important central Italian vineyards, the genetic variability of the fungus was investigated using a molecular approach. Infected clusters were collected from vineyards in all northern Italian regions, and cultures of *G. bidwellii* were obtained from the berries of each cluster. DNA was extracted from other berries of the same clusters, and that DNA extracted from the berries was analysed using microsatellites (SSR) obtained specifically from *G. bidwellii*. DNA was extracted also from the mycelium and ITS, and β -tubulin and calmodulin were sequenced and compared for the construction of phylogenetic trees. The genetic variability found suggests that there exists a possibility that the natural cross-breeding of different genotypes in Italy may lead to the development of other strains more resistant to pesticides and more dangerous to grapevine crops.

USE OF SEM X-RAY MICROANALYSIS FOR THE STUDY OF FUNGICIDE MODE OF ACTION: PRELIMINARY APPLICATION ON *ERYSIPHE NECATOR*. G. Russo, S.E. Legler, T. Caffi and V. Rossi. Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it

The knowledge of fungicide mode of action (MoA) provides useful information in modern plant disease management, for the targeted use of the fungicides and implementation of anti-resistance strategies. Nowadays, the MoA is well-defined for a restricted number of fungicides. The study of fungicide MoA requires the integration of microscopy and bio-molecular tools, which may be more difficult to apply when the target organism is a biotrophic fungus. In this work, we used the SEM X-ray microanalysis in order to find differences in the chemical composition of *Erysiphe necator* chasmothecia sprayed with meptyldinocap. SEM X-ray is a scanning electron microscope able to detect chemical elements by their scattered X-ray emissions from the specimen. The principal component analysis performed on element energy emissions recorded on control and treated chasmothecia, revealed a relevant association between Ca, P and K. The function obtained by linear discriminant analysis made it possible to correctly classify treated and untreated samples considering Ca and Al energy emissions as the most significant variables. Noteworthy, two way ANOVA performed on the semi-quantitative Ca percentage estimation, showed that relative amount of Ca was higher in treated chasmothecia and increased significantly from 24 to 48 h after treatment. These results, together with the negative correlation observed between Ca and Al ($r=-0,693$) and S and P ($r=-0,612$), suggest a pH change in treated chasmothecia wall and the occurrence of an oxidative stress inside the chasmothecia which involves the Ca-dependent signal

transduction pathway. These preliminary hypotheses need confirmation through further studies.

OCCURRENCE OF DIFFERENT *CITRUS TRISTEZA VIRUS* ISOLATES IN ALEMOW SEEDLINGS AND TAROCCO ORANGES GRAFTED ON CITRANGE IN SICILY. M. Russo, G. Licciardello, D. Raspagliesi, S. Fassari, A. Bertuccio and A. Catara. *Parco Scientifico e Tecnologico della Sicilia, Zona Industrial, Blocco Palma I, Str.le Lancia 57, 95121 Catania, Italy. E-mail: mrusso@pstsicilia.it*

There is evidence that mild and severe *Citrus tristeza virus* (CTV) isolates have been introduced into Sicily in independent events and were differently spread by humans and aphids (mostly *Aphis gossypii*) but the relative rates of spreading are unknown. Capillary electrophoresis analysis of single strand conformation polymorphism (CE-SSCP) has allowed to establish that the majority are single isolates grouping in different profiles based on p23 and p27 gene analysis which generated six and three profiles, respectively. A low percentage of mixed profiles has also been detected. To investigate the role of aphid and human contribution to virus spread in a highly infected area, we analyzed 112 alemow seedlings (18 to 24-month-old) and 362 trees of commercial clones of citrange-grafted Tarocco oranges. DAS-ELISA disclosed that 28 seedlings (25%) and 58 orange trees (16%) were infected by CTV. Aliquots (5 µl) of virus eluates from single positive wells or a mixture of aliquots from different wells were processed by PCR and analyzed by CE-SSCP. In both cases two different profiles, matching with those generated by SG29 (aggressive) and Bau282 (mild) isolates, were obtained. A third, non conventional, profile and a single case of mixed infection were also observed. The results agree with previous investigation showing that: (i) SG29 and Bau282 are prevalent in Sicily; (ii) both are aphid-transmitted at similar rates; (iii) the prevalent mild isolate is not of potential value for cross protection. Results show also that DAS-ELISA/CE-SSCP sequential method allows the discrimination in a single run of CTV profiles present in a mix of multiple infected samples.

PLANT PHYTOHORMONES AS PRIMING AGENTS IN RICE. B. Sabatini, L. Bertini, S. Proietti, C. Caporale and C. Caruso. *Department of Ecological and Biological Sciences, University of Tuscia, Via San Camillo de Lellis, 01100 Viterbo, Italy. E-mail: caruso@unitus.it*

Plant immunity against invading microbes relies on the quick perception of general elicitors from pathogens. These elicitors are conserved microbe-specific molecules, also referred as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs). The recognition of different MAMPs/PAMPs by their cognate pattern recognition receptors (PRRs) at the plant cell surface and subsequent signal transduction across the plasma membrane induce plant defense responses leading to basal resistance or innate immunity. Plants can also be "primed" for more rapid and robust activation of defense to biotic or abiotic stress. "Priming" is induced by a pre-exposure to a low concentration stress factors (PAMPs, effectors, chemicals, pathogen) and makes primed cells stronger and more reactive to later stress than non-primed cells. In this work we report priming induction in rice using two plant phytohormones, salicylic acid (SA) and methyl-jasmonate (JA) as priming agents. To verify treatment effectiveness, rice plants were either infected with *Fusarium culmorum* spores following SA spraying or wounded following JA spraying. The expression level of specific marker genes of SA and JA pathways revealed the involvement of genes coding

for PRRs, signal transducers, transcription factors, Pathogenesis-Related (PR) and antioxidant proteins in the priming phenomenon. Since recent findings correlate priming to chromatin modification, epigenetic studies on promoters of the more relevant genes will be carried out in order to deepen the molecular mechanism on which priming is based.

STUDIES ON A NEW GRAPEVINE DISEASE IN TRENTINO VINEYARDS. P. Saldarelli¹, R. Beber², L. Covelli³, P. Bianchedi³, R. Credi², A. Giampetruzzi¹, U. Malossini³, C. Pirolò¹, C. Poggi Pollini², C. Ratti², F. Terlizzi² and V. Gualandri³. ¹*Istituto di Virologia Vegetale del CNR, UOS Bari and Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy.* ²*Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 42, Bologna, Italy.* ³*Fondazione Edmund Mach, Centro Trasferimento Tecnologico Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy. E-mail: p.saldarelli@ba.ivv.cnr.it*

Symptoms of stunting, chlorotic mottling and leaf deformation with reduced yields and low quality of berries, were observed on cv. Pinot gris, in Trentino vineyards since 2003. Afterwards, similar symptoms were reported from cvs Traminer, Pinot noir in Trentino and in Friuli Venezia Giulia. Analysis by high throughput sequencing led to the discovery of a new trichovirus, named *Grapevine pinot gris virus* (GPGV), closely related to *Grapevine berry inner necrosis virus* (GINV). To define the aetiology and the biological features of the disease, field, greenhouse and laboratory studies were made. Field observations on a time span of three years (2010-2012) indicated a progressive increase of diseased vines from 14.7 to 33.9% in a cv. Pinot gris vineyard whereas the incidence in a cv. Traminer vineyard was stationary and lower (ca. 3%). RT-PCR assays showed that, in these vineyards, GPGV was present in all symptomatic but also in 70% of symptomless vines. Biological indexing demonstrated that GPGV is efficiently transmitted by grafting to *Vitis riparia*, and *V. vinifera* cvs Pinot gris and Traminer inducing specific symptoms. A screening on different varieties and vineyards in the area (Val d'Adige and Vallagarina) suggests a widespread distribution of GPGV. Its presence appears closely associated with a disease condition although latent infections in symptomless vines can also occur.

DISORDERS OF OLIVE CAUSED BY ENVIRONMENTAL STRESSES. S.M. Sanzani¹, L. Schena², F. Nigro¹, V. Sergeeva³, A. Ippolito¹ and M.G. Salerno¹. ¹*Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy.* ²*Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89122 Reggio Calabria, Italy.* ³*School of Natural Sciences, University of Western Sydney, Locked Bag 1797 Penrith South, DC NSW 2751 Australia. E-mail: antonio.ippolito@uniba.it*

Olive (*Olea europaea*) is an evergreen shrub or tree native to and widely spread in the Mediterranean area, but now grown in other warm-temperate regions of the world. Olive has long been considered a crop for marginal lands, unsuitable for intensive cultivation. However, a new upsurge of interest in extra-virgin olive oil, due to the awareness of its benefit for human health, has increased olive cultivation. A number of new olive groves are planted at high and super high density, irrigated, fertilized and trained for mechanical pruning and harvesting. Very little is known on the influence of these new forms of cultivation on olive biotic and abiotic diseases. The vast knowledge on parasitic diseases may readily adapt to the

new growing systems, but not equally well to the non-parasitic diseases which have been much less investigated. Non-parasitic diseases are particularly dangerous for olive trees, since they are often poorly understood or completely unrecognized, resulting in heavy economic losses. They may be due to a lack/excess of essential nutrients or an excess of non-essential elements; an unsatisfactory environment (too cold or hot, too wet or dry, or too windy); unsuitable soil characteristics (poor physical condition, water-logging, salinity, inappropriate pH). Also environmental pollution, spray and fire damage, and climatic extremes like lightning, hail, and snow can cause heavy losses to olive production. An investigation focusing on the most important disorders caused by environmental, physical, and chemical stresses affecting the tree normal physiological processes was conducted. Interactions among plant healthiness, production, and quality were also highlighted.

EFFICACY OF JETFIVE AND KARMA, TWO NOVEL FORMULATIONS, AGAINST POSTHARVEST DECAY OF CITRUS FRUITS. S.M. Sanzani¹, F. Garganese¹, K. Youssef², M. Zingaro, M.A. Dimartino³, A. Myrta⁴ and A. Ippolito¹. ¹Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. ²Agricultural Research Center, Plant Pathology Research Institute, 9 Gamaa St., 12619 Giza, Egypt. ³Certis Europe B.V., Via J.M.E. de Balaguer 6, 21047 Saronno, Italy. ⁴Certis Europe B.V., Boulevard de la Woluwe 60, 1200 Brussels, Belgium. E-mail: simonamarianna.sanzani@uniba.it

Alternative postharvest treatments to control fruit decay have become an essential requirement due to increasing concerns for the negative impact on human and environmental health and the development of fungicide-resistant strains. The efficacy of two formulations, JetFive (5% peracetic acid and 20% hydrogen peroxide) and Karma (85% potassium bicarbonate), commercialized by Certis Europe (Italy), against citrus postharvest pathogens was evaluated. JetFive was tested at two concentrations (500 and 1000 ml/hl), whereas Karma was tested just at a 3% rate, following previous preliminary trials. Fruits of sweet orange cv. Navelate were wounded on the two opposite sides of the equatorial zone, dipped in the treatment solutions for 2 min and incubated at 6°C for 2 weeks following nine days of shelf-life. Fruits dipped in water, in a postharvest phytofortifier made up of Ca, K and P salts (Fortisol Ca PLUS, 2%, Citrosol, Spain) or Imazalil (200 ml/hl, DeccoZil50, Decco, Italy) were used as controls. JetFive at the two concentrations and Karma, after storage plus two days of shelf-life, significantly reduced disease incidence by up to 95%, as compared to water control, similarly to Imazalil and Fortisol. No significant differences were recorded among JetFive and Karma. Whereas, concerning disease severity, at two days of shelf-life JetFive (1000 ml/hl) proved to be the best treatment with a 84% reduction as compared to water control, comparable to that obtained by Imazalil. Similar results were obtained when the microbial population was evaluated. In fact, up to two days of shelf-life, all treatments significantly reduced it, particularly JetFive at the highest concentration, that performed similarly to Imazalil. The results show the potential of JetFive and Karma as alternative to fungicides for controlling citrus postharvest rots.

MORPHOLOGICAL, GENETICAL AND PATHOGENETIC CHARACTERIZATION OF COLLETOTRICHUM ACUTATUM SENSU LATO FIRST RECORDED ON CARTHAMUS TINCTORIUS IN ITALY. S. Sarrocco¹, R. Baroncelli^{1,2}, A. Zapparata¹, S. Tavarini¹, L.G. Angelini¹ and G. Vannacci¹.

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Safflower (*Carthamus tinctorius* L.) is an asteraceous plant species which is receiving increasing attention in the Mediterranean environments as new oilseed crop. *Gloeosporium carthami* (Fukui) Hori et Hemmi, first described as the causal agent of leaf blight of safflower in Japan, was transferred into the genus *Gloeosporium* in 1919, then treated as synonym of *Colletotrichum gloeosporioides* and, in 2012, it was recombined with the genus *Colletotrichum* and named *C. carthami* based on its morphology, specific pathogenicity on asteraceous plants and because it was phylogenetically related to the *Colletotrichum acutatum* species complex. During 2012, *Colletotrichum* isolates were collected in field-grown safflower crops in central Italy (Pisa countryside 43.49N;10.51E) from plants showing typical anthracnose symptoms and it was detected also on and in safflower seeds. The genetic variability of these isolates was assessed by a multilocus (four genes) sequencing approach. Phenotypic characterization was carried out by the assessment of growth rates at different temperatures, morphology of colonies on PDA and shape and size of conidia. Data were compared with those from isolates of *C. acutatum sensu lato* from safflower from different geographic areas and other *C. acutatum* isolates. No attempts have been made to clarify the confusing taxonomy of *C. carthami* and *C. crysanthemi*, therefore our isolates have been provisionally assigned to *C. acutatum sensu lato*. Pathogenicity tests were performed both on seeds and plants. Results confirmed pathogenicity of our isolates and the seed-borne transmission of the pathogen. This is the first record of this pathogen on *C. tinctorius* in Italy.

BIOSTIMULATION AND BIOCONTROL OF TRICHODERMA HARZIANUM 6776 ON TOMATO. S. Sarrocco¹, L. Fiorini¹, L. Moncini², G. Pachetti² and G. Vannacci¹. ¹Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. ²Centro Ricerche Strumenti Biotecnici nel settore Agricolo-forestal, c/o ISIS "Leopoldo II di Lorena" Cittadella dello Studente, 58100 Grosseto, Italy. E-mail: lisa.fiorini@agr.unipi.it

Many *Trichoderma* isolates have been effectively used as biocontrol agents for a wide range of economically important plant pathogens. Besides protecting plants through antagonism, species of *Trichoderma* can induce resistance by colonizing, as endophytes, the root epidermis and outer cortical cells of their hosts and can also have a biostimulating effect on plant growth. *Trichoderma harzianum* 6776 is considered a new potential beneficial isolate to be employed as active ingredient in new biofertilizers and/or biopesticides as well as in new "tailor made substrates". Preliminary biocontrol experiments showed that *T. harzianum* 6776 was able to reduce plant mortality due to *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Rhizoctonia solani* and gave promising results against *Fusarium oxysporum* f. sp. *lycopersici*. The ability to significantly stimulate industrial tomato plant development was assessed by several experiments performed under greenhouse conditions according to standard procedures for the production of plantlets for transplanting. The biostimulating effects, expressed as higher stem height and diameter, increased fresh and dry weight compared with controls were further evaluated on different tomato cultivars, included MicroTom which is considered a model cultivar, and on tomato rootstocks, showing interesting results. *T. harzianum* 6776 biomass was obtained by fermentation of organic matter derived from the food industry and was then added to the peat-based tomato growth substrate at the final concentration of 10%. Since quality

and quantity of fungal inoculum is a key factor on performance of beneficial fungi, different fermentation substrates were tested in order to improve the activity of *T. harzianum* 6776.

PHYTOPHTHORA PARVISPORA, A SERIOUS ROOT AND COLLAR ROT PATHOGEN OF ARBUTUS UNEDO. B. Scanu, B. Linaldeddu, L. Maddau and A. Franceschini. *Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Viale Italia 39, 07100 Sassari, Italy. E-mail: bscanu@uniss.it*

Arbutus unedo, family Ericaceae, is one of the main components of the Mediterranean scrub biome and a valuable ornamental plant for gardening and landscaping that is increasingly cultivated in commercial nurseries. Since 2008, a severe dieback of *A. unedo* associated with root and collar rot has been observed on potted plants in a forest nursery and on free standing plants in two locations along the north-east coast of Sardinia (insular Italy). Several isolates of *Phytophthora* were obtained from both infected tissues and rhizosphere soil samples. A multi-gene sequence analysis of two nuclear (ITS, β -tubulin) and three mitochondrial (*cox1*, *cox2*, COI) gene regions combined with morphology, growth-temperature relationships and pathogenicity, revealed that all *Phytophthora* isolates belonged to a distinct monophyletic taxon, which was subsequently described as *Phytophthora parvispora* Scanu et Denman. *P. parvispora* falls within *Phytophthora* ITS Clade 7a, closely related to *P. cinnamomi* but differing by its smaller-sized sporangia, chlamydospores, oogonia and oospores, higher oospore wall index, single-celled antheridia, higher minimum and maximum temperatures for growth and faster growth at optimum temperature. In soil infestation tests, all isolates of *P. parvispora* were as aggressive as *P. cinnamomi* to the root system of *A. unedo*. This is the first report of *P. parvispora* causing root and collar rot on *A. unedo* worldwide. It is most plausible that these findings in Sardinia are linked to the trade of plants-for-planting, which is considered the major pathway of *Phytophthora* species into ornamental, horticultural and natural environments.

MYCORRHIZA HELPER BACTERIA IN CORYLUS AVELLANA: IN VITRO INTERACTIONS WITH ROOT FUNGAL PATHOGENS. L. Scattolin¹, M. Marcolin¹ and A. Squartini². ¹Dipartimento Territorio e Sistemi Agro-forestali, Università degli Studi di Padova, Via dell'Università 16, 35020 Legnaro (PD), Italy. ²Dipartimento Biotecnologie Agrarie, Università degli Studi di Padova, Via dell'Università 16, 35020 Legnaro (PD), Italy. E-mail: linda.scattolin@unipd.it

In a wide range of terrestrial ecosystems, different symbiotic mycorrhizal associations between plants and fungi occur. Among these, the ectomycorrhizal association (ECM) is based on complex mutualistic relationships which have been shown to comprise not only plant root tips and ECM fungi, but also bacteria able to enhance the formation of mycorrhiza, so called mycorrhiza helper bacteria (MHB). Highly specific relationships exist between such bacteria and fungi, but their role in ectomycorrhization and plant health has not yet been completely understood. The aim of this study is to investigate *in vitro* the potential ability of 12 MBH, isolated from 30 common hazelnut trees (*Corylus avellana*), to prevent the development of eight common telluric pathogenic fungi in forests. The test, performed on Petri dishes, containing plate count agar medium, assessing the extent of inhibition zones of the fungus, showed different extents of antagonism exerted by the putative MHB, several of which hampered fungal growth. The potential benefits of an improved understanding of mycorrhizosphere

interactions in controlling plant diseases are evident from such experiments. However, many aspects of the mechanisms governing the symbiotal biocontrol capabilities offered by MHB are still awaiting adequate investigation.

NATURAL AND NATURAL-LIKE PHENOLIC COMPOUNDS INTERFERE WITH TRICHOHECENE BIOSYNTHESIS GENE EXPRESSION IN FUSARIUM CULMORUM. B. Scherm¹, G. Pani¹, F. Spanu¹, V. Balmas¹, P. Carta^{1,2}, D. Fabbrì², M.A. Dettori², E. Azara², G. Delogu² and Q. Migheli¹. ¹Dipartimento di Agraria Sezione di Patologia ed Entomologia Agraria and Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. ²Istituto di Chimica Biomolecolare del CNR, Traversa La Crucca 3, 07100 Sassari, Italy. E-mail: scherm@uniss.it

Fusarium culmorum is an ubiquitous soil-borne fungus able to cause foot and root rot and Fusarium head blight (FHB) on different small grain cereals, particularly wheat and barley. It causes significant yield and quality losses and results in contamination of the grain with trichothecene mycotoxins. Some secondary plant metabolites, present in larger amounts in FHB-resistant plants, were shown to inhibit fungal growth *in vitro* and/or mycotoxin production by *Fusaria*. These are phenolic and polyphenolic compounds belonging to benzoic and cinnamic acids, furanocoumarins, phenylpropanoids, chromenes and flavones. We are focusing our research on natural and natural-like phenolic molecules (phenolic acids, acetophenones, benzaldehydes, phenylpropanoids, cinnamic acids and hydroxylated biphenyls) able to inhibit trichothecene production *in vitro* without affecting fungal growth. The effect of different inhibitory compounds on the expression of key genes in the biosynthetic pathway of trichothecenes (*TRI3*, *TRI4*, *TRI5*, *TRI6*, *TRI10*), and isoprenoids (*FPP*, farnesyl-pyrophosphate synthase) is being tested by qRT-PCR in *F. culmorum* (strain ISPaVe MCf 21 wild type; syn. strain INRA 117) grown *in vitro*, under toxin-inducing conditions. Among three potential reference genes tested (*GAPDH*, *TUB*, *18S*) only the *18S* gene met qualifications of stability. All tested compounds decreased the *TRI5* gene expression to 3-60% compared to the untreated control. A significant decline of trichothecene production down to 1.6% with no effect on fungal growth was observed for most compounds over a concentration range between 0.5 and 1.0 mM. Multiple genes of the trichothecene pathway were affected, especially in the presence of strong inhibitors. Interestingly, when partial or no inhibition occurred, *TRI5* and *TRI6* expression was less affected, but the expression of *TRI10* (157-240%), *TRI3* (133-177%) and *FPP* (133-169%) was triggered. Threshold concentrations were identified, below which even the most active compounds were unable to influence the expression of genes in the trichothecene pathway and, consequently, trichothecene production by the pathogen.

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SOYBEAN SEEDLINGS AS A NEW MODEL FOR STUDYING VIRULENCE FACTORS OF FUSARIUM GRAMINEARUM. L. Sella¹, K. Gazzetti¹, C. Castiglioni¹, R. Marcato¹, M.C. Paccanaro¹, W. Schäfer² and F. Favaron¹. ¹Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. ²BioCenter Klein

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Recent evidence has shown that soybean is a new host of *Fusarium graminearum* mostly in the early phase of seedlings development. Some *F. graminearum* mutants previously characterized for virulence in wheat spike infection were tested by using a rapid virulence assay in order to establish their behavior in soybean. The disrupted mutants used were affected in DON ($\Delta tri5$), lipase ($\Delta Fgl1$) or in two MAPK signaling pathways, regulating cell wall degrading enzymes production ($\Delta gpmk1$) and osmotic balance ($\Delta FgOS-2$), respectively. The ability of these mutants to colonize soybean mirrors their behavior in wheat. In fact, the mutants that show reduced virulence ($\Delta tri5$, $\Delta Fgl1$ and $\Delta FgOS-2$) or non pathogenicity ($\Delta gpmk1$) on wheat spike are, correspondently, less virulent or non-pathogenic in soybean seedlings. However, a different ranking of symptom severity occurs in the two hosts: in wheat the $\Delta tri5$, $\Delta Fgl1$ and $\Delta FgOS-2$ mutants give similar reduction of symptom severity in comparison to the WT while in soybean the $\Delta FgOS-2$ mutant was less virulent than $\Delta tri5$ and $\Delta Fgl1$. The anatomy of the infected organs may partially explain these differences. In wheat the rachis node represents a histological barrier which stops the $\Delta tri5$ and $\Delta Fgl1$ mutants since DON and lipase are necessary to overcome this barrier. In soybean the absence of discontinuity between root and hypocotyl tissues allows the progress of infection of the $\Delta tri5$ and $\Delta Fgl1$ mutants, thus the absence of DON and lipase seem only to slow down seedling colonization. This soybean infection assay could be effectively used to rapidly screen *F. graminearum* mutants for their virulence, possibly anticipating results of wheat infection.

BROWN ROT OUTBREAK IN CHESTNUT NUTS CAUSED BY GNOMONIOPSIS sp. IN SOUTHERN ITALY. L. Sigillo¹, D. Zito², V. Senape¹, C.M. Oliveira Longa³, R. Bugiani⁴ and G. Maresi⁵. ¹Consiglio di Ricerca e Sperimentazione in Agricoltura, Centro per la Sperimentazione e Certificazione delle Sementi, SS18 km 77.700, 84091 Battipaglia (SA), Italy. ²Associazione Castanicoltori Campani, Via Bosco Faiano 3, Torre Le Nocelle (SA), Italy. ³Fondazione Edmund Mach, Centro di Ricerca e Innovazione, Dipartimento di Agroecosistemi Sostenibili e Biorisorse, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy. ⁴Servizio Fitosanitario, Regione Emilia-Romagna, Via Corticella 133, 40054 Bologna, Italy. ⁵Fondazione Edmund Mach, Centro Trasferimento Tecnologico, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy. E-mail: l.sigillo@ense.it

"Brown rot" of chestnut nuts causes browning and mummification of kernels. Its causal agent was previously identified as *Phoma endogena* or as *Phomopsis endogena*, but recently the same symptoms were correlated with the presence of *Gnomoniopsis smithogilvyi* in Australia and *Gnomoniopsis castanea* in Piedmont (northern Italy), which are homologous to *Gnomoniopsis* sp. isolated from *Dryocosmus kuriphilus* galls. In 2012 a brown rot outbreak was observed on cv. Marrone di Roccadaspide in Salerno province (Campania, southern Italy), where a survey conducted in 10 orchards recorded up to 50% of yield loss. Fruits showed typical disease symptoms and light brown mycelium colonies with orange-coloured conidial masses were isolated on potato dextrose agar from symptomatic fruits, healthy branches and old wasp galls. Molecular identification of four isolates was carried out by amplification of the ITS1, 5.8S and ITS2 regions. A GenBank BLAST search with ITS sequences showed that *G. smithogilvyi* (accession No. KC145863.1) is the closest match, with a 99-100% homology with isolates previously obtained in Italy and Australia. *Gnomoniopsis* sp. and *D. kuriphilus* infections seemed correlated but disease detection on chestnut in Australia, where the insect is not present, suggests that the fungus might live as endophyte, whereas stress factors could be responsible

for damage appearance. So far, no effective disease control methods are known: only copper compounds and tebuconazole are authorized on chestnut in Italy. Since the level of the observed damages cause great concern in Campania, where chestnut has high economic and cultural values, more epidemiological investigations of this emerging pathogen along with effective agronomic practices to reduce its potential in chestnut orchards are needed.

A NOVEL NEPOVIRUS FROM AEONIUM sp. R. Sorrentino¹, A. De Stradis², M. Russo², D. Alioto¹ and L. Rubino². ¹Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. ²Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: l.rubino@ba.ivi.cnr.it

A virus was isolated from potted plants of an unidentified species of *Aeonium* (Crassulaceae), a succulent ornamental very common in Southern Italy, showing faint chlorotic spots and rings on both leaf surfaces. The virus was successfully transmitted by sap inoculation to a limited range of hosts, and propagated in *Nicotiana benthamiana* which was used for ultrastructural observations and virus purification. Virus particles are isometric, ca. 30 nm in diameter, have a single type of coat protein (CP) subunits 54 kDa in size, that encapsidate single-stranded positive-sense RNA species of 7,549 (RNA1) and 4,010 (RNA2) nucleotides. A third RNA molecule 3,472 nts in size entirely derived from RNA2 was also detected in infected *Aeonium* plants. The structural organization of both genomic RNAs and the cytopathological features were comparable to those of nepoviruses. In addition, amino acid sequence comparisons of CP and the Pro-Pol region (a sequence containing parts of the proteinase and polymerase) with those of other nepoviruses showed that the *Aeonium* virus belongs to the subgroup A of the genus *Nepovirus* and is phylogenetically close to *Tobacco ringspot virus* (TRSV). Comparison of each single domain of TRSV and the *Aeonium* virus polyproteins disclosed a relatively low percentage of identity throughout. Moreover, the *Aeonium* virus showed to be serologically distinct from TRSV. Based on the species demarcation criteria for the family *Secoviridae*, the virus under study appears to be a novel member of the genus *Nepovirus* for which the name of *Aeonium ringspot virus* (AeRSV) is proposed.

NEW MICROBIAL ANTAGONISTS AND THERMOTHERAPY AS SEED TREATMENTS AGAINST FUSARIUM FUJIKUROI ON RICE. D. Spadaro^{1,2}, S. Matic^{1,2}, M.L. Gullino^{1,2} and A. Garibaldi¹. ¹AGROINNOVA, Centre for Agro-Environmental Innovation, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. ²Department of Agricultural, Forestry and Food Sciences, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it

Sixty-two isolates of yeasts and yeast-like fungi were obtained from the rice seeds. Four of the yeast isolates were selected as the most efficient against *Fusarium fujikuroi*, causal agent of the bakanae disease. Isolates R23 and R26 were identified as *Metschnikowia pulcherrima*, the isolate R9 as *Pichia guilliermondii*, and the isolate SB1 as *Sporidiobolus pararoseus* using morphological and molecular parameters. Rice seeds, treated with *P. guilliermondii* R9, *M. pulcherrima* R23 and R26, had a significantly lower infection rate by *F. fujikuroi*. Four selected yeasts suppressed *F. fujikuroi* infection in rice plants *in vivo* under greenhouse conditions. Immersion of rice seeds in the yeast cell suspension (1×10^8 /ml) resulted in reduction of the infection from 93% in the non-treated control to 20% in *P. guilliermondii* R9 treatment, and to 28.5% in *M. pulcherrima* R23 treatment. Four selected antagonists were also used in combination

with thermotherapy which increased their biocontrol efficacy. Thus, *P. guilliermondii* R9 and *M. pulcherrima* R23 combined with thermotherapy at 60°C for 10 min decreased the bakanae disease index below 5%, and ameliorated the germination rate compared to the single treatment. This study presents the first report of use of *M. pulcherrima*, *P. guilliermondii* and *S. parvoseus* on rice seeds, either singly or combined with hot water treatment, for the control of *F. fujikuroi*.

A NEW METHOD FOR AN EARLY DETECTION OF BROWN ROT ON PEACH FRUIT: DA METER TEST. A. Spadoni, I. Cameldi, E. Bonora, M. Noferini and M. Mari. *Dipartimento di Scienze Agrarie, Università degli Studi, Bologna, Viale Fanin 42, 40127 Bologna, Italy. E-mail: alice.spadoni3@unibo.it*

Brown rot is the most important disease for peach fruit in post-harvest. In order to provide a 'brown rot susceptibility index' for peach, experiments were carried out using a hand-held nondestructive instrument, the DA Meter (Sinteleia, Bologna, Italy). The instrument measures the chlorophyll content and gives an index (index of absorbance difference - I_{AD} -) related to fruit ethylene production. In order to demonstrate a different brown rot susceptibility level between peaches belonging two different I_{AD} index, cvs Springbelle and Redhaven were previously classified by DA meter in two classes: high I_{AD} value (H) and low I_{AD} value (L). Firmness, acidity, soluble solid content and ethylene production were analyzed at harvest and after three and seven days of storage at 20°C for each class. H and L classes were divided in two equal lots one of which was evaluated for the percentage of *Monilinia* natural infections after seven days at 20°C. Fruit of the second lot were wounded and artificially inoculated with 20 µl of a *Monilinia fructicola* spore suspension (5×10² spores/ml) and stored at 20°C. After three days the lesion diameter of infected fruit was recorded. Naturally infected fruits of H I_{AD} class (less ripe) showed a significant brown rot reduction (80%) with respect to L I_{AD} class (more ripe). The same behavior was observed in fruit artificially infected, with a 26% of lesion diameter reduction. According to our results, the I_{AD} appears be an useful tool for screening peach fruits susceptibility to *Monilinia* rots.

A NOVEL FUSARIUM CULMORUM GENE INVOLVED IN FOOT AND ROOT ROT ON DURUM WHEAT IDENTIFIED BY TRANSPOSON-MEDIATED INSERTIONAL MUTAGENESIS. F. Spanu^{1*}, I. Camboni^{1*}, B. Scherm¹, V. Balmas¹, M. Pasquali² and Q. Migheli¹. *¹Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia and Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. ²CRP - Gabriel Lippmann, 41 rue du Brill, 4422 Belvaux, Luxembourg. E-mail: qmigheli@uniss.it*

Fusarium culmorum is a major fungal pathogen of wheat, causing foot and root rot (FRR) and fusarium head blight (FHB). FRR symptoms include pre- and post-emergence death of seedlings, brown discoloration on coleoptiles, roots and pseudostem, brown lesions on the basal portion of the stem, tiller abortion and formation of whiteheads, resulting from premature death of the plant. The genome of *F. culmorum* is being sequenced but for many genes the function is yet unknown. Therefore, the characterisation and identification of pathogenicity-related genes is essential in the build-up of alternative control methods against this disease. Here we report on the application of a transposon tagging approach with the *mimp1/impala* double component system to select mutants of *F. culmorum* altered in their metabolic or morphological processes and

impaired in their aggressiveness during the first step of interaction between this fungus with the host plant. *In vitro* bioassays were carried out to identify altered phenotypic characters of revertants growing on potato dextrose agar (PDA) amended with 2 M sorbitol, 1 M NaCl (osmotic stress), 30 mM potassium persulphate (oxidative stress) and 0.02% sodium dodecylsulphate (SDS). To test thermal stress resistance of revertants, radial colony growth was tested on PDA at 37°C and 8°C. An *in vitro* pathogenicity test was performed by placing durum wheat seeds on 10 mycelium plugs in a Petri dish and incubating for three days in the dark at 25°C. To confirm the result obtained *in vitro*, *in planta* assays were performed in greenhouse conditions. Several *F. culmorum* mutants were obtained with altered phenotypic characters, including stunted vegetative growth and loss of pathogenicity towards wheat stem base/root tissue. Cloning of sequences flanking the *mimp1* element obtained by splinkerette PCR in one of the selected revertants (R38) allowed to identify an hypothetical gene with orthologs only in the fungal domain. The first *mimp1*-tagged gene involved in FRR pathogenicity adds to the few other genes known to play a role in this disease. Given that this gene contains a leucine zipper domain, it may likely have a regulatory role that will be further investigated by RNA seq.

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HYDROGEN CYANIDE SYNTHESIS AND ANTAGONISTIC ACTIVITY OF TWO PSEUDOMONAS STRAINS. C.P. Strano¹, R. Zago¹, P. Bella¹, G. Licciardello² and V. Catara¹. *¹Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli Studi, Via Santa Sofia 100, 95131 Catania, Italy. ²Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Blocco Palma I, Str. le Lancia 57, 95121 Catania, Italy. E-mail: vcatara@unict.it*

Bacterial strains of the genus *Pseudomonas* are capable of suppressing a range of plant diseases due to their ability to biosynthesize antimicrobial metabolites. Antibiotics, cyclic lipopeptides (CLPs) with antimicrobial activity, siderophores and hydrogen cyanide are the main secondary metabolites to which the biological control is attributed. Using two strains of *P. corrugata* and *P. mediterranea* impaired in CLP production we observed that their antagonistic activity was also the result of the production of other substances such as diffusible siderophores, an additional unknown antimicrobial substance and volatile compounds (VOCs). Putative genes encoding hydrogen cyanide (HCN) synthase have been found during genome annotation of *P. corrugata* CFBP 5454. HCN is a VOC produced by many antagonistic *Pseudomonas* species and it was shown that three contiguous structural genes, *hcnABC* operon, encoding together a membrane-bound HCN synthase complex, are sufficient for cyanogenesis. Sequence analysis revealed that the genetic organization of this locus is high similar to other *Pseudomonas* HCN synthase cluster. The putative *pcobcnA* and *pcobcnC* genes were insertionally inactivated by integration of the suicide vector pKNOCK-Km into *P. corrugata* CFBP 5454. Genomic mutants were characterized and the role of this compound in biocontrol activity was investigated. Test paper (Cyantemo) for the detection of HCN production showed that in *pcobcnA* and *pcobcnC* mutants metabolite production in completely abolished. *In vitro* experiments against the phytopathogenic fungus *Botrytis cinerea* also showed that HCN production is mainly involved in conidia germination inhibition.

ANALYSIS OF BACTERIAL CELLS INTO THE SAP OF *ACTINIDIA* spp. PLANTS AFFECTED OR NOT BY *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE*. V. Tagliavento, L. Gallipoli, A. Mazzaglia and G.M. Balestra. *Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia, Università degli Studi della Tuscia Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: balestra@unitus.it*

Kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is a global and fatal threat to the cultivation of *Actinidia* spp. worldwide. It has been demonstrated in a recent past that this bacterium is able to colonize and move into the vascular system of kiwifruit plants (Renzi *et al.*, 2012, *Phytopathology* 102: 827-840). As a matter of facts, once the pathogen penetrates the host surface through both natural or artificial openings, it shows a typical endophytic behaviour. It seems furthermore able to invade both the host xylem and phloem. This research was aimed at analyzing both qualitatively and quantitatively the whole microbial populations retrievable from the sap of symptomatic and asymptomatic plants of *A. deliciosa* cv. Hayward and *A. chinensis* cv. Jintao. Samples of sap were collected monthly from pre-selected plants and plated on different media in order to obtain a complete analyses of the bacterial charge in the sap along the different seasons. All the strains isolated during repeated samplings were firstly characterized physiologically, identified by 16S ribosomal typing and, those different from *Psa*, were also tested for their antagonistic ability with respect to known *Psa* strains. Moreover, they were characterized also for their potential ability to produce fluorescent pigments as well as biofilm. The experimental data are still under evaluation. However, preliminary results suggest the presence of few recurring bacterial genotypes with some difference between diseased and healthy plants.

THE EPIPHYTIC LIFE OF *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* ON KIWIFRUIT AND OTHER CULTIVATED AND SPONTANEOUS PLANTS. R. Tontou, D. Giovanardi, C. Facchini and E. Stefani. *Dipartimento di Scienze Agrarie e degli Alimenti, Via Amendola 2, Pad. Besta, 42122 Reggio Emilia, Italy. E-mail: emilio.stefani@unimore.it*

Bacterial canker of kiwifruit is the most destructive disease of cultivated *Actinidia* spp. The causal agent is the Gram-negative bacterium *Pseudomonas syringae* pv. *actinidiae* (*Psa*). Several surveys have been carried out in kiwi orchards in Romagna (northern Italy) and proved that *Psa* may have a significant epiphytic life, mainly on flowers, pollen and leaves. On the fruits of affected orchards *Psa* was detectable until early August. In Romagna, kiwifruit is cultivated in areas where also other fruit crops are grown, often side by side. A two-year survey confirmed that *Psa* is present as a leaf epiphyte in neighbouring stone fruit orchards (apricot and plum), when an affected kiwi orchard is nearby. Several other spontaneous plants and weeds present in kiwifruit orchards have been checked for the epiphytic presence of *Psa*. Stinging nettle was constantly associated with the epiphytic presence of *Psa*, thus providing good conditions for bacterial growth and survival. *Psa* was also detected on, or isolated from other weeds, like amaranth, common mallow, purslane, dandelion and smartweed. The time range during which *Psa* was detected/isolated on other plants was the same as *Psa* could be traced on kiwi plants. These results suggest that *Psa*, like other pathogenic and non pathogenic pseudomonads, might spend a significant lapse of time on non-host plants. The role in the epidemiology of the bacterial canker of kiwifruit of such epiphytic population is not known. As a good practice for kiwifruit orchards, control of weeds, especially in case of *Psa* presence, is particularly advised.

GENETIC STRUCTURE ANALYSIS OF ITALIAN *FUSARIUM FUJIKUROI* POPULATIONS BY SSR MARKERS AND SCREENING FOR DISEASE RESISTANCE. M.T. Valente¹, F. Desiderio², A. Infantino¹ and M. Aragona¹. ¹*Consiglio per la Ricerca e Sperimentazione in Agricoltura, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy.* ²*Consiglio per la Ricerca e Sperimentazione in Agricoltura, Centro di Ricerca per la Genomica e la Post-genomica Animale e Vegetale, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy. E-mail: maria.aragona@entecra.it*

The heterothallic ascomycete *Fusarium fujikuroi* Nirenberg, (teleomorph: *Gibberella fujikuroi* Sawada), is the causal agent of Bakanae of rice, a disease that in the last years has become of increasing economical concern, in many Italian rice-growing areas. This research is the first study of the genetic structure of Italian *F. fujikuroi* populations. Within the framework of the Project RISINNOVA, a collection of 331 isolates of *F. fujikuroi* coming from seven Italian rice-growing areas has been analysed for mating type frequency and for genetic variation in 16 novel specific polymorphic simple sequence repeats (SSR) loci. The estimated average number of alleles was 5.25 for each locus. In total 110 different haplotypes were detected with 85 private haplotypes distributed in all populations. The total Nei's genetic diversity was 0.5. The clonal fraction ranged from 17% to 62% among populations and the four most frequently haplotypes represented 40% of the collected isolates, suggesting that clonal reproduction plays an important role. Nevertheless, the potential of sexual recombination was confirmed by the balanced mating idiomorph ratio in five of seven populations analysed and by high values of genotyping diversity ($H_e = 0.92$, by stepwise model). The AMOVA revealed a small (5%) level of genetic differentiation among populations while the remaining 95% of genetic variation occurred within populations. All the populations were characterized by a small frequency of private alleles, suggesting that the fungus could have a good adaptation fitness due to its genetic diversity and its sexual recombination potential. Preliminary results of the screening of a collection of Italian rice germplasm for the identification of Bakanae-resistant genotypes are reported.

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PEROXYNITRITE PRODUCED DURING THE HYPERSENSITIVE RESPONSE COULD QUESTION THE FUNCTIONAL REDUNDANCY OF ATMKK4 AND ATMKK5 VIA SELECTIVE NITRATION. E. Vandelle, B. Sottocornola and M. Delle-donne. *Dipartimento di Biotecnologie, Università degli Studi, Strada Le Grazie 15, 37134 Verona, Italy. Email: massimo.delledonne@univr.it*

The hypersensitive response (HR) triggered by an avirulent pathogen in resistant plants is characterized by the simultaneous production of nitric oxide (NO) and reactive oxygen species (ROS), both involved in the onset of cell death. Among other things, NO can react with O_2^- in a diffusion-limited reaction to produce peroxynitrite, the increase of which has been recently shown in Arabidopsis plants challenged with an avirulent pathogen (Gaupels *et al.*, 2011, *Nitric Oxide* 25: 222-228) with a timing that correlates with an increase in tyrosine-nitrated proteins (Cecconi *et al.*, 2009, *Electrophoresis* 30: 2460-2468). In plants, peroxynitrite is not responsible for NO-mediated cell death as observed in animals and till now its physiological function is poorly understood. However, it is emerging as a potential signaling molecule during the induction of defense responses against pathogens. In order to decipher the role of

peroxynitrite during the HR, we attempted to identify specific targets of nitration displaying signaling functions during this process. In particular we focused our interest on MAPK cascades, a complex network of phosphorylation events involved in plant defense responses and known to be regulated by nitration in animals. We identified AtMKK4 as specifically nitrated by peroxynitrite, leading to an inhibition of its activity *in vitro* as well as *in vivo*. Interestingly, despite 78% of sequence homology with AtMKK4, AtMKK5 is not nitrated by peroxynitrite and its activity is not modulated by such a treatment. Therefore peroxynitrite produced during the HR could block selectively the activity of AtMKK4, raising the question of AtMKK4 and AtMKK5 function redundancy in mediating defense signals in plants.

LACCASE IN *TRICHODERMA VIRENS*: ENZYMATIC ACTIVITY AND GENE EXPRESSION IN RESPONSE TO DIFFERENT SUBSTRATES. M. Vergara^{1,2}, C. Fattorini¹, L. Guglielminetti¹ and G. Vannacci¹. ¹Dipartimento di Scienze Agrarie, Alimentari e Agro-Ambientali, Università degli Studi, Via del Borghetto 80, Pisa, Italy. ²Scuola Normale Superiore, Pisa, Italy. E-mail: rvergara@agr.unipi.it

Laccases, the most extensively studied among ligninolytic enzymes, are phenol-oxidase metalloenzymes, widely distributed in nature. Thanks to their highly unspecific oxidation ability on many diverse substrates, laccases are useful biocatalysts for several biotechnological applications. In fungi they are engaged in several biological processes such as lignin degradation, development associated pigmentation (melanin synthesis in appressorium), detoxification and pathogenesis. Laccase activity in some *Trichoderma* spp. is also related to the production of the green pigment in conidial spores. Six laccase genes were previously identified in *Trichoderma virens*, and one of them was deleted and proved to be involved in the mycoparasitic action against *Botrytis cinerea* sclerotia. Laccase enzymatic activity has been recorded on conidial suspensions by growing *T. virens* at different times on a specific substrate (solid Hölker medium), especially formulated to induce sporulation. In the same experimental scheme, the addition of several phenolic compounds to the growth medium has been checked in order to ascribe a possible substrate specificity to *T. virens* laccase. Different levels of enzymatic activity were displayed in response to specific substrates. The same analysis was performed by growing on equivalent media the *T. virens* transformant, in which one laccase gene was deleted, and the activities recorded were different from the wild type. On the other side the expression of the six *T. virens* laccase genes in response to those substrates is in progress, both on wild type and knockout mutant, in order to detect an association between single genes and substrate specificity.

METABOLITES PRODUCED BY *GNOMONIOPSIS CASTANEA* ASSOCIATED WITH NECROSIS OF CHESTNUT GALLS. F. Vinale¹, M. Ruocco¹, U. Bernardo¹, E. Guerrieri¹, M. Giorgini¹, P. Cascone¹, R. Marra^{1,2}, S. Lanzuise^{1,2}, S. Woo^{1,2}, N. Lombardi^{1,2}, G. Manganiello¹, R. Varlese¹, A. Pascale^{1,2}, P. Mazzei³, A. Piccolo³, S. Caira⁴ and M. Lorito^{1,2}. ¹Istituto per la Protezione delle Piante del CNR, UOS Portici 80055 Portici (NA), Italy. ²Dipartimento di Agraria, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. ³Centro Interdipartimentale di Spettroscopia di Risonanza Magnetica Nucleare, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. ⁴Istituto di Scienze dell'Alimentazione del CNR, Avellino, Italy. E-mail: f.vinale@ipp.cnr.it

Many cultivated varieties of chestnut (*Castanea* spp.) are subjected to severe infestations caused by the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera:Cynipidae), which can disrupt the fruiting process and reduce a tree's yield by up to 70%. In addition to pest infestation, necrotic symptoms on leaves and galls are often observed due to the development of a gall colonizer identified as the fungus *Gnomoniopsis* sp. The new species *Gnomoniopsis castanea* has been recently described, based on the association with *Castanea sativa*, the morphology and phylogenetic analysis of the internal transcribed spacer (ITS) region of ribosomal DNA and the *EF1- α* locus. The fungus has been associated with nut rot and caused disease symptoms when artificially inoculated on fruits or flowers. Here we report the isolation and characterization of two novel secondary metabolites produced in liquid culture by a *Gnomoniopsis* strain isolated in Campania (southern Italy) from chestnut galls infested with *D. kuriphilus*. Sequence analysis of the fungal ITS rDNA indicated a 99% similarity with GenBank sequences of *G. castanea*. The isolated compounds were purified from the crude ethyl acetate extract of the culture filtrate (while absent in the mycelium extract), by using silica gel chromatography and preparative HPLC-DAD. The compounds, named Gnomonicine A and B, were characterized by spectroscopic methods, including UV, LC-MS/MS, and 1D and 2D NMR analyses, and showed an indole derivative structure. The roles that *G. castanea* secondary metabolites may have in the development of nut brown rot, and their involvement in the chestnut gall wasp infestation, are being investigated.

CONTROL OF *ALTERNARIA* BLIGHT DISEASE ON TOMATO BY *BACILLUS* STRAINS ISOLATED FROM SOLANACEOUS PHYLOPLANE. M. Zaccardelli and C. Pane. *Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Orticoltura, Via dei Cavalleggeri 25, 84098 Pontecagnano (SA), Italy.* E-mail: massimo.zaccardelli@entecra.it

The use of living natural enemies of pathogens to replace chemical fungicides in conventional agriculture is a good eco-compatible method. Some spore-forming bacteria strains isolated from the tomato, eggplant and pepper phylloplane, were selected for their ability to control early blight disease caused by *Alternaria* spp. *In vitro* and *in vivo* activity and M13-PCR DNA-fingerprinting led to the selection of five new *Bacillus* spp. strains useful as biological control agents, from a wide collection of 93 candidates. All isolates were Gram-positive and physiologically characterized for production of endospore, fungistatic volatile and siderophore-like compounds. The potential antagonists were identified by 16S-rDNA gene sequence analysis, and results assigned them to *Bacillus thuringiensis* group-related strains. The evident inhibition zone seen in dual culture plates suggested antibiosis-like antagonisms as the main mechanisms used by these bacterial isolates in interaction with the pathogens. Moreover, light microscopy revealed *Bacillus*-induced malformations on hyphae that showed excessive vacuoles, swelling of cells and morphological changes of the spores. By *in vivo* tests, spore-forming bacteria provided substantial biocontrol of *Alternaria* disease on tomato. In particular, five strains proved to be very effective. *Bacillus* strains isolated from solanaceous phylloplane are promising biocontrol agents that have potential for practical application as biofungicides.

HOW ENVIRONMENTAL STRESSES AFFECT *ASPERGILLUS FLAVUS* METABOLISM. M. Zaccaria, A.A. Fabbri, C. Fanelli and M. Reverberi. *Department of Environmental Biology,*

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Aspergillus flavus is a saprophytic fungus responsible for worldwide spreading of harvest and post-harvest infections to crops, mainly cereal grains and legumes. *A. flavus* is one of the main producers of aflatoxins, the most dangerous carcinogenic metabolites in nature. In order to effectively address the economic and sanitary consequences of *A. flavus* contamination, a detailed and extensive knowledge of the pathogen metabolism, and of the environmental conditions that trigger the different biological processes, is of paramount importance. We intended to investigate the biological processes of the synthesizing fungus that lead to an increase in toxin production and conidiogenesis, the environmental effects that trigger such processes and, in particular, the specific stresses that underlie such a response. In our work, we analyzed the biological response due to four different induced stresses: carbon starvation, hypoxia, pH, and reactive oxygen species. We evaluated the expression of different molecular markers in order to pinpoint the stimuli that the four different conditions induce, and analyzed the outcomes on a phenotypic level. The parameters taken into consideration were: mycelial growth, conidiogenesis, aflatoxin synthesis; intracellular reactive oxygen species (superoxide anion, peroxynitrite, hydrogen peroxide); synthesis of catalase, superoxide dismutase, glutathione peroxidase; genetic expression. The genetic markers investigated are sequences underlying major metabolic processes: cellular respiration (aconitase, succinate dehydrogenase), pentose phosphate pathway (glucose-6-phosphate dehydrogenase, transaldolase, trehalose-6-phosphate-synthase). Moreover, we believe C6 transcription factor to be an hypoxia induced factor akin to SREBPs of mammals and yeast. Such gene was also part of our analysis.

MALDI-TOF MS ANALYSIS OF TOMATO PLANTS TREATED WITH ACYBENZOLAR-S-METHYL E. Zingariello¹, M. Larocca¹, A. Fanigliulo², R. Rossano¹ and A. Crescenzi¹. ¹Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano Campus Macchia Romana, 85100 Potenza (PZ), Italy. ²Bioagritest, Centro Interregionale di Diagnosi Vegetale. Zona PIP lotto E2., 85010 Pignola (PZ), Italy. E-mail: aniello.crescenzi@unibas.it

Acybenzolar-S-methyl (ASM) is a synthetic inducer and a functional analogue of salicylic acid, involved in systemic acquired resistance (SAR). In our study, we assessed the expression of proteins, in *Solanum lycopersicum* leaves treated with ASM. Tomato leaves were ground into a fine powder with liquid nitrogen and stored at -70°C. For protein extraction, the powder was washed twice with ethanol and deionized water, after centrifugation the final pellets were dried in speed-vac and dissolved in 1% (w/v) SDS, 20% (v/v) glycerol, 40 mM DTT, 50 mM Tris-HCl, pH 7.5. Protein content was determined according to the method of Bradford. Aliquots of extracts from untreated and ASM-treated samples were precipitated with chloroform/methanol and the protein pellets were dried and analyzed by 2-D electrophoresis. IEF (Isoelectrofocusing) steps were performed on IPG dry-strips of 13 cm in non-linear pH gradient of 3-11 (GE-Healthcare), proteins were separated in the 2nd dimension (SDS-PAGE) in a 13% (w/v) polyacrylamide gel. Gels were coomassie stained and analyzed by the ImageMaster 2D Elite software (Amersham Biosciences). Three gels were produced for untreated and ASM-treated samples, respectively. Protein spots present on at least two gels were used for comparison of the obtained spots. Image analysis of 2-DE gels revealed that 116±8 and 124±11 spots were extracted from control and ASM-treated samples, respectively. Proteins were considered differentially expressed when spot intensities (normalized volume) varied of at least

a two-fold differences (ANOVA; $P < 0.05$) in up or down-regulation respect to the untreated sample (control). Protein spots that significantly changed in response to the ASM-treatment with respect to the control, were successfully identified by MALDI-ToF analysis. Twentyeight spots identified as corresponding to 17 different proteins were classified in three groups. on the basis of their function, Of these, the majority (18 spots) were involved in defense mechanisms/stress responses, seven spots in photosynthetic metabolism and three spots as proteins associated to energy metabolism.