CHARACTERIZATION OF A PR-10 PROTEIN FROM *VITIS RIPARIA*. **M. Alessandrini, A. Lovato, L. Bortesi, G. Zoccatelli, S. Zenoni, M. Pezzotti and A. Polverari.** *Dipartimento di Biotecnologie, Università degli Studi, Strada le Grazie 15, 37134 Verona, Italy. E-mail: annalisa.polverari@univr.it*

We have started a project aimed at characterizing the expression profile and protein activity of pathogenesis-related proteins of the PR-10 family in grapevine. According to a previous microarray analysis, the expression of several genes, annotated as similar to PR-10 proteins, is strongly increased in resistant *Vitis riparia* and, to a lesser extent, in *V. vinifera,* following infection with *Plasmopara viticola*. PR-10 proteins are induced by pathogen infection and abiotic stresses in a number of species; because they often show ribonucleolytic activity it has been suggested that they may be involved in antiviral defence; their antimicrobial activity has been tested with controversial results. The coding sequence of a homologous gene, denoted *Vr-PR10*, has been cloned from the resistant species *V. riparia* and the corresponding protein expressed in *E. coli*. The purified recombinant protein showed ribonucleolytic activity *in vitro* and was used to raise antibodies which recognized the native protein from leaf tissue in Western blot analyses. The protein was tested *in vitro* and *in vivo* against *P. viticola, Botrytis cinerea* and *Grapevine virus A* (GVA), without showing any antimicrobial or antiviral activity. The possibility that PR-10 proteins have different or indirect roles in defense should be considered in further investigations.

DISTRIBUTION OF *BOTRYOSPHAERIACEAE* SPECIES ASSOCIATED WITH GRAPEVINE DECLINE IN WESTERN SICILY. **A. Alfonzo, C. Conigliaro, V. Mondello and S. Burruano.** *Dipartimento DEMETRA, Università degli Studi, Viale delle Scienze 4, 90128 Palermo, Italy. E-mail: santella@unipa.it*

Recently, the pathogenicity of different Botryosphaeraceae species on grapevines has been reported from many European and extra-European areas. During summer 2008, in a Sicilian vineyard located in the Marsala area (TP), we observed a decline induced by *Lasiodiplodia theobromae*. The occurrence and quite rapid spread of the disease seemed to support the regional variability of the species in question, which is retained a tropical and subtropical pathogen to different trees. In the last two years, the decline syndrome has been observed in the following other grape-growing areas of western and central Sicily: Alcamo and Salemi (TP), Montevago (AG) and Milena (CL). From declining plants showing typical external and internal symptoms of spurs, canes and trunks, fungal isolates were obtained in pure and/or mixed culture and were identified, by morphological and molecular analysis, as *L. theobromae*, *Diplodia seriata* and *Neofusicoccum luteum*. These results, even if preliminary, show the rapid spread of the grapevine decline by Botryosphaeraceae species in Sicily confirming, at the same time, their different distribution in relation to environmental conditions of the surveyed areas. Pathogenicity tests are in progress to ascertain the role of these fungi in the evoking of the decline syndrome.

EVALUATION OF THE ANTIFUNGAL ACTIVITY OF MORPHOLOGICALLY DISTINCT COLONIES OF *BACILLUS SUBTILIS* STRAIN ET-1. **A. Ambrico and M. Trupo**. *Unità Tecnica Tecnologie Trisaia, Laboratorio Biotecnologie, C.R. ENEA Trisaia, ss106 Jonica km 419.5, 75026 Rotondella (MT), Italy. E-mail: alfredo.ambrico@enea.it*

Bacillus subtilis differentiates into distinct adaptive subpopulations of specialized cells that coexist within highly structured communities. Here we report how phenotypic dissociations in *B. subtilis* can induce a significant reduction of the secondary metabolites which are associated with antifungal activity. Strain ET-1 initially has a rough, dense, colony morphology phenotype (R-form) with undulated edges, which dissociated into a S-form (smooth, soft, whitish) and into a M-form (slimy, translucent, amoeba-shaped colony). Correlations between colony forms, spore formation and *in-vitro* antifungal activity of cell-free supernatants were studied. Germinal inhibition over 95% of *Penicillium digitatum* conidia was induced only by S and R forms when cell-free supernatants were diluted 1:16 and 1:128 respectively. Biosynthesis of antifungal molecules in broth was significantly reduced when an old R-form colony was used for inoculation because it was already dissociated in M and S phenotypic cells. After 48 h of growth in liquid medium a comparison of the spore-forming colony and an examination by phase-contrast microscopy revealed a reduced ability of the dissociant M-form to sporulate (10^4 CFU/ml). A complete spore release from the vegetative cells was observed only for the R-form (10^9 CFU/ml), whereas the S-form contained a mixture of endospores (10^8 CFU/ml) and few vegetative cells. Different levels of *Podosphaera fusca* control on melon by the three form colonies were obtained in *in-vivo* trials. To reduce secondary metabolite production problems, the use, as starter, of a pasteurized spore suspension by young isolated R-form colony of the ET-1 strain is recommended.

***AGROBACTERIUM*-MEDIATED TRANSFORMATION OF *PYRENOCHAETA LYCOPERSICI* FOR INVESTIGATING MOLECULAR DETERMINANTS OF VIRULENCE.** **M. Aragona, M.T. Valente and A. Infantino.** *CRA-PAV, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: maria.aragona@entecra.it*

Pyrenochaeta lycopersici is a soil-borne fungus causing an economically important disease known as corky root rot on tomato and other hosts. A gene coding for an extracellular endo-1,4-β-glucanase has recently been cloned and, in order to explore its putative role in virulence, an ATMT (*Agrobacterium tumefaciens*-mediated transformation) system was established. *P. lycopersici* does not produce conidia in culture so the transformation method currently available for this species involves protoplast transformation in presence of PEG. Despite of the low efficiency, a GUS-expressing transformant has been obtained and used for investigating the timing of infection and colonization of *P. lycopersici* inside tomato roots. To increase the transformation efficiency and to obtain a larger number of transformants for gene replacement experiments, we tested both mycelium and protoplasts as recipients for an ATMT-based method. Preliminary results showed that only the protoplasts but not the mycelium were successfully transformed by using the pRF-HU2 vector, harbouring promoter and terminator genes from *Aspergillus nidulans* and hygromycin coding gene as fungal marker. The average transformation frequency obtained with the first experiments was up to 30 transformants per 10^7 protoplasts, all showing the DNA vector insertion. We are going to test the applicability of this technique for targeted gene replacement in *P. lycopersici*, after cloning the endo-1,4-β-glucanase sequences into the pRF-HU2 vector.

MONITORING FACTORS AFFECTING EPIDEMIOLOGY OF ALTERNARIA BROWN SPOT OF CITRUS. **P. Bella[1], M. Russo[2], M. Tomasello[2], V. Catara[1] and A. Catara[2].** *[1]Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli*

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Alternaria brown spot of citrus, caused by *Alternaria alternata,* causes considerable damage mainly to hybrids of Dancy tangerine. The fungus induces brown to black spots on leaves, fruit and young twigs resulting in reduced fruit yield and quality. Following the studies carried out primarily in Florida, Brazil, Israel and Spain, disease epidemiology observations were performed in an orchard of cv. Fortune mandarin grafted onto Citrange in southern Italy (38°09'06.82"N- 16°09'57.40"E). Surveys, carried out over the last eight years (2003-2011), have shown that for eight months per year (March to November) temperatures are sufficient for infection, however the annual rainfall distribution of 600-1000 mm, favours infection in March-April (65-236 mm; mean 121 mm) and in September-October (121-462 mm; mean 252 mm). Flower and fruit infections, occurring before fruit set and fruit growth, directly affect fruit yield and quality, whereas infections of the leaves and twigs in September-October increase the fungal inoculum, which produces heavier infections the following year. In 2010, the percentage of fruit with symptoms was 71% whereas in March 2011 it was 98%. After the heavy rains of 2011 in the last two weeks of April (152 mm) the incidence of the disease on twigs (20-25 cm) was 96% with a disease index (DI) of 0.72. However, in cv. Fortune plants growing randomly in plots with other citrus cultivars, only 53% of the shoots showed symptoms and the DI was 0.16. The inoculum level, expressed as the average concentration of air-borne conidia, was 26 conidia/m^3 in early spring and decreased to 8 conidia/m^3 in May.

FIRST REPORT OF *IMPATIENS NECROTIC SPOT VIRUS* INFECTING *ISOTOMA AXILLARIS*. M.G. Bellardi[1], L. Cavicchi[2], M. Pirini Casadei[2], V. Vicchi[3] and G. Bozzano[4]. *[1]Dipartimento di Scieze e Tecnologie Agroalimentari -Patologia Vegetale, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. [2]Plesso Didattico "G. Scarabelli", Università degli Studi di Bologna, Viale G. Ascari 17, 40026 Imola (BO), Italy. [3]Servizio Fitosanitario Regione Emilia-Romagna, Via di Corticella 133, 40129 Bologna, Italy. [4]Società Cooperativa "L'Ortofrutticola", Via Dalmazia 169, 17031 Albenga (SV), Italy. E-mail: mariagrazia.bellardi@unibo.it*

Isotoma axillaris Lidt. (sin. *Laurentia axillaris*; also known as blue stars or rock isotome) is a perennial species of Campanulaceae native to Australia, typically cultivated as annual in Liguria (northern Italy). Leaves are ovate or obvate in outline, with pinnatified lamina; solitary flowers blooming in April-May at the leaf axils. In 2011, severe symptoms resembling those caused by tospoviruses occurred in a farm located in the Albenga area (Savona). Small necrotic concentric rings and necrosis of the leaf lamina were present in almost 7% of blooming plants grown outdoors. Twenty symptomatic plants collected during inspections were maintained in insect-proof greenhouse at Bologna University. Virus was isolated by mechanical transmission to Chenopodiaceae species. Symptomatic *I. axillaris* was negative for *Tomato spotted wilt virus* (TSWV) and positive for *Impatiens necrotic spot virus* (INSV) in DAS-ELISA. To further confirm the presence of INSV, total RNA was extracted and used for RT-PCR. Nucleotide sequences obtained were found to be closely related to published sequences (98% nucleotide identity with INSV, GenBank accession Nos DQ523598, L20886, AB109100, and L20885). To date, INSV has been found in 1991 naturally infecting *I. fluviatilis* (sin. *L. fluviatilis*), another species of *Isotoma*, weed in Canada. Our record is thought to be the first of natural occurrence of INSV in *I. axillaris.* Sueveys for the presence of thrips vectors of INSV on the farm were conducted and many populations were found, suggesting that thrips are the main responsible for INSV spreading and widening its natural host range.

FIRST REPORT OF *HOSTA VIRUS X* INFECTING *HOSTA* IN ITALY. M.G. Bellardi[1], L. Cavicchi[2] and S. Davino[3]. *[1]Dipartimento di Scienze e Tecnologie Agroalimentari, Patologia Vegetale, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. [2]Plesso Didattico "G. Scarabelli", Università degli Studi di Bologna, Viale G. Ascari 17, 40026 Imola (BO), Italy. [3]Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche agrarie e Zootecniche, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze edificio 5, 90128 Palermo, Italy. E-mail: mariagrazia.bellardi@unibo.it*

Species of *Hosta* (Liliaceae) are herbaceous perennials more often grown for their foliage (blue, gold, green or variegated) than for their flowers. Virus diseases reported elsewhere in the word on cultivated hosta species include *Hosta virus X* (HVX), first described in USA in 1996, and considered to be the main pathogen of this genus. In October of 2010, the following systemic virus-like symptoms were observed on the leaves of three varieties cultivated in Emilia-Romagna (northen Italy), i.e. Gold Standard, Substance and Whirlwind: irregular green blotches scattered on the yellow lamina, green bands running along the veins having an "ink-bleeding" look, malformations, different leaf thickness. Electron microscopic observations revealed the presence of virus particles *ca*. 560-600 nm in length, which in ISEM and PAS-ELISA tests clearly reacted with the antiserum to HVX (provided by University of Minnesota, MN, USA). Mechanical inoculations carried out using symptomatic hosta leaf sap made it possible to transmit HVX to *Chenopodium murale* L. (necrotic pin-point lesions) and *Gomphrena globosa* L. (necrotic red spots 2-3 mm in size). RT-PCR with specific primers HVXCP+ 5'-ATGGCAAGTGACGCACCAACTCCACC-3' and HVXCP- 5'-TCAACTTGAGCCTTCCGGG-3' amplified the expected fragment of 663 bp the coat protein gene. HVX appeared closely related to a Polish isolate FJ821705 (98% nucleotide identity). To prevent HVX spread, it is important to minimize mechanical contact between plants during cultivation, and immediately remove those showing visible symptoms. Considering that two of the infected cultivars tested have been imported from The Netherlands certified virus-free propagation material must be secured.

STREPTOMYCETES AS POSSIBLE BIOCONTROL AGENTS AGAINST SOIL-BORNE PATHOGENS. M. Bonaldi, A. Kunova, P. Sardi, P. Cortesi and M. Saracchi. *Dipartimento di Protezione dei Sistemi Agroalimentare e Urbano e Valorizzazione delle Biodiversità, Università degli Studi, Via G. Celoria 2, 20133 Milano, Italy. E-mail: marco.saracchi@unimi.it*

Streptomycetes are well known as soil and rhizosphere inhabitants. Their role as plant pathogens has been extensively studied, whereas their activity as endophytes, that could influence plant development and health, is less known. Streptomycetes can also act as plant growth promoters or as biocontrol agents against soil-borne pathogens. Our aim was to search for *Streptomyces* active against soil-borne pathogens. Twenty-six *Streptomyces* spp. strains were chosen among a collection of some hundreds actinomycetes isolated from surface-sterilized roots of healthy plants. They were first tested *in vitro* against isolates of soil-borne phytopathogenic fungi belonging to six genera: *Alternaria*, *Botrytis*,

Colletotrichum, *Fusarium*, *Rhizoctonia* and *Sclerotinia*. The results showed different patterns of antifungal activity, and the seven most bioactive strains, named AZ112I, CMJ58I, CMJ60I, ER08W, MRX13R, VT111I, ZEA17I, were used for seed-dressing of some species (e.g. onion, tomato, etc.). Their growth on seed-coats and root endophytic colonization were confirmed by microscopic observation and back isolation from surface-sterilized roots. Treated seeds showed significant improved percent germination, and lower saprophyte contamination and seedlings had longer main roots and more lateral roots than the untreated ones.

FAIRY RINGS BY KILLER FUNGUS AFFECT PLANT DIVERSITY, SOIL QUALITY AND MICROBIAL FUNCTIONALITY OF SPECIES-RICH GRASSLAND. G. Bonanomi[1], G. Incerti[3], A. Mingo[1], S. Mazzoleni[1] and M. Allegrezza[2]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. [2]Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy, [3]Dipartimento di Scienze della Vita, Università degli Studi, Via Giorgieri 10, 34127 Trieste, Italy. E-mail: giuliano.bonanomi@unina.it*

More than 50 species of basidiomycetes can produce the so called fairy rings through networks of mycelia radially growing below ground. In this study we addressed the following questions: do the fairy rings by *Agaricus campestris* affect the spatial distribution of coexisting plant species? Is the ring development related to changes of soil physical, chemical, and microbiological properties? Fairy rings located in a species-rich grassland were monitored for plant biomass, species richness and composition. Seventeen soil parameters were analysed including physico-chemical (water holding capacity, pH, electrical conductivity, organic C, P, total N, NH_4^+, NO_3^-, hydrophobicity and cyanide concentration), and microbiological properties (enzymatic activity by FDA, community-level physiological profile by BIOLOG EcoPlates™, microbial biomass, fungal mycelium, culturable actinomycetes, bacteria and fungi). A bioassay was performed to evaluate the response of three coexisting species (*Bromus erectus*, *Cynosurus echinatus* and *Centaurea ambigua*) growing on soils collected from fairy ring underneath four different concentric belts. Plant species composition dramatically changed in response to fairy ring development with disappearance of grassland species from the most external belt. Profound changes of soil properties were found after fungal passage with consistent reduction of C and N contents and increases of FDA and microbial functional diversity. Soil from the fungal zone showed remarkable increases of mineral N forms, electrical conductivity and hydrophobicity. The bioassay showed species-specific responses to different soil types. This study provides evidence that the spread of fairy ring fungi, by killing dominant perennial plants, creates empty niches for many short-lived species, thus allowing coexistence.

BIOCHEMICAL CHANGES OF ORGANIC MATTER EXPLAIN FUNGAL SAPROPHYTIC ABILITY: EVIDENCE FROM SOLID STATE ^{13}C-CPMAS NMR SPECTROSCOPY. G. Bonanomi[1], M. Capodilupo[1], G. Incerti[2], V. Lanzotti[3], F. Scala[1] and S. Mazzoleni[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy, [2] Dipartimento di Scienze della Vita, Università degli Studi, Via Giorgieri 10, 34127 Trieste, Italy, [3]Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. E-mail: giuliano.bonanomi@unina.it*

Soil-borne fungal pathogens are among the major limiting factors for the productivity of agro-ecosystems. However, the ecology of the saprophytic life phase and the role of fungal ecological succession in the process of pathogenesis are still poorly understood. By a litter-bag decomposition experiment, we investigated the saprophytic ability of seven fungal species (*Aspergillus niger*, *Botrytis cinerea*, *Ganoderma lucidum*, *Mucor* sp., *Pythium ultimum*, *Trichoderma harzianum* and *Umbelopsis* sp.) grown over 45 organic matter types decomposing for 180 days. Organic matter substrates were characterized throughout the decomposition process by classic proximate chemical analyses (total C and N, labile C, cellulose and lignin content) and, at molecular level, by solid state ^{13}C-CPMAS NMR. The saprophytic ability of all species decreased during the decomposition process over all organic matter types, being more rapid for *A. niger*, *B. cinerea* and *Mucor* sp.. This finding is consistent with the early disappearance of such species during fungal succession. The biochemical quality of organic matter progressively decreased during decomposition, both considering proximate parameters and C types corresponding to spectral regions from ^{13}C-CPMAS NMR. The latter were also consistently predictive of fungal growth over the 45 substrates. Our results support the hypothesis that changes in organic matter quality controls the dynamics of fungal succession.

DEGRADATION OF ELASTO-MECHANICAL PROPRIETIES OF WOODEN POLES USED IN SOIL BIOENGINEERING STRUCTURES DUE TO WOOD-DECAY FUNGI. L. Bosso[1], G. Menegazzi[2], M. Brigante[3], G.B. Chirico[4], G. Cristinzio[1] and N. Romano[4]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Parco Nazionale del Vesuvio, Via Palazzo del Principe, 80044 Ottaviano (NA), Italy. [3]Dipartimento di Ingegneria Strutturale, Università degli Studi "Federico II", Via Claudio 21, 80125 Napoli, Italy. [4]Dipartimento di Ingegneria Agraria e Agronomia del Territorio, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: luciano.bosso@unina.it*

Wood is degraded by a variety of organisms. The major agents for wood degradation are the fungi, especially those belonging to the "wood-decay" group. These fungi produce lignocelluloses enzymes that degrade cell wall components causing a variation of some properties of the wood. This paper discusses the results of an experiment carried out on chestnut wooden poles collected from soil bioengineering structures in some areas of the Vesuvio National Park (Naples, Southern Italy). We analyzed the effects exerted on the decay in wood physical and mechanical characteristics by fungi belonging to the following four families: Meruliaceae, Strophariaceae, Polyporaceae and Corticiaceae. Significant correlations were found between the presence of wood-decay fungi (in percent), wood moisture content and ultimate stress. Specifically, conditions of large fungi attack and high wood moisture can affect even severely the strength of a wood element and hence the behavior of the entire structure.

FUNGI AND BACTERIA IN THE BIOLOGICAL CONTROL OF CHESTNUT INK DISEASE. L. Bosso[1], T. Marras[2], C. Caprari[2], G. Naclerio[2] and G. Cristinzio[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territo-*

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Ink disease, one of the most dangerous diseases of chestnut has been reported from many European countries, from Portugal to Romania. Two species of *Phytophthora* are responsible for ink disease, *P. cambivora* and *P. cinnamomi*. In this work we carried out some *in vitro* experiments, as reduction of radial growth and mycelial dry weight, of these two Oomycetes, using two fungi belonging to genus *Byssochlamys* and two bacteria belonging to genus *Bacillus*, all isolated from agricultural and forestry soil. Our results showed a high reduction in radial growth and mycelial dry weight by both fungi and bacteria. The *Byssochlamys* strains inhibited the radial growth of *P. cambivora* and *P. cinnamomi* by *ca.* 80% and *ca*. 83%, respectively, whereas *Bacillus* inhibited *P.cambivora* and *P.cinnamomi*. by *ca.* 83% and *ca.* 73%, respectively. Mycelial dry weight reduction was also remarkable, i.e. *Byssochlamys* by *ca.* 90% for both *Phytophthorae* while *Bacillus* by *ca.* 60% for *P. cambivora* and *ca.* 50% for *P. cinnamomi*.

ENDOPHYTIC BACTERIAL DIVERSITY ASSOCIATED WITH GRAPEVINE PLANTS AND THEIR POTENTIAL APPLICATIONS. D. Bulgari[1], P. Casati[1], F. Quaglino[1], P.A. Bianco[1,2] and F. Faoro[1,2]. *[1]Dipatimento di Protezione Vegetale-Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. [2]Istituto di Virologia Vegetale del CNR, UOS Milano, Via Celoria 2, 20133 Milano, Italy. E-mail: franco.faoro@unimi.it*

Endophytic bacteria can be defined as those colonizing the interior of plants without inducing disease. Though their relationship with the host is not well understood, they may reduce disease severity by activating systemic resistance, antibiosis, competition of nutrients and niches. Due to these abilities, bacterial endophytes are candidates for biological control of plant diseases. To identify potential biocontrol agents of phytoplasmas, endophytic bacterial communities associated with healthy, phytoplasma-diseased and recovered grapevine were characterized by cultivation-dependent and independent methods. Endophytes were isolated directly from grapevine leaf metagenome by an optimized DNA extraction methods. Sequencing of 16S rRNA gene libraries highlighted the presence of five operational taxonomic units (OTUs) showing best sequence matches with γ-Proteobacteria, family *Enterobacteriaceae*, with a dominance of the genus *Pantoea*. Furthermore, the genera *Bacillus, Stenotrophomonas*, *Methylobacterium*, *Pectobacterium*, *Enterobacter*, *Brevundimonas* e *Agrobacterium*, *Brevibacillus*, *Staphylococcus* e *Sphingomonas* were identified by cultivation-dependent methods. Some of these bacteria, particularly *Pantoea agglomerans* are already known as biocontrol agents of several plant diseases. *P. agglomerans* was also localized in grapevine leaf tissues by fluorescence *in situ* hybridization (FISH) and immunomicroscopy. In detail, this bacterium was detected in the phloem, xylem and parenchyma of grapevine leaf veins, where it could play a role in modulating phytoplasma infection and recovery.

MYCOTOXIN REDUCTION IN MAIZE AND WHEAT: A PREDECTIVE APPROACH. M. Camardo Leggieri[1], V. Rossi[1], A. Logrieco[2] and P. Battilani[1]. *[1]Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29100 Piacenza, Italy. [2]Istituto di of Sciences of Food Production, del CNR, Via Amendola 122/0, 70126 Bari, Italy. E-mail: paola.battilani@unicatt.it*

MYCORED is a 7° FP large collaborative project funded by EC aimed at developing strategic solutions to reduce mycotoxin contamination in economically important food and feed chains. Wheat and maize are important crops considered in the project due to relevance for human and animal consumption and as they cause major mycotoxin safety concerns worldwide. In particular, deoxynivalnol (DON) and zearalenone (ZEA) are secondary metabolites associated with *Fusarium* head blight (FHB), a wheat ear disease caused by a complex of *Fusaria*. Fumonisins (FUM) and aflatoxins (AFs), whose main producers are respectively F*usarium verticillioides* and *Aspergillus flavus*, are crucial problems in maize. A specific work package in MYCORED is aimed at developing and validate predictive models for these relevant fungi to forecast the risk of mycotoxin contamination in different geographic areas and years, mainly based on meteorological conditions. A rational approach was elaborated and adopted by all countries involved (Italy, United Kingdom, Hungary, Russia, Egypt, Mexico, Nigeria, Argentina) in order to collect samples and data useful for models validation. Specific protocols were developed for crop sampling at harvest, appropriate questionnaires were prepared to collect cropping system data, and suitable stations were selected for meteorological data. A data base was prepared and data storage is ongoing. A web access to predictive models is under development. Based on the first 2-year data (2009-2010), FHB model validation gave encouraging results, with good predictions in all the countries involved. More efforts are requested regarding maize, because the contribute of meteorological data seems not sufficient for a reliable prediction of FUM and AFs contamination at harvest.

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OCCURRENCE OF *TOMATO SPOTTED WILT VIRUS* ON TOBACCO IN CAMPANIA. R. Carrieri[1], R. Sorrentino[2], E. Lahoz[1] and D. Alioto[2]. *CRA, Unità di Ricerca per le Colture Alternative al Tabacco, Via P. Vitiello 108, 84018 Scafati (SA), Italy. [2]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: raffaele.carrieri@unina.it*

During a survey on the sanitary status of tobacco carried out in Campania (southern Italy) in 2010, *Tomato spotted wilt virus* (TSWV) was detected on Burley tobacco cv. F 3119. The analyses were performed by DAS-ELISA with a specific polyclonal antiserum. Symptoms appeared as severe necrosis on the stem and leaves and death of the plant. Total RNA was extracted from leaves with the SV total RNA isolation system kit and used as template for RT-PCR, employing primers specific to the TSWV nucleocapsid (N) and movement protein of the middle segment (NSm) genes. The amplified fragments (754 and 670 bp, respectively) were aligned and edited using BlastN. The analysis of the 754 bp N fragment showed the closest identity with p105/2006RB strain (DQ915946), identified on pepper in Italy, in 2006 (99.9% nucleotide identity). The 670 bp fragment, corresponding to NSm gene, showed the closest identity with the PED-1 isolate identified on pepper in Campania in 2009 (99.4% nucleotide identity). Phylogenetic analysis, based on the multiple alignments of NSm gene sequences of the tobacco isolate from Campania and those present in databases, revealed that the viral isolate under study belongs to A-like subgroup. A-like isolates were also identified in TSWV-infected lettuce and chrysanthemum from Campania. These findings, together with the previous

report from pepper, seem to indicate a threatening spread of TSWV isolates A-type in Campania, even though they do not seem to belong to the resistance-breaking (RB) biotype, as evidenced by the analysis of amino acids at positions 118 and 120 of NSm protein.

GENETIC CHARACTERIZATION OF *SCLEROTIUM ROLFSII* ISOLATES FROM SEVERAL ORNAMENTAL CROPS IN SICILY. R. Carrieri[1], A. Carella[1], R. Caiazzo[2], G. Polizzi[3] and E. Lahoz[1]. *[1]CRA, Unità di Ricerca per le Colture Alternative al Tabacco, Via Pasquale Vitiello 108, 84018 Scafati (SA), Italy. [2]Tree Fruit Research and Extension Center, Washington State University 1100 N Western Ave, Wenatchee WA 98801, USA. [3]Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Sez. Patologia vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: raffaele.carrieri@unina.it*

Sclerotium rolfsii is one of the most devastating plant pathogenic fungi throughout the world. It causes southern blight disease in a wide variety of agricultural and horticultural crops. Recently, southern blight has caused large losses in ornamental plants and turfs in eastern Sicily (southern Italy). A previous report showed that 37 isolates of *S. rolfsii*, collected from various ornamental hosts in eastern Sicily over two years, varied considerably in some morphological features: growth rate at different temperatures, number of sclerotia per plate, dry and fresh weight. Moreover, based on pairings among isolates to establish mycelial compatibility, five vegetative compatibility groups (VCGs) were found that showed significant differences in pathogenicity. On the contrary, polygalacturonase patterns showed no variations within the isolates. In the present work, the genetic variability among the 37 Sicilian isolates is investigated. Genomic DNA was purified and analyzed by PCR and analysis of the sequence of ITS1-5.8S-ITS2 ribosomal subunit region and elongation factor-1α gene. The purpose of this study was to detect a possible relationship between genetic diversity and pathogenicity of specific VCGs of the analyzed *S. rolfsii* isolates. The study of variability of *S. rolfsii* strains in a particular geographical area is important for documenting the origin of isolates and the changes occurring in the population.

INFECTION PATTERNS OF *FUSARIUM OXYSPORUM* f. sp. *MELONIS* IN MELON PLANTS. V. Catalano[1], L. Luongo[1], A. Haegi[1], M. Scotton[2], N. Ficcadenti[3] and A. Belisario[1]. *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. [3]CRA, Unità di Ricerca per l'orticoltura, Via Salaria 1, 63030 Monsampolo del Tronto (AP), Italy. E-mail: a.belisario@ispave.it*

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melonis* (FOM) is the most important and widespread infectious disease of melon. The four known races of this pathogen can be distinguished only by infection on appropriate cultivars. At the moment, no molecular tools are available to discriminate races, and the disease progression is poorly understood. Resistance to races 1 and 2 is controlled by a single dominant gene, whereas only partial polygenic resistance to race 1,2 has been reported. The analysis of the colonization patterns of FOM race 1 and FOM race 1,2, the two main races present in Italy, was performed in compatible and incompatible binomials to identify differences in colonization along the stem in consideration to the type of resistance. A systematic reisolation procedure on infected stems coupled with PCR assays allowed to delineate the fungal growth along the stem. Moreover, a quantitative assay was set up in order to estimate the amount of the pathogen both in compatible and incompatible host-pathogen combinations, e.g. with cv. Charentais FOM-2-inoculated as either FOM race 1 or race 1,2.

TEMPERATURE RESPONSE OF *BOTRYOTINIA FUCKELIANA* SUB-POPULATIONS. N. Ciliberti, S.E. Legler, T. Caffi, L. Languasco 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*

Botryotinia fuckeliana (de Bary) Whetzel (anamorph: *Botrytis cinerea* Pers.:Fr.), the causal agent of grapevine grey mould, has two sub-populations (*transposa* and *vacuma*) with different combinations of the transposable elements Boty and Flipper: *transposa* has three combinations (Boty+Flipper+; Boty+Flipper-; Boty-Flipper+), and *vacuma* lacks transposable elements (Boty-Flipper-). The effect of temperature (5 to 30°C) was determined for a representative isolate of each of the four combinations. The isolates were obtained from the culture collection of the University of Bari. Colony growth rate was lower for the *vacuma* isolate than the *transposa* isolates at 30°C but the opposite was true at 5°C. Between 10 and 25°C, colony growth was similar for all four isolates. Only the *transposa* isolates produced sclerotia between 5 and 20°C, and produced more conidia than the *vacuma* isolate, with an optimum at 15 and 20°C. Conidial germination after 24 h was lower for the *vacuma* isolate than for the *transposa* isolates at 5 and 10°C, did not differ among isolates between 15 and 25°C, and was lower for the Boty+Flipper+ isolate at 30°C. Inoculation of bunches at different growth stages (inflorescence swelling, flowering, groat-sized berries, ripening, and maturation) indicated that the incidence of *vacuma* isolate was low before flowering while the it was very high and similar for all isolates at flowering. Occurrence of latent infection in berries was also investigated. The observed differences suggest that different sub-populations of *B. fukeliana* require different infection models.

SPREAD OF AN ATYPICAL POPULATION OF *PSEUDOMONAS SAVASTANOI* ON *MYRTUS* sp. IN SARDINIA. T. Cinelli[1], V. Ligios[2], G. Marchi[1], L. Mugnai[1] and M. Fiori[2]. *[1]Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. [2]Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi di Sassari, Via E. De Nicola, 07100 Sassari, Italy. E-mail: fiorim@uniss.it*

Virulent strains of the phytopathogenic bacterium *Pseudomonas savastanoi* induce parenchymatous galls (knots) at the site of infection. Knots are mostly seen on the young stems, branches and twigs of different plants belonging to Oleaceae (*Olea*, *Jasminum*, *Forsythia*, *Phillyrea*, *Ligustrum*), Fabaceae (*Retama*), Rhamnaceae (*Rhamnus*), Myrtaceae (*Myrtus*) or to Apocinaceae (*Nerium, Mandevilla*). On ash (*Fraxinus* spp.) *P. savastanoi* causes wart-like excrescences. During surveys carried out in the spring 2010 in six locations of north-western Sardinia (insular Italy) on different selections of myrtle (*Myrtus communis*) and also on spontaneous plants, canker-like lesions of about 1 cm long were observed on branches and twigs. From the tissues below the bark, which appeared dark-brown, dried and cracked, a bacterium was constantly isolated that, based on the results of biochemical tests and molecular typing methods, was identified as

Pseudomonas savastanoi. Pathogenicity tests were performed with 2 isolates by placing a bacterial suspension containing 10^8 CFU/ml on wounds made in the bark of twigs of M*yrtus communis* var. *tarentina*. After 4 to 6 weeks the centre of the wound was surrounded by an irregular mound of new tissue. Occasionally after 2-3 months these newly formed tissues had joined together over the surface of the wound. Typical "knots" were never observed. Further characterization is now in progress to better evaluate the differences that exist between the myrtle population of *P. savastanoi* found in Sardinia and knot inducing strains isolated from *Myrtus* sp. in other countries of the Mediterranean basin.

ARMILLARIA MELLEA HYPHAL WALL POLYSACCHARIDES ELICIT PHENOL ACCUMULATION AS A DEFENSIVE RESPONSE IN _GENISTA MONOSPERMA_. **F. Clematis[1], S. Fascella[1], B. Cangelosi[1], J. Tedeschini[2], M. Dolci[3] and P. Curir[1].** *[1]CRA, Unità Ricerca Floricoltura e Specie Ornamentali, Corso Inglesi 508, 18038 Sanremo, Italy. [2]Agriculture University of Tirana, Durres, Albania. [3]Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali. Università degli Studi di Torino, Via L. da Vinci 44, 10025 Grugliasco (TO), Italy. E-mail: p.curir@istflori.it*

Polysaccharides obtained as a crude wall extracts (CWE) from *Armillaria mellea* hyphae grown *in vitro* proved their ability to trigger a defensive response in the host plant *Genista monosperma*. Experiments were performed on 3-month-old herbaceous plants treated for 48 h with various concentrations of deproteinated CWE. This latter consisted of proteins, neutral carbohydrates, amino sugars and ashes. *G. monosperma* tissues, elicited by CWE, displayed a defensive response consisting both in a quantitative increase of vacuolar phenol accumulation and in thickening of parenchyma cell walls, which could be appreciated by microscopic examination. Histochemical analyses disclosed that phenolic acids (e.g. ferulic acid) were the main responsible of plant cell wall thickening, due to a progressive molecule encrustation by esterification. Our data confirm therefore the importance of phenolics in the plant response to fungal attacks and, moreover, they indicate that hyphal polysaccharides may behave as elicitors of the *G. monosperma* defensive response.

A TRANSCRIPTOMIC APPROACH TO UNDERSTAND INDUCED RESISTANCE TO BACTERIAL CANKER OF STONE FRUITS ELICITED THROUGH TREATMENTS WITH COPPER GLUCOHUMATES. **D. Dallai[1], L. Dondini.[2] and E. Stefani[1].** *[1]Dipartimento di Scienze Agrarie e degli Alimenti, Università degli Studi di Modena e Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy. [2]Dipartimento di Colture Arboree, Università di Bologna, viale G. Fanin 46, 40127 Bologna, Italy. E-mail: emilio.stefani@unimore.it*

Bacterial canker of stone fruits, caused by *Xanthomonas arboricola* pv. *pruni*, is a recurrent disease in Italian peach and plum orchards. Several field and glasshouse trials on peach and plum were done, with the aim to effectively control the disease by using some novel molecules, such as glucohumates. The most remarkable results were obtained with copper glucohumates (with a reduction of the disease by *ca.* 80%). Results are very promising and suggest the possibility to implement effective control strategies, where copper compounds and novel molecules are both used in commercial orchards. In order to study and understand the effect of the biomolecules used, untreated and glucohumate-treated peach plants were subject to further molecular analyses in order identify possible genes/sequences involved in the induction of disease resistance. A transcriptomic approach was developed for detecting the transcripts present in plant tissues, after elicitation of an induced protection state. Total RNA was extracted, retro-transcribed and c-DNA-AFLP was done to identify different sequence fingerprints in the protected plant tissue. Discrimination of newly expressed sequences was performed with DHPLC, and comparison of transcripts was done on the complete peach genome database in order to identify the genes or sequences involved in the elicitation of induced resistance. Preliminary data showed, in treated plants, the presence of nine putative genes like a putative senescence protein, already described as being involved in the induced resistance to fire blight in other Rosaceae. Thus, copper glucohumates might be considered possible candidates for the elicitation of resistance to bacterial diseases.

ULTRASTRUCTURAL MODIFICATIONS BY _CHALARA FRAXINEA_ IN COMMON ASH. **E. Dal Maso, G. Fanchin, S. Mutto Accordi, L. Scattolin and L. Montecchio.** *Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. Email: montecchio@unipd.it*

Chalara fraxinea, responsible for ash dieback, is present in many European countries where it constitutes a severe threat not only to forests, but also to urban areas and nurseries. To examine the *C. fraxinea* infection strategies in common ash tissues, an ultrastructural investigation (ESEM, TEM) was performed. Artificial infections in wounded stems demonstrated that *C. fraxinea* develops intracellularly, moving through the cells and colonizing easily wood cortex, phloem, paratracheal parenchyma and parenchymatic rays. The possible involvement of enzymes or toxins in parenchymatic cells disruption are discussed.

SUPPRESSIVE EFFECT OF EXPLODED BIOMASS OF _ARUNDO DONAX_ AND WHEAT STRAW AGAINST SOIL-BORNE PLANT PATHOGENS. **U. De Corato[1], N. Sharma[2], O. Maccioni[2] and E. Viola[3].** *[1]Unità Efficienza Energetica dell'ENEA, Servizio Agricoltura, Ufficio Territoriale di Bari, Via R. Da Bari 119, 70122, Bari, Italy. [2]Unità Tecnologie Trisaia dell'ENEA, Laboratorio di Biotecnologie, Centro Ricerche Trisaia, s.s. 106 Jonica Km. 419.500, 75026 Rotondella (MT), Italy. [3]Unità Tecnologie Trisaia dell'ENEA, Laboratorio di Biomasse e Solare Termico, Centro Ricerche Trisaia, s.s. 106 Jonica Km. 419.500, 75026 Rotondella (MT), Italy. E-mail: ugo.decorato@enea.it*

A suppressiveness of exploded biomasses of *Arundo donax* and wheat straw, a renewable energy sources commonly used for industrial production of ethanol by steam-explosion, was tested in a greenhouse against soil-borne plant pathogens, with respect to exploded biomass of *Miscanthus sinensis* and commercial compost. The tests were performed at three doses (10, 20 and 30% of potting mix) on seven horticultural pathosystems (cucumber/*Pythium ultimum*, tomato/*Phytophthora nicotianae*, bean/*Rhizoctonia solani*, fennel/*Sclerotinia sclerotiorum*, eggplant/*Verticillium dahlie*, melon/*Fusarium oxysporum* f.sp. *melonis* and lettuce/*Fusarium oxysporum* f.sp. *lactucae*). A disease suppression was evaluated by index $Ds=[(N_p-N_{cb})/N_p]\times100$ and analyzed by Tukey's HSD test ($P<0.05$). Suppressiveness of *M. sinensis* ranged from 97% in eggplant/*V. dahliae* to 25% in lettuce/*F. oxysporum* f. sp. *lactucae*. It was higher of the compost and wheat straw in all the pathosystems and of *A. donax* in two pathosystems. A suppressiveness of *A. donax* ranged from 85% in eggplant/*V. dahliae*

to 18% in lettuce/*F. oxysporum* f. sp. *lactucae*, and it was higher of the compost and wheat straw in five pathosystems. A wheat straw suppressiveness ranged from 45% in eggplant/*V. dahliae* to 13% in melon/*F. oxysporum* f. sp. *melonis*. It was lower of *M. sinensis* in all pathosystems, of *A. donax* in six pathosystems and of the compost in five pathosystems. In conclusion, we confirm that the exploded of *Miscanthus* is the most suppressive on all pathosystems at the highest dose tested. Moreover, the eggplant/*V. dahliae* and lettuce/*F. oxysporum* f. sp. *lactucae* resulted more and less sensitive to a suppressive effect, respectively.

ACTIVITY OF ELECTROLYSIS IN REDUCING MICROBIAL POPULATION AND CONTROLLING POSTHARVEST DISEASES OF CITRUS FRUITS. F. Fallanaj[1], S.M. Sanzani[1], C. Zavanella[2] and A. Ippolito[1]. *[1]Dipartimento di Biologia e Chimica Agro-forestale ed Ambientale, Università degli Studi "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. [2]Adamant Technologies SA Eplatures-Grise 17, 2300 La Chaux-de-Fonds, Switzerland. E-mail: simona.sanzani@agr.uniba.it*

Electrolysis has recently gained particular attention in the food industry as a novel technology for preventing fruit infection in the postharvest environment. Furthermore, the application of electrolyzed oxidizing (EO) water as alternative to chemical fungicides against fruit spoilage gave also promising results. The present investigation was carried out to evaluate the potential application of electrolysis in reducing the main postharvest rots of citrus fruits. Preliminary laboratory-scale trials with wash water from different packing houses were performed. The water was added with a conidial suspension of *Penicillium digitatum* (10^5 CFU/ml) and subjected to electrolysis. Microbiological evaluation of *P. digitatum* vitality in orange fruit wash water showed nearly 100% inhibition of spore germination after 30 min, whereas, the complete suppression of the fungal population in lemon wash water was observed after 15 min of electrolysis. On the basis of these preliminary results, further trials were conducted in two packing houses located in Sicily (insular Italy). During the commercial trials, fruits were dipped in electrolyzed water to evaluate its ability to prevent infections during storage and shelf-life. Treatment of wounded fruits with electrolyzed water compared with tap water, reduced the incidence of infection by 70% after 15 days storage at room temperature. These results suggest that the application of electrolysed water in conventional postharvest treatments may increase citrus fruit shelf-life, particularly given the current need for alternatives in the global context of organic and integrated disease management strategies.

INFLUENCE OF LIGHT ON GROWTH, CONIDIATION AND FUMONISIN PRODUCTION BY *FUSARIUM VERTICILLIOIDES*. F. Fanelli[1], M. Schmidt-Heydt[2], M. Haidukowski[1], A. Susca[1], R. Geisen[2], A. Logrieco[1] and G. Mulè[1]. *[1]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/0, 70126 Bari, Italy. [2]Max Rubner Institut, Department for Safety and Quality of Fruit and Vegetables, Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany. E-mail: giuseppina.mule@ispa.cnr.it*

Light is a very important signal for fungi since it influences many different physiological responses. We analyzed the influence of light of varying wavelength and intensity on the growth, conidiation and fumonisin B1 (FB1), B2 (FB2) and B3 (FB3) biosynthesis by *Fusarium verticillioides* ITEM 10027. Wavelengths from both sides of the spectrum, from red (627 nm) to blue (470-455 nm), had a stimulating effect on the growth and fumonisin production, with an increase of up to 150% of total fumonisins analyzed, compared with dark incubation. If the intensity of the 455 nm blue light was increased, fumonisin biosynthesis was reduced. Furthermore the incubation under a short wave blue light (390 nm) had an inhibitory effect on the fungal growth and the fumonisin production was reduced by 85%. White pulsing light had an inhibitory effect on fumonisin production, with a reduction of about 50%, but not on the fungal growth, comparable with the dark incubation. The regulation of fumonisin production was analyzed by real time RT-PCR, measuring the expression level of fum1, fum21 and fvVE1 transcripts, that encode protein involved in fumonisin biosynthesis, revealing a correlation between gene expression and fumonisin production.

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SERIOUS OUTBREAK OF BACTERIAL CANKER OF TOMATO IN SOUTHERN ITALY. A. Fanigliulo[1], A. Viggiano[1], G. Piegari[1] and A. Crescenzi[2]. *[1]Bioagritest Srl, Zona PIP lotto E2, 85010 Pignola (PZ), Italy. [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano, Campus Macchia Romana 3A310, 85100 Potenza, Italy. E-mail: aniello.crescenzi@unibas.it*

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is the causal agent of canker and wilt disease of tomato. This disease causes severe economic losses that may become devastating to tomato production. *Cmm* bacteria penetrate the plant through wounds or natural openings, find their way into the xylem, and develop a massive systemic infection. Typical disease symptoms include wilting of the leaves, leaflet necrosis, development of canker lesions on the stem and "Bird's eyes" on fruits (spots with raised brown centers surrounded by an opaque white halo). During summer 2010, an outbreak of bacterial canker was observed in tomato fields in the main tomato-growing area of Apulia and Basilicata (south-east Italy). The disease was observed starting 20 to 30 days from transplant and reached at the beginning of ripening, a 100% incidence, with complete crop loss. Detection assays were performed on symptomatic plants in order to ascertain the etiological agent. The diagnostic procedure comprised isolation from infected tissues, presumptive diagnosis with a rapid test, identification of presumptive isolates and determination of pathogenicity (2005 OEPP/EPPO, Bullettin OEPP/EPPO Bullettin 35, 271-273. PM 7/42). All symptomatic plants were infected with the bacterium under investigation. They all belonged to the tomato cv. Uno Rosso, deriving from the same tomato seed batch. This let us to believe that bacterial infection was associated with tomato seed.

EVALUATION OF THE EFFICACY OF TANNING PRODUCTS ON THE PRODUCTS' DISTRIBUTION ON THE SEED SURFACE AND DISPERSION OF THE ACTIVE SUBSTANCE AT VARIOUS STAGES OF MANIPULATION OF TANNED SEED. A. Fanigliulo[1], A. Viggiano[1], V. Filì[1] and A. Crescenzi[2]. *[1]Bioagritest Test Facility, Bioagritest Srl. Zona PIP lotto E2. 85010 Pignola (PZ), Italy. [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata. Viale dell'Ateneo Lucano Campus Macchia Romana 3A310, 85100 Potenza (PZ), Italy. E-mail: aniello.crescenzi@unibas.it; info@bioagritest.it*

A study was carried out in 2010 by the Bioagritest test facility

with the purpose of evaluating the efficacy of tanning products on the product distribution on the surface of the seed and dispersion of the active substance at various stages of the manipulation of tanned seed. Seeds under investigation were those of *Zea mais*, the tanning product was a formulate whose a.i. was Clothianidin. The formulate was compared with the standard Poncho 600 FS (treatment 1) or used with the addition of: (i) fungicide and adjuvant (ii) fungicide, (iii) fungicide, adjuvant and glue. An aliquot of 333 grams of tanned seed from each treatment, in 3 replicates, plus the untreated control were subjected to the following: the mass of seeds was subjected to stripping at the amplitude of 1.20 mm/g for three different time intervals (45, 90 and 180 min) in appropriate disposable containers. The amplitude of 1.20 mm/g simulates very strong stripping conditions. This choise was made in order to simulate the extreme (overestimated) conditions to which the seeds may be subjected after tanning. The powder was then re-suspended in an appropriate buffer and the amount of active ingredient released and/or dispersed was determined, using a method developed in this study. This method allows the quantitative determination of clothianidin by LC-DAD (liquid chromatography with photodiode array detection) in plastic containers (for tanned seeds): the sample extracted with appropriate solvent and diluted in mobile phase was filtered and then analyzed in LC-DAD in reversed phase, in a chromatography column C18 type, and the signal was read at 266 nm. The work was planned, performer and recorded according to UNI CEI EN ISO IEC 17025 and EN ISO 900: 2008 standards. The treatments were applied using electronic vibrators, at programmed speed and times, in disposable containers. The three intervals analyzed were the simulation of the three main phases of seed manipulation: packaging, transport and sowing by seeder with hopper. The choice of the three time intervals was also a tools to assess the tightness of the tanning product on the surface of the tanned seed. For the evaluation of the distribution of the active ingredient on the surface of tanned seed, 50 tanned seeds for each of the 4 treatments, in 4 replicates, were used. The degree of uniformity of the product on the treated seeds was determined by image acquisition and analysis of the surface of the treated seed corn. Quantification of the active ingredient (Clothianidin) released from the seeds after tanning treatment (drift of the a.i.) and % of the surface of colored kernels of corn were assessed. Data were statistically analyzed using the XLSTAT statistical software.

PRELIMINARY INVESTIGATION ON THE USE OF ESSENTIAL OILS TO CONTROL ROSE POWDERY MILDEW. S. Fascella, B. Cangelosi, P. Lucido, P. Curir and C. Pasini. *CRA, Unità Ricerca Floricoltura e Specie Ornamentali, Corso Inglesi 508, 18038 Sanremo, Italy. E-mail: c.pasini@istflori.it*

The purpose of this study was to evaluate the efficacy of essential oils from five vegetable species (oregano, thyme, clove, geranium, carvacrol) and a commercial solution of fertilizer KH_2PO_3 (50% w/w) for the control of powdery mildew of roses (*Podosphaera pannosa* var. *rosa)*. The experiments were carried out on the rose cv. Top Meillandina, grown in pots and kept under shade. The essential oils were sprayed at the dose of 0.5 ml/l and the mineral salt KH_2PO_3 at 3 ml/l. Rose plants were treated also with Nimrod (Bupirimate 23,8 % a.i.) at the dose of 3 ml/l, as a standard reference. Disease severity was assessed seven days after the fourth weekly application. All the treatments reduced powdery mildew severity, in comparison to untreated roses. The preliminary results showed a remarkable antifungal action, in particular the essential oils provided a 80% disease reduction. Further studies will be aimed at assessing the antifungal activities of these substances on cut rose cultivars, to provide a natural alternative to synthetic products in the control of the pathogen. No phytotoxic effect was observed with natural products.

MOLECULAR IDENTIFICATION OF XYLOPHAGUS FUNGI ASSOCIATED WITH *QUERCUS ILEX* IN URBAN LANDSCAPES. A. Francini, E. Pellegrini and C. Nali. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "Giovanni Scaramuzzi", Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: cristina.nali@agr.unipi.it*

Studies on wood-decay fungi and their putative relationships with pollution, availability of nutrients, mechanical injuries and disturbances due to constructions have recently been improved in order to help decisions related to landscape planning and management. It is important to remember that decay does not necessarily mean immediate death of trees, as the process may extend over several years. In urban areas the risk of accidents involving people or properties could be important. In this work, the presence and identity of wood decaying fungi affecting *Quercus ilex* were evaluated for setting up ready-to-use diagnostic protocols. The survey was carried out in 2011, in the municipality of San Giuliano Terme (Pisa, central Italy). Morphological descriptions of the fungi detected were done. Moreover, detection and identification of four *Basidiomycota* (*Ganoderma*, *Fomes*, *Phellinus* and *Trametes*) and one *Daldinia* (*Ascomycota*) were performed by PCR-based methods using mitochondrial ribosomal DNA (rDNA) loci. The ITS1 and ITS2 regions from fungal cultures were amplified and sequenced. The nucleotide sequence of *Fomes fomentarius* was deposited in the GenBank (accession No. JF927882, 604 bp). Further developments will take into account the correlation between tree age, presence and identity of fungi and their degradative potential.

SOIL DISINFESTATION BY OZONE FOR CONTROLLING VERTICILLIUM WILT OF ARTICHOKE. M. Gallo, C. Fumarola, S.A. Rocca and F. Ciccarese. *Dipartimento di Biologia e Chimica Agroforestale e Ambientale, Università degli Studi "A. Moro", Via G. Amendola 165/A, 70126 Bari, Italy. E-mail: fciccare @agr.uniba.it*

According to the European legislation, which has revised and reduced the number of pesticides with the objective of stimulating new tools with low environmental impact, the need to identify effective and environmentally friendly technologies for soil disinfestations has emerged. In the course of studies aimed at identifying alternatives to the use of methyl bromide, a procedure for soil disinfestation by ozone (Patent No. 05017372.3) has been developed. The results of a trial for soil disinfestation using ozone for controlling Verticillium wilt of artichoke are reported. The trials were carried out in field naturally infested by *Verticillium dahliae* on a 3-year-old artichoke plot cv. Violetto di Provenza. Ozonated water, at 5 ppm concentration, was applied before (one day) and after (twenty days) transplanting with a sub irrigation system. Chemically treated (Azoxystrobin at 0.5 ml/m^2 concentration applied before and after transplanting), untreated and healthy controls were included. Verticillium wilt severity was expressed as McKinney index of external symptoms (IES) and vascular discolorations (IVD). Every year the yield was also assessed. IES and IVD on artichoke grown on infested and untreated soil were 82.55% and 72.15%, respectively. Ozone applications proved effective in the control of Verticillium wilt before (IES=16,3%; IVD=5,6%) and after transplanting (IES=18,6%; IVD=5,8%). The application of Azoxystrobin in sub irrigation

confirmed its efficacy against Verticillium wilt. Compared with infested and untreated control, ozone and Azoxystrobin determined a yield increase that nearly matched that of healthy crops.

EFFICIENT ELIMINATION OF VIROIDS FROM GRAPEVINES BY SOMATIC EMBRYOGENESIS. G. Gambino[1], B. Navarro[2], R. Vallania[1], I. Gribaudo[1] and F. Di Serio[2]. *[1]Istituto di Virologia Vegetale del CNR, UOS Grugliasco, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy.[2]Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: f.diserio@ba.ivv.cnr.it*

Five different viroids have been recorded from grapevines, frequently co-infecting the same plant in various combinations. *Grapevine yellow speckle viroid-1* (GYSVd-1) and *Grapevine yellow speckle viroid-2* are the causal agents of a disease by the same name, while *Hop stunt viroid* (HSVd), *Citrus exocortis viroid* and *Australian grapevine viroid* cause symptomless infections. Their presence is threatening anyhow, since infected vines may represent inoculum sources for other susceptible crops. Viroid spread can be restrained by prophylactic measures based on the use of viroid-free propagation material. This strategy, however, is not applicable to grapevines because the traditional sanitation techniques based on thermotherapy and meristem tip culture, which are effective against most, if not all viruses, do not work with viroids. With the aim of finding an appropriate procedure for getting rid of these pathogens, we have tested somatic embryogenesis, a technique known for its efficiency against grapevine viruses, as a means for GYSVd-1 and HSVd elimination from four grape cultivars. While both viroids were detected by RT-PCR in embryogenic and non-embryogenic calli from anthers and ovaries of infected grapevines, somatic embryos differentiated from infected calli and embryo-derived plantlets were viroid-free. *In situ* hybridization, used for ascertaining the spatial distribution of GYSVd-1 and HSVd within proliferating calli, showed that both viroids had the expected nuclear localization. The present results support the use of somatic embryogenesis as a proficient method for generating viroid-free material and for further investigating plant-viroid and plant-virus interactions in grapevine.

CO-OCCURRENCE OF FUNGAL ENDOPHYTES AND OOMYCETES IN FOREST ECOSYSTEMS AND THEIR ROLE IN TREE DECLINE. B. Ginetti, A. Ragazzi and S. Moricca. *Dipartimento di Biotecnologie Agrarie, Sezione Protezione delle Piante, Università degli Studi, Piazzale delle Cascine 2, 50144 Firenze, Italy. E-mail: beatrice.ginetti@unifi.it*

In the Boscoincittà Park of Milano (northern Italy), trees of *Acer pseudoplatanus* and *Quercus* display bark cankers, tarry exudates from the lower trunk, stunted growth, and crown decline. From these trees some fungi of the family Botryosphaeriaceae were isolated, including pathogens such as *Botryosphaeria dothidea* and *Neofusicoccum parvum.* Trees were also positive for the presence of *Phytophthora* in serological tests performed *in situ*. Infected trees exhibited greenish- and/or reddish-brown flame-shaped blotches and stripes below the bark. From these areas various *Phytophthora* isolates were recovered on a V8A-PARPNH selective medium. The same agents also occurred in the soil around the trees and in nearby water sources. Isolates from the soil and water were recovered by an apple bait and cultured on a selective medium. To induce fruiting bodies, mycelial portions were transferred to frozen pea medium and then to filtered and sterilized pond water; they were found to belong to species of *Phytophthora*. These species, which are more vital and active in the winter season, may have impaired tree physiology, gradually weakening the trees and predisposing them to the Botryosphaeriaceae in the following spring and summer, when these endophytes become most active. The co-occurrence of these two fungi and oomycetes on the same host and their complementary pathogenic action may well represent a new pathogenic complex causing the decline of the trees in question.

INFLUENCE OF SUBSTRATE AND ECOLOGICAL CONDITIONS ON *ASPERGILLUS FLAVUS* SCLEROTIA SPORULATION. P. Giorni and P. Battilani. *Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy. E-mail: paola.battilani@unicatt.it*

Aspergillus flavus is one of the most dangerous fungi to maize crops as it produces aflatoxins (AFs), i.e. mycotoxins with high toxicity for animals and humans and with immunosopressive and cancerogenic properties. Many studies have been conducted to improve the knowledge on the infection cycle of this fungus, however little information is available on sporulation, the crucial stage for the beginning of fungal infection. Sclerotia represent the main source of inoculum in the field since they can resist for long time in the soil or on debris also in extreme conditions of temperature (T) and water activity (a_w). Both these ecological factors result important for sporulation. An artificial inoculum with sclerotia produced by a strain of *A. flavus* was conducted both on corn stalks pieces (3 cm long) and on Czapek agar (CZ). Substrates were inoculated with a single sclerotium and incubated in different conditions of temperature (5-45°C, with 5°C step) and a_w (0.85-0.99 a_w, with 0.02 a_w step). Sporulation was checked between 2 and 20 days of incubation. Data were analysed as function of T, a_w and as daily sporulation rate. High variability was recorded among sclerotia regarding the amount of spores produced. Nevertheless, sporulation was never observed at 5 and 45°C, while spores were produced at 0.85 a_w. The maximum amount of spores was obtained at 30-35°C and 0.99 a_w after 8 days incubation both on stalks and CZ media. The number of conidia produced was higher on the artificial media than on stalk pieces.

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AN INSIGHT IN SOME POPULATION FEATURES OF *XANTHOMONAS ARBORICOLA* pv. *JUGLANDIS*. D. Giovanardi[1], D. Dallai[1], S. Bonneau[2], M. Le Saux-Fischer[2], C. Manceau[2] and E. Stefani[1]. *[1]Dipartimento di Scienze Agrarie e degli Alimenti, Università di Modena e Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy. [2]Plant Pathology Department, UMR PaVé-INRA Angers, France. E-mail: d_giova81@yahoo.it*

Xanthomonas arboricola pv. *juglandis* (*Xaj*) is the causal agent of bacterial blight of walnut, an emerging disease, which has the potential to severely affect walnut orchards. An Italian strain collection of *Xaj*, obtained during the past 3 years from affected orchards in Romagna, was first assayed with conventional PCR with XajF/XajR primer pair developed to confirm strain identity. The population structure of the collection of *Xaj* isolates, confirms the presence of different genetic groups identified by rep-PCR (using the REP, BOX and ERIC primers) and by multilocus sequence typing (MLST) and multilocus variable number analysis of tandem repeat (MLVA). *Xaj* and *Xaj*-like bacterial isolates from the

Italian collection are currently being analysed by MLSA (multi locus sequence analysis), using 7 primers for 7 different housekeeping genes, with the purpose to better characterise the Italian isolates for phylotyping. The study of copper resistance on a wide collection of over 150 *Xaj* strains frequently showed high resistance (up to 500 ppm Cu^{++}): two strains have been further studied confirming the presence of chromosomal genes *copA* and *copB* involved in the general *copABCD* copper resistance structure, as described for *Pseudomonas syringae*. Sequencing and comparing with other *Xanthomonads* were done. The elucidation of *Xaj* population structure may help to deeper investigate some additional aspects of the molecular epidemiology of the disease, thus allowing a better control strategy in the field.

A COMPREHENSIVE APPROACH TO STUDY THE IMPACT AND THE EPIDEMIOLOGY OF THE INVASIVE FOREST PATHOGEN *HETEROBASIDION IRREGULARE* IN ITALY. P. Gonthier[1], N. Anselmi[2], P. Capretti[3], F. Bussotti[3], M. Feducci[3], M. Garbelotto[4], L. Giordano[1], F. Guglielmo[1], T. Honorati[2], G. Lione[1], N. Luchi[3], V. Mancini[3], S. Michelotti[1], M. Michelozzi[5], G. Nicolotti[1], B. Paparatti[6], M. Pollastrini[3], S. Speranza[6], and A.M. Vettraino[2]. *[1]Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. [2]Dipartimento per l'Innovazione nei Sistemi Biologici, Agroalimentari e Forestali, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. [3]Dipartimento di Biotecnologie Agrarie, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. [4]Department of Environmental Science, Policy and Management, Ecosystem Sciences Division, University of California at Berkeley, 137 Mulford Hall, 94720, Berkeley CA, USA. [5]Istituto di Genetica Vegetale, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy. [6]Dipartimento di Scienze e Tecnologia per l'Agricoltura, le Foreste, la Natura e l'Energia, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: paolo.gonthier@unito.it*

The North American forest pathogen *Heterobasidion irregulare* was introduced in Italy in 1944. The fungus is invasive and is currently distributed in pine stands over about 105 km of coast approximately centred around Rome, often in association with significant mortality of *Pinus pinea* trees. Little is known on the impact and the aggressiveness of this exotic pathogen on native tree species, as well as on its epidemiology and interactions with the closely related native pathogen *Heterobasidion annosum*. The Italian Ministry of Education, University and Research, within the PRIN program, funded a research aimed at: (i) investigating the ecology and epidemiology of *H. irregulare* in Italy, including its level of pathogenicity on native tree species, its spreading ability, its interactions with abiotic factors or related fungal species, and (ii) defining, based on the above information, the contents of a proposal for its control. Several sites outside the current area of invasion have been investigated for the presence of *H. irregulare*. Preliminary data indicate that a northward expansion of its range in the last six years did not occur. Investigations on the role of insect vectors and pruning wounds in the spread of the disease are in progress. Comparative inoculation experiments using *H. irregulare* and *H. annosum* genotypes failed to detect a disproportionate pathogenicity of the exotic pathogen on native pine species. However, preliminary results from pine logs inoculations indicate a higher saprotrophic ability of *H. irregulare* versus *H. annosum*, which may be consistent with the observed invasion process.

RECOMBINANT *PLUM POX VIRUS* ISOLATES RECOVERED IN EMILIA-ROMAGNA REGION. P. Grillini, A. R. Babini, A. D'Anniballe, P. Fini and V. Vicchi. *Servizio Fitosanitario Regione Emilia-Romagna, Via di Saliceto 81, 40128 Bologna, Italy.*

Plum pox virus (PPV) is the causal agent of Sharka, the most dangerous disease of stone fruits, reducing fruit quality and yield. In Italy Sharka has been detected since 1973 but in Emilia-Romagna (northern Italy) it became epidemic on peach only after 1995. Both PPV-D (Dideron) and PPV-M (Marcus) strains are present in our region as well as the recombinant strain of both (PPV-Rec). Since 2007, the strains of 135 PPV isolates from different host species and geographical areas of Emilia-Romagna, were characterized using PCR-RFLP targeting two genome regions (CP and P3-$6K_1$). Twenty-seven isolates from 10 different orchards were identified as PPV-Rec strain. To investigate their genetic diversity in more details, the (Cter)P3-$6K_1$-(Nter)CI and (Cter)NIb-(Nter)CP regions of few isolates were partially sequenced. Nucleotide sequence alignment showed that analysed isolates are closely related to previously characterised recombinant strain and share the same recombination breakpoint in the 3' terminal part of the NIb gene. PPV-Rec isolates were mainly found on European and Japanese plum varieties (Grossa di Felisio, Stanley, Black Top, Angeleno). Only one apricot cultivar (Caldesi 2) and one rootstock (MRS2/5) resulted infected.

CHARACTERIZATION OF *CITRUS LEAF BLOTCH VIRUS* ISOLATES COLLECTED IN OLD ITALIAN CITRUS COLLECTIONS. M. Guardo, T. Marletta, G. Sorrentino, G. Varrica and A. Caruso. *Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale (CT), Italy. E-mail: maria.guardo@entecra.it*

Citrus leaf blotch virus (CLBV), the type species of the genus *Citrivirus,* family *Betaflexiviridae* recently found in Italy, causes a bud union disorder of Nagami kumquat (*Fortunella margarita*) and Calamondin (*Citrus mitis* or *madurensis*) grafted on trifoliate rootstocks. The virus is symptomless on sour orange rootstocks. A survey was carried out with the aim to investigate the presence and the origin of CLBV in old Italian citrus collections. Samples of different citrus species from field trees, were collected in Palermo (botanical garden and San Giovanni degli Eremiti garden) in Naples (botanical garden) in Florence (gardens of Medici's villas of Boboli and Castello) and in a private collection of Pescia (Pistoia). The tests were carried out using one step RT-PCR with specific primers to amplify part of coat protein (CP) and the RNA-dependent RNA polymerase (RdRp) genes; each positive sample was analysed by single-strand conformation polymorphism (SSCP) and samples with different electrophoretic pattern were cloned and sequenced. Among 61 samples only some Nagami kumquat gave positive reaction to CLBV infection. All positive samples showed a predominant SSCP profile belonging to ISA 9-ME-I isolate, found in Sicily in 2006. This suggest that the infected Italian CLBV population could derive from a single origin and that virus introduction may have occurred recently.

TIME COURSE ANALYSIS OF THE EXPRESSION OF GENES POSSIBLY CORRELATED TO THE DIFFERENT SUSCEPTIBILITY OF UNRIPE AND RIPE STRAWBERRY FRUITS TO *COLLETOTRICHUM ACUTATUM*. M. Guidarelli, M. Maradeo, L. Zoli, P. Bertolini and E. Baraldi. *CRIOF, Dipartimento di Produzione e Valorizzazione Agroali-*

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Many fungal pathogens interact with fruits of their hosts at pre-harvest unripe stages and remain quiescent during ripening, causing severe economic losses for post-harvest fruit production. *Colletotrichum acutatum* causes anthracnose symptoms only on ripe strawberry (*Fragaria* × *ananassa*) fruits, whereas, on white unripe fruits, it becomes quiescent as melanized appressoria. A previous microarray experiment revealed that ripe and unripe strawberries, interacting with *C. acutatum*, regulate differently the expression of several genes. Among these genes, a few code for proteins with important regulatory roles in plant response to pathogens. In this study, qRT-PCR was used to make a narrow time scale analysis of the different activation of these genes in white and red fruits challenged with the pathogen. In particular, the expression of *lectin*, *WRKY*, *brassinosteroid insensitve receptor kinase 1 (BR1)*, and phenylpropanoid and flavonoid genes was monitored in fruits after 8-16-20-24 h interaction with *C. acutatum*. Differently from phenylpropanoid and flavonoid genes, the expression of *lectin*, *WRKY* and *BR1* genes was regulated exclusively on white fruits at 24 h post-interaction, when the *C. acutatum* becomes quiescent. Our results suggest that these genes play a specific role in *C. acutatum* quiescence.

MULTIPLEX RT-PCR FOR DETECTING FIVE VIROIDS INFECTING GRAPEVINES. **M. Hajizadeh[1], B. Navarro[2], N. S. Bashir[1], E. M. Torchetti[2] and F. Di Serio[2].** *[1]Plant Protection Department, University of Tabriz, 29 Bahman, 51664 Tabriz, Iran. [2]Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: f.diserio@ba.ivv.cnr.it*

Grapevine is the natural host of at least five viroid species, i.e. *Grapevine yellow speckle viroid 1* (GYSVd-1), *Grapevine yellow speckle viroid 2* (GYSVd-2), *Australian grapevine viroid* (AGVd), *Hop stunt viroid* (HSVd) and *Citrus exocortis viroid* (CEVd). Some species (HSVd, GYSVd-1, GYSVd-2) are of worldwide occurrence, whereas others (AGVd and CEVd) seem to be more restricted geographically. The lack of fast and reliable laboratory procedures for the contemporary detection of multiple viroidal infections is an important limitation for studying the distribution and relative incidence of these viroids in grapevine-growing areas. To fill this gap, a multiplex RT-PCR (mRT-PCR) assay has been developed for the contemporary detection of the five viroids and the simultaneous amplification of the cDNA fragment of a host-derived mRNA (actine mRNA), as an internal positive control. The method was validated testing 57 grapevine plants from Iran infected by one or more viroid species. Concordant results were obtained by our mRT-PCR protocol and single RT-PCR (sRT-PCR) tests conducted using primers previously reported for amplifying separately cDNAs of the monomeric full-length genomic RNA of each targeted viroid. Negative samples were further assayed by Northern-blot hybridization. Except for CEVd, in this survey HSVd, AGVd, GYSVd-1, and GYSVd-2 were detected in 100, 95, 93 and 65% of the tested samples, respectively, confirming their wide distribution in Iran. The present mRT-PCR protocol has the potential to be used routinely for large-scale surveys and certification programs.

PLANT DISEASES IN EXOTIC PURPLE PASSION FRUIT IN THE ANDEAN ZONE **L. Hoyos-Carvajal[1], S. Castillo[1], S. Benítez[1], E. Ortiz[1], L. Farfán[1] and D. Riascos[2].** *[1]Laboratorio de Sanidad Vegetal, Facultad de Agronomía, Universidad Nacional de Colombia, Bogotá, Colombia. [2]Facultad de Ciencias Agropecuarias, Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia. E-mail: limhoyosca@unal.edu.co*

Several species of passion fruits are marketed as exotic fruits in Europe and North America, which has a great impact on local economies in Andean zones. In Colombia, purple passion fruit (*Passiflora edulis*) in one of the most important passionfruits, whose diseases are not well known. Through sampling on main producing areas of the country (9 regions), different causal agents were detected by microbiological, histological and molecular tools, yielding a collecting of *ca.* 200 strains of fungi and bacteria. Among these, *Xanthomonas axonopodis* detected by molecular methods (specific primers) did not correspond to *X. axonopodis* pv. *passiflorae* commonly reported in this family of plants and widely distributed in other passionfruit-producing countries. Histopathological analysis showed systemic distribution in plant of bacterial masses, colonizing vascular tissues, leafs and fruits. *X. axonopodis* can attack other hosts such as *P. ligularis, P. edulis flavicarpa, P. mollissima* and *P. maliformis*. By gene sequencing (ITS, *tef*) from fruits, *Cladosporium cladosporiodes, Colletotrichum boninense* and *C. acutatum* were detected as causal agents of diseases, but not *C. gloeosporioides* which is reported as a common pathogen of fruits. *F. oxysporum* and *F. solani* were detected, which cause diseases in different *Passiflora*, and may attack other hosts such as cape gooseberry (*Physalis peruviana*). The detection of these causal agents and knowledge of their biology allows the appropriate management of diseases in this crop, keeping it sustainable and safe to producers and consumers.

GENOTYPING OF *CLAVIBACTER MICHIGANENSIS* subsp. *MICHIGANENSIS* BY HOUSEKEEPING GENE SEQUENCING AND HIGH RESOLUTION DNA MELT-CURVE ANALYSIS. **G. Ialacci[1], G. Licciardello[2], P. Bella[1], K. H. Garteman[3], R. Eichenlaub[3] and V. Catara[1].** *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia, Zona industriale. Blocco Palma I, 95131, Catania, Italy. [3]2 Lehrstuhl für Gentechnologie/Mikrobiologie, Universität Bielefeld, 33594, Germany. E-mail: vcatara@unict.it*

High-resolution melting (HRM) analysis is emerging as an efficient and robust method for discriminating DNA sequence variants. The objective of this study was to apply HRM analysis to genotyping *Clavibacter michiganensis* subsp. *michiganensis,* causal agent of tomato bacterial canker. The first approach was to identify suitable gene fragments able to distinguish the strains at the subspecies level. To this aim a number of *C. m.* subsp. *michiganensis* strains, isolated in Italy previously genotyped by BOX-PCR and f-AFLP markers, were characterised using the sequences of internal fragments of five house-keeping genes (*gyrB*, *sdhA*, *ligA*, *bipA*, *kdpA*). Approximately 600-800 bp internal fragments of each gene were sequenced on both strands using an automated DNA capillary sequencer for a total of about 100 sequences. Nucleotide sequences were aligned in MEGA4 using CLUSTALW**.** Sequences of *C. m.* subsp. *michiganensis* strains from other countries were included in the *in silico* analysis. The *kdpA* and *sdhA* gene fragment analysis allowed to discriminate the highest number of strains and two primer pairs were designed on the highest variable regions to be used for HRM analysis. The results for HRM of the *kdpA* PCR products showed a better reproducibility. The *C. m.* subsp. *michiganensis* strains were clearly distinguishable from reference strains belonging to other *C. michiganensis* subspecies. The analysis allowed also to detect infrasubspecific variability. HRM analysis provided a resolving

power similar of the other fingerprinting methods and it is a promising approach to easily genotyping bacterial population of plant pathogenic bacteria.

EFFECT OF COMPOST ON ENZYME ACTIVITY, MICROBIAL BIOMASS AND HEALTH OF SOIL IN AN ORGANIC SYSTEM. G. Innocenti[1], M. Montanari[1], C. Ciavatta[2] and S. Scagliarini[3]. *[1]Dipartimento di Protezione Valorizzazione Agroalimentare, Università degli Studi, Viale Fanin 46, 40127, Bologna, Italy. [2]Dipartimento di Tecnologie Agroambientali, Università degli Studi, Viale Fanin 40, 40127 Bologna, Italy. [3]Centro Agricoltura Ambiente Giorgio Nicoli, via Argini Nord 3351, Crevalcore (BO) Italy. E-mail: gloria.innocenti@unibo.it*

The response of soil biota to compost amendment is a very complex phenomenon. There is little information available on microbial density, enzyme activity and suppressive ability of soil after compost addition under field conditions. In particular, there is a lack of sufficient information on compost-microbiota interaction in organic systems. The study site was an experimental long-term organic pear orchard located in the Po Valley near Bologna (northern Italy), on a sandy-loam soil. The soil was amended with a commercial municipal solid waste at 8 and 16 t ha^{-1} $year^{-1}$ in March from 2006 to 2008. Control treatments consisted in a commercial Nitrogen organic product (Fertil) and no fertilisation treatment. Each plot was 232 m^2, with 29 plants, and each treatment was replicated four times in a randomised block design. Soil samples were collected using a soil sampler (30 cm long, 8 cm diameter) in early autumn from 2006 to 2008. Numbers of cultivable fungi and bacteria, and enzymatic activity were determined by plate count technique on selective media and FDA test, respectively. Soil health was assessed using the patho-system *Pythium-Beta vulgaris*. Physico-chemical parameters were also measured. The soil amendment with the highest rate of compost increased the microbial activity, and did not influence the density of microbial groups. Probably the microbial groups were in equilibrium, and the compost as a source of nutrients, was able to stimulate their activity. The suppressive ability was favoured by the highest rate of compost, even if differences with other treatments were not significant.

***STAGONOSPORA NODORUM* BLOTCH: AGRONOMIC AND QUALITY DATA OF THREE DURUM WHEAT VARIETIES. A. Iori, A. Niglio, P. Cacciatori, C. Cecchini, C. Cristofori and M. Chierico.** *CRA, Unità di Ricerca per la Valorizzazione Qualitativa dei Cereali, Via Cassia 176, 00191 Roma, Italy. E-mail: angela.iori@entecra.it*

Stagonospora nodorum (teleomorph *Phaeosphaeria nodorum*) causes stagonospora nodorum blotch on wheat. This pathogen can attack all aerial parts of the plant including the seeds, causing considerable yield losses. The objectives of this preliminary study were to: (i) evaluate the resistant or susceptible behaviour at the adult stage of three durum wheat varieties (Ciccio, Svevo and Simeto) subjected to *S. nodorum* artificial inoculation in the field and (ii) examine the effect of the pathogen on some quantitative and qualitative traits. Field trials were carried out on varieties grown in an experimental field at Montelibretti (Rome) with a split-plot design. Different experimental conditions were used: untreated control, treated control with commercial fungicide, twice inoculated plants at different stages with *S. nodorum* spore suspension (1×10^6 spore/ml), once inoculated plants with the same fungal suspension. Disease severity was evaluated directly in the field considering the percentage of area affected by *S. nodorum* on flag leaf and ear. Grain yield, 1,000 kernel weight, hectolitric weight, protein content and SDS sedimentation test were determined on all samples. Results show that all the varieties at the adult stage were susceptible to the isolate used. Generally the inoculated samples showed values of grain yield, hectolitric weight and 1,000 kernel weight lower than the treated control, while data of the untreated control were variable. Grain protein content was quite similar in all the samples. For SDS sedimentation test no differences between inoculated samples and controls were observed, except for the cv. Ciccio.

ECTOMYCORRHIZAL FUNGAL SPECIES AS SENTINEL ORGANISMS OF TREE HEALTH STATUS. E. Lancellotti, A. Schiaffino and A. Franceschini. *Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via De Nicola 9, 07100 Sassari, Italy. E-mail: afran@uniss.it*

Root colonization by ectomycorrhizal fungi (ECM) is strictly driven by host tree metabolism. Therefore, the alteration of physiological status of the host tree, for instance as a disease consequence, may cause changes in the presence and distribution of ECMs. For this reason some ECMs could be selected as sentinel organisms of tree health status. With this aim in mind a study was carried out to evaluate the influence of tree health status on the ECMs detected in a *Quercus suber* stand where many trees are affected, with different degree, by cork oak decline. The ECM guilds characterizing differently damaged cork oak trees were evaluated, as well as the relationships among tree health status and distribution of genera, phylogenetic assemblages of ECMs and single ECM. The results showed that: (i) *Cortinarius* and *Sebacina* genera colonized trees in the worst health conditions; (ii) some ECM phylogenetic assemblages detected into *Inocybe* and *Tomentella* genera were linked only to healthy trees or diseased trees; (iii) some ECMs (i.e. *Cenococcum geophilum*) colonized mainly diseased trees, while other ECMs (i.e. *Lactarius chrysorrheus*) the healthy ones.

EXPRESSION ANALYSIS OF DEFENSIN-LIKE GENES (*DEFL)* IN GRAPEVINE IN RESPONSE TO *BOTRYTIS CINEREA* AND PLANT DEFENSE HORMONES. L. Lenzi[1], L. Giacomelli[1], C. Zadra[2] and C. Moser[1]. *[1]Edmund Mach Foundation, San Michele all'Adige (TN), Italy. [2]Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi, Borgo XX Giugno 74. 06121 Perugia, Italy. E-mail: claudio.moser@mail.ismaa.it.*

Defensins are a class of small, basic, cysteine-rich peptides found in plant, insect and vertebrates, which share a common tertiary structure and show broad antimicrobial activity. Using a combination of HMM and BLAST searches to scan the *Vitis vinifera* cv. Pinot noir genome, 79 defensin-like sequences (*DEFLs*) have been identified, which correspond to 46 putative defensin genes.To better understand the involvement of this gene family in the signalling and defense against *Botrytis cinerea*, we investigated the changes in expression of 14 grapevine defensins in berries and inflorescences following artificial inoculation with the fungus. In parallel, we measured the content of some jasmonates (jasmonate, methyl jasmonate, OPDA) and salicylic acid (SA) in berries infected with *Botrytis cinerea* and tested whether these defensins are modulated by treatments with these typical mediators of defense. Among the 14 *DEFLs*, five were significantly induced upon *B. cinerea* infection either in inflorescences or in berries. Analytical measurements revealed that SA, JA and OPDA, but

not MeJA accumulated in infected inflorescences and berries. Preliminary data on berries treated with different hormones showed that none of the tested defensins was induced by treatment with MeJA and BTH (an analogue of salicylic acid), whereas one defensin gene was up-regulated after treatment with ethephon, a precursor of ethylene. Our results suggest a role of specific defensin genes in the grapevine defense against *B. cinerea* and the involvement of both MeJA and SA in the plant response. Preliminary data on the regulation of defensin genes by hormones have also been acquired.

SANITARY SELECTION OF CALABRIAN GRAPEVINE GERMPLASM. G. Leo[1], D. Luison[2], A. Roschetti[1], G. Albanese[1] and F. Faggioli[2]. *[1]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università degli Studi Mediterranea, Località Feo di Vito, 89122 Reggio Calabria, Italy. [2]CRA, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: giovanna.leo@unirc.it*

Many old autochthonous grapevine varieties of high value are widely spread in Calabria (southern Italy). The improvement of these cultivars requires the registration of qualified clones after an accurate genetic and sanitary selection, according to European and national rules. Grapevine sanitary selection, essential to exclude the presence of viral diseases, was conducted on the following cultivars: Arvino, Gaglioppo, Greco bianco, Greco nero, Guardavalle, Lacrima, Magliocco dolce, Mantonico bianco, Parmisana, Pecorello and on the rootstock 17-37. One hundred and eight-two biotypes of these cultivars, with optimal agronomic and productive characteristics, were identified in the best grapevine-growing areas of the region. Sanitary selection was carried out inspecting the plants, in spring and autumn, to detect viral symptoms. Woody material, collected from symptomatic and symptomless vines were tested by serological (ELISA) and molecular (Multiplex RT-PCR) analysis to detect the following viruses: *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll associated virus*-1 (GLRaV-1), *Grapevine leafroll associated virus*-2 (GLRaV-2), *Grapevine leafroll associated virus*-3 (GLRaV-3), *Grapevine virus A* (GVA), *Grapevine virus* B (GVB), *Grapevine fleck virus* (GFkV). During field inspections widespread symptoms of leafroll and infectious degeneration were found. The serological and molecular analysis revealed the presence of viruses in 172 samples out of 182 tested (94%). Infected samples showed mostly mixed infections of different viruses (105 out of 172 equal to 61%). The incidence of the identified viruses was: 67%, 60%, 23%, 13%, 13%, 3% and 0.6% respectively for GVA, GLRaV-3, GFLV, GLRaV-1, GFKV, GLRaV-2 and GVB.

GENETIC ANALYSIS OF STEM RUST RESISTANCE IN ELITE DURUM WHEAT GERMPLASM THROUGH ASSOCIATION MAPPING. T. Letta[1,4], I. Terracciano[1], M. Maccaferri[1], A. Badebo[2], K. Ammar[3], J. Crossa[3], P. Oliveira[5], Y. Jin[6], M.C. Sanguineti[1] and R. Tuberosa[1]. *[1]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. [2]Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia. [3]CIMMYT, Int. Apdo Postal 6-641, Mexico D.F., Mexico 06600. [4]Sinana Agricultural Research Center, Bale-Robe, Ethiopia. [5]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA. [6]USDA-ARS, Cereal Disease Laboratory, St.Paul, MN 51108, USA. E-mail: tuberosa@agrsci.unibo.it*

Stem rust (SR), caused by *Puccinia graminis* f. sp. *tritici*, is a highly destructive fungal disease. To identify resistance sources, a panel of 183 elite durum wheat cultivars was characterized for SR response in four consecutive seasons (off- and main-seasons 2009 and 2010) under field condition in Ethiopia, a 'hotspot' site for SR epidemics. The panel was artificially inoculated using isolates comprising Ug99 and a local mixture of urediniospores of durum-specific races prevalent in Ethiopia. The recorded maximum disease severity scores were then converted into coefficient of infection (CI). In average, CI in the off-seasons was markedly higher than in the main-seasons. About 13 and 30 cultivars had CI values <10 in off-seasons and main seasons, respectively. The CI phenotypic distributions indicate that the overall genetics of resistance in the panel is complex. The molecular profiles of the panel (323 SSR and 900 DArT markers) were subjected to association mapping analysis using mixed linear model taking into account population structure and familial relationships. Several chromosome regions putatively involved in SR response were identified. The four regions with the largest effect (R^2 values ranging from 2.3 to 5.7%) were mapped on chromosones 1B, 5A, 6A (coincident with *Sr13*) and 7B. Significant associations were also detected in other chromosone regions not known to harbor SR resistance genes. Our study has identified a number of novel regions for resistance to Ug99 and other Ethiopian races potentially useful to contribute to the improvement of SR resistance in durum wheat.

EFFECT OF SEVERE AND MILD *CITRUS TRISTEZA VIRUS* ISOLATES ON SELECTED GENES EXPRESSION OF DIFFERENT CITRUS HOSTS. G. Licciardello[1], M. Tessitori [2], R. La Rosa[2], G. Nobile[1] , A. Catara[1] and S. Rizza[2] . *[1]Laboratorio di Diagnosi e Biotecnologie Fitosanitarie, Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Blocco Palma I, 95121 Catania, Italy. [2]Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Sezione Fitopatologia e Genetica Vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: glicciardello@pstsicilia.org*

Seven genes, involved in plant defence response (metallothionein, MT and peroxidase, PRX, alcool-dehydrogenase ADH), signal transduction (CONSTANS-like, CLP and ethylene-responsive binding protein, EREBP), aminoacid transport (aminoacid permease, AP) and regulation of gene silencing (rgs-CaM), were selected to evaluate their modulation in different citrus hosts inoculated with *Citrus tristeza virus* (CTV) isolates. Plants with different susceptibility to CTV, as Mexican lime, sour orange, pineapple sweet orange, Duncan grapefruit and Etrog citron were tested 18 months after inoculation with a severe isolate (SY568 like), whereas sour orange and Mexican lime seedlings were also challenged with a mild isolate (T30 like) to compare relative expression levels. Real-time RT-PCR results showed that the selected genes exhibited different patterns of expression depending on the citrus host. ADH and PRX genes were down-regulated in all citrus hosts. Rgs-CaM, AP and CLP were down-regulated in Etrog citron, Duncan grapefruit and sweet orange but were over-expressed in sour orange. Moreover, seedlings of sour orange and Mexican lime, the most susceptible hosts, showed a similar expression pattern for ADH, EREBP and PRX (down-regulated), as well as for MT, rgs-CaM and AP (up-regulated) when infected with either SY568 or T30 like isolates. Nevertheless, changes induced in rgs-CaM gene by the severe viral isolate were 11 and 6 times higher than the mild isolate, in sour orange and Mexican lime, respectively. Over expression of rgs-CaM gene occurred only in highly susceptible plants showing severe symptoms. This gene appears to act as host specific suppressor of RNA silencing, enabling CTV to evade host RNA silencing activity.

TRICHODERMA VIRIDAE **MAY BE A PLANT PATHOGEN. M.G. Li Destri Nicosia, S. Mosca and L. Schena.** *Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università degli Studi Mediterranea, Località Feo di Vito, 89122 Reggio Calabria, Italy. E-mail: lschena@unirc.it*

Trichoderma viridae is commonly known as a biocontrol agent of soil-borne pathogens and is frequently utilized as an active ingredient in bio-formulations. It is also known as a causal agent of postharvest rots on stored lemons, however it has never been reported as a root and/or crown rot pathogen. A strain of *T. viridae* (Fv2) isolated from the basal trunk of young decaying *Pinus nigra* trees with symptoms of decline was re-inoculated on both cuttings and eight-year-old trees of the same pine species. A plug of PDA with actively growing mycelium of *T. viridae* was inserted under the bark and caused extended necrotic lesions around the inoculation site on both cuttings and trees. On two-years-old pine seedlings the same strain, inoculated as previously described, caused the death of 80 and 90% of the plants in one and three months, respectively. *T. viridae* was always re-isolated from symptomatic tissues around the inoculation site. Further studies are needed to evaluate the range of hosts species and the actual role of this strain in causing the decline of pine trees in natural conditions.

EFFECTS OF ESSENTIAL OILS, *BACILLUS AMILOLIQUEFACIENS* **AND ACIBENZOLAR-S-METHYL ON BACTERIAL WILT INCIDENCE AND *RALSTONIA SOLANACEARUM*** **POPULATIONS IN TOMATO PLANTS. V. Ligios and M. Fiori.** *Dipartimento di Protezione delle Piante, Università degli Studi, Via E. De Nicola, 07100 Sassari, Italy. E-mail: fiorim@uniss.it*

The efficacy of 5 Sardinian essential oils from *Helichrysum* sp., *Mentha piperita, M. pulegium, Myrtus communis, Thymus herbabarona*, thymol (Fluka), *Bacillus amyloliquefaciens* and a SAR inducer acibenzolar-S-methyl was evaluated *in vitro* and on tomatoes plants grown in greenhouse for bacterial wilt control. The soil was first infested with a mixture of an equal amount of two virulent *Rs* strains. After 1 h incubation, essential oils and thymol were incorporated into the soil. To evaluate the antagonistic activity of *B. amyloliquefaciens*, the suspensions of *Rs* were incorporated into soil 24 h after the antagonist. Acibenzolar-S-methyl was applied on tomato plantlets as a foliar spray at a concentration of 25 mg/l before transplanting and increased to 50 mg/l after transplanting. The trials were repeated twice. Seven days after essential oils treatments, *Rs* populations density were determined on SMSA. Wilted plants and latent infection, detected by direct isolation and ELISA, were determined one month after transplanting. Bacterial wilt incidence and Rs concentration, compared with control, were reduced considerably on tomato plants transplanted in soil treated with 1 g/l of essential oils, thymol and *B. amyloliquefaciens*. However, the effectiveness of oils and thymol was reduced when application rates were lowered (0.5 g/l). At two concentrations (0.5 and 1 g/l), significantly more wilt occurred in tomato plants grown in soil treated with *M. piperita* oil. Plants treated with acibenzolar-S-methyl were apparently healthy but diagnostic tests revealed *Rs* systemic infection in all plants. The possibility to test essential oils, together with antagonists and SAR inducers are now in progress.

EVALUATION OF ACTIVITY OF LIQUID ORGANIC FERTILIZERS, HEMOZYM NK4.5-6 AND HEMOZYM BIO N5, ON THE GROWTH OF BIOCONTROL AGENTS. A.M. Ligorio, M. Ferrara, F. Nigro and A. Ippolito. *Dipartimento di Biologia e Chimica Agro-Forestale ed Ambientale, Università degli Studi "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. E-mail: ippolito@agr.uniba.it*

Hemozym NK4.5-6 and hemozym BIO N5 are liquid-nitrogen based organic fertilizers, obtained by processing beef blood for pharmaceutical and food industry purposes. In this study, laboratory assays were conducted to determine the effects of both fertilizers on the growth of several biocontrol agents (BCAs).The growth rate of the fungal BCAs *Trichoderma harzianum* F178 and *Clonostachys rosea* F114 was determined on agar plate containing glucose (0.5%) and amended with 0.5 and 5% NK4.5-6. After 6 days incubation at 24±2°C, NK4.5-6 at 5% inhibited the growth of *T. harzianum* F178, whereas at 0.5% growth was enhanced and an abundant sporulation was observed. *C. rosea* F114 was not inhibited by the two concentrations of NK 4.5-6 and at 0.5% the colony diameter was greater as compared to the control. *Bacillus megaterium* EL050, *B. licheniformis* EL90, and *B. subtilis* EL80 were cultured in a minimal medium (MM) amended with 5% of NK 4.5-6 and bacterial growth was monitored 4, 6, 24, and 36 h after inoculation. *B. licheniformis* EL90 and *B. subtilis* EL80 showed a significant increase of the growth rate as compared to the control, whereas no significant variations were observed for *B. megaterium* EL050. The effects of NK4.5-6 and BIO N5, on the growth of fungal BCAs F178 and F114 was also investigated in the soil. After 5 days incubation at 25±2°C, the population of F178 was higher in the soil amended with NK 4.5-6 and BIO N5 as compared to the un-amended control. Based on the results obtained, the use of both formulations can be suggested to improve the performance of antagonistic microorganisms artificially introduced and those naturally present in the soil.

BRANCH DIEBACK OF PHOENICEAN JUNIPER CAUSED BY *DIPLODIA AFRICANA* **IN ITALY. B.T. Linaldeddu, B. Scanu, L. Maddau, S. Seddaiu and A. Franceschini.** *Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: ben@niss.it*

Since autumn 2009, a progressive dieback of shoots and branches of Phoenicean juniper has been observed in a natural area on the island of Caprera (Sardinia, Italy). Necrotic lesions frequently girdled the main stems, causing a rapid death of the upper crown. The fungal isolates obtained in culture from symptomatic tissues were identified according to morphological features as *Diplodia africana*. On PDA at 25°C, all isolates developed a moderately aerial mycelium, initially white then turning dark grey from the centre to the margin after 5 to 6 days. All isolates sporulated well in culture and produced hyaline, aseptate, thick-walled, oblong to cylindrical conidia measuring 30.1±1.8× 11.7±1.1 µm. Identification was confirmed by analysis of the ITS sequence of a representative isolate (DA1). BLAST searches in GenBank showed 100% similarity with reference sequences of *D. africana* including the ex-type isolate (CBS 120835). The representative sequence of isolate DA1, was deposited in GenBank (accession No. JF302648). Its pathogenicity was verified by inoculation on stems of ten 3-year-old seedlings of Phoenicean juniper with PDA plugs colonized by mycelium. Two months after inoculation, all seedlings displayed canopy dieback and inner bark necrosis in the stem, from which the pathogen was successfully reisolated, thus fulfilling Koch's postulates. Five control seedlings remained symptomless. *D. africana* was originally thought to be associated with disease symptoms on stone fruit trees in South Africa. This is the first report of *D. africana* in

the northern hemisphere and also as a fungal pathogen of Phoenicean juniper.

EPIDEMIOLOGICAL AND AETIOLOGICAL INVESTIGATIONS ON THE SEVERE OUTBREAKS OF OLIVE ANTHRACNOSE IN APULIA. A. Longo, M. Ferrara, I. Pentimone, A. Ippolito and F. Nigro. *Dipartimento di Biologia e Chimica Agro-forestale ed Ambientale, Università degli Studi "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. E-mail: nigrof@agr.uniba.it*

Olive anthracnose, caused by *Colletotrichum* spp., is an important disease responsible for major yield losses and poor oil quality. The severe epidemic outbreaks of the disease occurring in Apulia (south-east Italy) during the last years, induced the regional government to declare the emergency status for the provinces of Lecce and Brindisi, in which the disease incidence was 50% in average, frequently reaching 90%. To collect new data on the disease, samples from various olive orchards located in the provinces of Brindisi and Lecce, were analyzed. In October 2010 the incidence of drupe latent infections ranged from 20% to 70%, depending on the cultivar. The epiphytic population of *Colletotrichum* spp. on leaves and flowers showed an increasing trend from February to the end of April 2011; the infection rate on 1-year-old branches varied from 25% to 80%. Isolates of *Colletotrichum* spp. (about 100) collected during the surveys were characterized by morphological characters and molecular assays. Although differences in spore morphology, growth rate and colony colour do not allow a conclusive identification of the species, preliminary data from molecular analysis suggests that the *C. acutatum* species complex is more common than *C. gloeosporioides*. Data from restriction fragment profiles of amplified *Tub*2 gene, showed a prevalence of *C. acutatum* subgroup A4. Isolates showing a restriction profile similar to the molecular group A6 were also found.

FIRST REPORT OF *STEMPHYLIUM* sp. ON OLIVE TREES IN SICILY. S. Lo Piccolo, G. Conigliaro, V. Mondello, L. Torta and S. Burruano. *Dipartimento DEMETRA Università degli Studi, Viale delle Scienze 4, 90128 Palermo, Italy. E-mail: santella@unipa.it*

In the last five years, we have observed a new decline of *Olea europaea* in north-western and southern Sicily (insular Italy). The syndrome consists in more or less extensive leaf chlorosis, sometimes with irregular necrotic marginal or apical spots. Apical defoliation of twigs showing cortical necrosis and withering has often been observed. Isolations, carried out in 2007/08, yilded a complex of fungal genera associated with the syndrome. Among these, *Stemphylium* sp., a well-known foliar pathogen of other plants, was constantly isolated from all the alterations observed. In December 2010, single spore colonies of this fungus were used for artificial inoculations on healthy 3-year-old olive plants (cv. Biancolilla). Necrotic foliar spots, extending from the inoculum site to the apex leaf, and twig cortical necrosis with darkening of internal tissues were, observed from 15/40 and 90/120 days, respectively, after inoculations. Colonies of *Stemphylium* sp. were always re-isolated from all symptomatic organs, thus fulfilling Koch's postulates. To our knowledge, this is the first report of *Stemphylium* sp. as a pathogen to olive trees in Sicily. Further morphological and molecular studies are in progress to achieve specific identification.

BACTERIAL CANKER OF KIWIFRUIT BY *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE*: DETECTION OF THE BACTERIUM IN FRUITS AND POLLEN. S. Loreti, A. Gallelli, A. L'Aurora and S. Talocci. *CRA, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: stefania.loreti@entecra.it*

Bacterial canker of kiwifruit induced by *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is causing serious damages to *Actinidia deliciosa* and *A. chinensis* in Italy. The severe outbreak of the disease raises important questions about trade of kiwifruit materials like pollen and fruit subjected to import-export among countries. The presence of *Psa* in asymptomatic fruits and pollen was investigated from October 2009 to October 2010. Symptomless fruits and pollen samples were analysed by bacterial isolation and two conventional PCR protocols. The presence of the bacterium in fruits samples was investigated, for its endophytic presence, in 50 fruits in bulk. *Psa* was detected in 6 of 49 fruit bulk-samples by isolation, and in 10 of 49, by PCR. One bulk fruit sample, was PCR-positive. The PCR performed for each single fruit showed a faint signal by both the PCR assays in 5-7 of 50 fruits assessed. Concerning pollen samples, *Psa* was detected in 8 of 11 pollen samples by isolation, and in 10 of 11 samples by PCR. The obtained results showed that fruit and pollen of kiwifruit can harbour this emerging bacterium. *Psa* detection from bulk fruit samples is probably due to the summation of a low charge of *Psa* distributed over more fruits; as a consequence, the risk of introducing the bacterium in *Psa*-free areas could be negligible. The role of the pollen in the dissemination of the disease is object of discussion and of development of further studies.

TOWARDS THE IDENTIFICATION OF RESISTANCE-RELATED GENES IN *VITIS RIPARIA*: *AGROBACTERIUM*-BASED FUNCTIONAL GENOMICS. A. Lovato, L. Bortesi, S. Dal Santo, T. Pandolfini, M. Pezzotti and A. Polverari. *Dipartimento di Biotecnologie, Università degli Studi, Strada le Grazie 15, 37134 Verona, Italy. E-mail: annalisa.polverari@univr.it*

Establishment of functional genomics in *Vitis* spp. is still difficult, due to the lack of reliable high-throughput tools for grapevine transformation. We are presently approaching two *Agrobacterium*-mediated strategies in *Vitis riparia*, which is resistant to *Plasmopara viticola,* to study the function of genes possibly related to the defense response. Genes have been identified in a previous microarray analysis, on the basis of their specific expression in *V. riparia* at early stages of downy mildew infection. The first strategy is based on vacuum agroinfiltration of grape leaves. The technique was optimized for the expression of the GUS reporter gene and for the silencing of the grapevine phytoene desaturase (PDS) gene. In the second strategy we used *Agrobacterium rhizogenes* hairy root production to test whether the silencing signal triggered by expression of the anti-PDS hairpin construct can spread systemically from hairy roots to the leaves, resulting in down-regulation of target genes. *V. riparia* plantlets were infected with *A. rhizogenes* ARQA1 transformed with a pRedRoot vector containing a 35S-hairpin construct against the grapevine PDS gene and a marker gene encoding the red fluorescence protein (DsRed1) under the control of a constitutive root promoter. This strategy should allow us to select plantlets with transformed roots based on red fluorescence emission and to observe the possible chlorophyll degradation caused by PDS gene silencing, if any.

FUSARIUM HEAD BLIGHT RESPONSE QTLs IN DURUM WHEAT. M. Maccaferri[1], G. Ferrazzano[2], M. Pascale[3], K. Ammar[4], M.A. Canè[1], P. Mantovani[2], A. Prodi[1], P. Nipoti[1], M.C. Sanguineti[1] and R. Tuberosa[1]. *[1]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. [2]Produttori Sementi Bologna, Via Macero 1, 40050 Argelato (BO), Italy. [3]Istituto di Scienze delle Produzioni Alimentari, CNR, Via Amendola 122, 70126 Bari, Italy. [4]CIMMYT, Int. Apdo Postal 6-641, Mexico City, 06600 Mexico. Email: marco.maccaferri@unibo.it*

Fusarium head blight (FHB) is a serious challenge for durum wheat production in environments subjected to recurrent epidemics, such as northern and central Italy. Effectiveness of breeding for quantitative trait (e.g. FHB response) can be improved through quantitative trait loci (QTL) mapping and subsequent marker-assisted selection activities. The presence of QTLs for FHB response was investigated in the cv. Kofa x Svevo population which includes 249 recombinant inbred lines (RILs) with known genotype at *ca.* 200 simple sequence repeat markers. In 2010, the RILs were evaluated in three field trials carried out in Italy (Argelato and Cadriano, Bologna) and Mexico (El Batan). The trials were mist-irrigated and artificially inoculated with local, highly virulent strains of *Fusarium graminearum*. FHB incidence and severity, percentage of damaged kernels, grain weight, test weight and DON content were recorded. The final infection levels were always high with the susceptible parent Kofa consistently showing an FHB incidence score (70-100%) higher than the medium-resistant parent Svevo. Heritability of FHB incidence and severity ranged from 15 to 60%. Some RILs showed considerably lower FHB disease scores than Svevo. In total, ten QTLs for FHB response were identified. Two main QTLs affecting all the FHB-related traits were located in chromosomes 2BL and 3BS. Some minor QTLs (chromosones. 1A, 1B, 6A and 7B) showed specificity for FHB-responses, while others (chromosones. 2A, 2B, 4A and 4B) affected FHB-responses and either heading date or plant height. These results indicate the presence of novel QTLs for FHB-response in the elite durum germplasm.

GENETIC CONTROL OF RESISTANCE TO *SOIL-BORNE CEREAL MOSAIC VIRUS* IN DURUM WHEAT. M. Maccaferri[1], R. Francia[1], C. Ratti[1], C. Rubies Autonell[1], A. Massi[2], S. Stefanelli[1], R. Tuberosa[1] and M.C. Sanguineti[1]. *[1]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. [2]Produttori Sementi Bologna, Via Macero 1, 40050 Argelato, Bologna, Italy. E-mail: marco.maccaferri@ unibo.it*

Soil-borne cereal mosaic virus (SBCMV) is the causal agent of a viral disease which affects wheat in Europe. In durum wheat, the cultivated germplasm shows responses ranging from susceptibility to almost complete resistance. Genetic analysis of SBCMV resistance was carried out using two recombinant inbred line (RIL) populations of 180 lines each, obtained from the crosses Meridiano (resistant)×Claudio (moderately susceptible) and Simeto (susceptible) ×Levante (resistant). The RILs were characterized for SBCMV response (symptom severity scores and virus concentration in the leaves) in the field in 2007, 2008 and 2009 under severe and uniform SBCMV infections and were genotyped with SSR and DArT markers. Transgressive segregation was observed for the disease response traits, with high heritability values, consistently >80%. A major QTL (*QSbm.ubo-2BS*) in the distal chromosome 2BS region was identified in both populations; it accounted for 60-85% of the phenotypic variation for symptom severity, 40-55% for virus concentration and 15-30% for grain yield. The meta-QTL analysis carried out using the entire dataset suggested that in both populations SBCMV resistance is likely to be controlled by the same QTL, with the favorable allele being contributed by cvs Meridiano and Levante. Our results confine *QSbm.ubo-2BS* in a *ca*. 2 cM-wide interval flanked by SSR markers that are being used for marker-assisted selection. Several QTLs with minor effects were also detected. The presence of *QSbm.ubo-2BS* was validated in a panel of 111 cultivated durum wheat accessions. The reported results open to the possibility to further characterize *QSbm.ubo-2BS* and suggest its feasibility for a positional cloning approach.

RESPONSE TO *SEPTORIA TRITICI* IN THE DURUM WHEAT GERMPLASM INVESTIGATED THROUGH ASSOCIATION MAPPING. M. Maccaferri[1], A. Ricci[1], K. Ammar[2], R. Talebi[3], T. G. Mahmod[3], G. Kema[3], S. Corneti[1], M. C. Sanguineti[1] and R. Tuberosa[1]. *[1]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. [2]CIMMYT, Int. Apdo Postal 6-641, Mexico City, Mexico 06600. [3]Plant Research International B.V., 6700 AA Wageningen, The Netherlands. E-mail: marco.maccaferri@ unibo.it*

Durum wheat production in the Mediterranean basin is plagued by a range of biotic stresses. Among these, *Septoria tritici* blotch (*Mycosphaerella graminicola*) has become important in southern Europe and north Africa. The high genome plasticity of the pathogen and its specialization features (durum *vs*. bread wheat) complicates the identification of valuable resistance genes (e.g. durable and effective across diverse regions). The genetic variation for the response to *Septoria* and the chromosomal location of resistance factors were studied using a germplasm collection of 183 durum accessions of diverse origin suitable for association mapping (Maccaferri *et al.*, 2006, *Plant Genetics Resources* **4**: 79-85). The panel was evaluated for two consecutive years (2008 and 2009) in Tunisia (Beja), Mexico (Toluca) and Italy (Argelato and Ferrara). The accessions were then inoculated under controlled conditions with *Mycosphaerella* isolates collected from durum wheat in a range of Mediterranean countries, as well as with bread wheat isolates. The germplasm collection was genotyped with *ca.* 300 simple sequence repeat (SSRs) markers of known map position and *ca*. 900 durum DArT markers. Highly diversified marker-trait association patterns were obtained based on the field and seedling (as % of necrotic leaf area and picnidia production). A preliminary analysis highlighted some chromosome regions consistently involved in *Septoria* resistance, particularly in chromosomes 1BL, 2AL and 4AL that accounted for a sizeable portion of phenotypic variation among accessions.

DISSECTION OF THE POWDERY MILDEW-RESISTANT RESPONSE PRESENT IN THE DURUM WHEAT cv. CLAUDIO. M. Maccaferri[1], A. Ricci[1], F. Bini[1], F. Chen[1], S. Stefanelli[1], S. Vecchi[1], S. Corneti[1], A. Massi[2] and R. Tuberosa[1]. *[1]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. [2]Produttori Sementi Bologna, Via Macero 1, 40050 Argelato, Bologna, Italy. E-mail: marco.maccaferri@ unibo.it*

Powdery mildew (Pm) is one of the main foliar diseases affecting durum wheat in southern Europe. To date, a few resistance sources have been characterized and mapped in durum wheat. Based on repeated field observations, the durum wheat cv. Claudio shows a resistance response consistent over the years. To identify the Pm resistance loci carried by cv. Claudio, a population of 181 recombinant inbred lines from the cross Claudio (re-

sistant) × Meridiano (susceptible) has been evaluated in two replicated field trials carried out under artificially inoculated conditions in 2007 and 2008 at Bologna (northern Italy). In parallel, a linkage map based on SSR and DArT molecular markers was produced. The frequency distribution of the data collected over two years showed that the genetic control of Pm response in this population is rather complex. Two major quantitative trait loci (QTLs) have been located on chr. 6BL (*QPm.ubo-6B*) and 7BL (*QPm.ubo-7B*) with both resistance alleles contributed by cv. Claudio. The effect of *QPm.ubo-7B* against Pm infection declined as the disease progressed, while *QPm.ubo-6B* showed an increased effectiveness. Additional minor QTLs with lower and less consistent effects across the years were found on chromosomes 2BS, 2BL, 6AS and 7AS, with both parents contributing resistance alleles. *QPm.ubo-7B* is tagged by *barc340*, *gwm146* and *gwm344*, possibly located in the homoeologous position of *Pm1*. Claudio is thus characterized by a polygenic resistance based on two main loci and several minor genes.

LACCASE ACTIVITY IN *TRICHODERMA VIRENS*. **L. Mannella[1], L. Guglielminetti[2], G. Vannacci[1] and M. Vergara[3].** *[1]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. [2]Dipartimento di Biologia, Università degli Studi, Via Mariscoglio 34, 56124 Pisa, Italy. [3]Scuola Normale Superiore, Piazza dei Cavalieri 7, 56126 Pisa, Italy. E-mail: rvergara@agr.unipi.it*

Fungal laccases are involved in multiple functions, such as lignin degradation, pigments (melanin) synthesis and degradation, detoxification and pathogenesis. Furthermore, they are useful biocatalysts for several biotechnological applications.The laccase gene function was previously analysed in *Trichoderma virens*, an effective biocontrol agent: six genes were identified and one of them was deleted and shown to be involved in the mycoparasitic activity against *B. cinerea* sclerotia. Laccase activity in some *Trichoderma* spp. is also associated with the production of green pigment in conidial spores. Further investigations on the laccase gene family in *T. virens* were performed in order to explore mechanisms putatively involved in ligninolysis, conidiogenesis and industrial dyes decolorization. Laccase functions in lignocellulosic process and sporulation mechanisms were studied by growing *T. virens* on two different substrates: wheat straw liquid medium, containing lignocellulose as the only carbon source, or solid Hölker medium, formulated to induce spore formation. In addition, liquid cultures containing twelve commercial textile dyes were set up and *T. virens* efficiently decolorized three of them. Biochemical and expression analyses performed on these different experimental systems suggested different roles of the relative enzymes in regulating multiple mechanisms. In conclusion, information is gained about some properties of the *T. virens* laccase gene family. Six genes are shown to be differentially involved in physiological processes in *T. virens* some of which are important for its fitness or antagonistic attitude while others are exploitable in biotechnological applications related to ligninolysis or to textile dyes decolorization.

BIOCONTROL OF *PENICILLIUM DIGITATUM* AND *P. ITALICUM* OF ORANGE BY *AUREOBASIDIUM PULLULANS*. **C. Mantelli, A. Spadoni and M. Mari**. *CRIOF, Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale G. Fanin 46, 40127 Bologna, Italy. E-mail: marta.mari@unibo.it*

Penicillium italicum and *Penicillium digitatum* causing blue and green mould, respectively, are the most common and serious pathogens of citrus fruits in Mediterranean climates. Although fungicides are commonly used worldwide to control citrus postharvest moulds, alternatives to chemicals such as biological control agents (BCAs) are extensively investigated. The aim of the present work was to investigate the efficacy of yeast *Aureobasidium pullulans*, L1 and L8 strains, isolated from the surface of healthy peach as a putative BCA for citrus blue and green mould. In *in vivo* trials, cv. Valencia oranges were treated with washed or autoclaved cells (10^8 CFU ml^{-1}) or with sterile filtrate of L1 and L8 and were subsequently inoculated with a conidial suspension ($5x10^3$ conidia ml^{-1}) of each pathogen. After 10 days at 20°C, the washed cells completely inhibited *P. italicum* and *P. digitatum*, no control of decay was observed in fruits treated with sterile filtrates, while autoclaved cells of both BCAs reduced the incidence of disease (-50%) compared to the control. In *in vitro* experiments the production of volatile compounds by BCAs was evaluated by a dual culture technique. L1 and L8 strains reduced the *P. italicum* growth of 48% and 45%, respectively with respect to the control, while *P. digitatum* was inhibited by 29% (L1) and 28% (L8). In a previous work, L1 and L8 strains showed a good efficacy towards *Monilinia laxa, M.fructicola* and *M. fructigena* attacks to peach and the present results confirm the broad spectrum of activity of this BCA against fruit postharvest pathogens.

PRELIMINARY INVESTIGATIONS ON FUNGAL DISEASES OF GOLF COURSES IN NORTHERN ITALY. **C. Martini, L. Mazzarolo and M. Mari.** *CRIOF, Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Via Gandolfi 19, 40057 Cadriano (BO), Italy. E-mail: marta.mari@unibo.it*

The aim of this study was to monitor the fungal diseases in four golf courses located in northern Italy and to characterize the fungal isolates by testing the resistance to fungicides commonly used against turf diseases. The influence of temperature on resistance to propiconazole, tebuconazole and prochloraz of *Sclerotinia homoeocarpa* isolates was also investigated. Over 200 diseased turf samples were collected during the 2008, 2009 and 2010 summers. *S. homoeocarpa* was the main fungal pathogen isolated (33%) followed by *Microdochium nivale* (29%), *Curvularia inaequalis* (20%) and *Curvularia coicicola* (6%). The occurrence of *Colletotrichum graminicola*, *Rhizoctonia cerealis* and *Laetisaria fuciformis* was low compared to other fungi. Ninety isolates of *S. homoeocarpa* were tested for sensitivity to the demethylation-inhibiting (DMI) fungicides (propiconazole and tebuconazole) and the inhibiting ergosterol biosynthesis (BSA) fungicides (prochloraz). Both DMI and BSA fungicides are registered for turfgrass in Italy. The pathogen's sensitivity to fungicides was assessed using the discriminatory concentrations of 0.2 mg/l for propiconazole, tebuconazole, and prochloraz. The EC_{50} was estimated on relative mycelial growth on fungicide-amended medium confirming the presence of resistance to propiconazole, tebuconazole and prochloraz in Italian *S. homoeocarpa* isolates. In addition, the isolates were inoculated on medium amended with the EC_{25} fungicide concentration and incubated at different temperatures (10°C, 20°C and 30°C) for 72 h. At 30°C the reduction of fungal growth on the fungicide amended medium was higher than that observed at 20°C (60% against 20%). More investigation are in progress to elucidate the molecular bases of this phenomenon.

SENSITIVITY TO TEBUCONAZOLE AND THIOPHANATE METHYL OF *MONILINIA LAXA* FROM EMILIA ROMAGNA ORCHARDS. C. Martini[1], G. Schnabel[2] and M. Mari[1]. *[1]CRIOF, Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Via Gandolfi, 19, 40057 Cadriano (BO), Italy. [2]Department of Entomology, Soils, and Plant Science, Clemson University, Clemson SC 29634, USA. E-mail: marta.mari@unibo.it*

Demethylation inhibitor (DMI) and to a lesser degree methyl benzimidazole carbamate (MBC) fungicides are used in many European production areas to control stone fruit diseases and to avoid economic losses. Symptomatic blossoms, twigs, and fruits were collected in summer from stone fruit orchards in Emilia Romagna (northern Italy) between 2009 and 2010. Over 100 single-spore isolates were generated; 61 were *Monilinia laxa*, 34 were *M. fructicola* and 5 were *M. fructigena*. Sensitivity of *M. laxa* to DMI fungicide (tebuconazole) and MBC fungicide (thiophanate methyl) was determined. Sensitivity was assessed with the spiral gradient endpoint method developed in 2004. The concentration of the fungicide is highest at the center of the plate and lowest at the edge of the plate. The EC_{50}values were calculated using SGE software. The range of EC_{50} values for tebuconazole was 0.004 to 0.32 µg/ml and for thiophanate methyl 0.03 to 2.37 µg/ml. Four *M. laxa* isolates were medium resistant to tebuconazole with EC_{50} values of 0.12, 0.16, 0.18 and 0.32 µg/ml and 57 isolates were sensitive to tebuconazole with EC_{50} values ranging from 0.004 to 0.063 µg/ml. Six *M. laxa* isolates exhibited low resistance to thiophanate methyl with EC_{50} values ranging from 0.88 to 2.37 µg/ml and 55 isolates were sensitive to thiophanate methyl with EC_{50} values ranging from 0.03 to 0.69 µg/ml. The sensitivity phenotypes of selected isolates were verified with discriminatory doses of 0.1 and 0.3 µg/ml for tebuconazole and 1 µg/ml for thiophanate methyl.

***ACIDOVORAX AVENAE* subsp. *CATTLEYAE* ON ORCHID IN LIGURIA. P. Martini[1], M. Odasso[1], L. Repetto[1], E. Biondi[2], S. Mucini[2] and U. Mazzucchi[2].** *[1]Istituto Regionale per la Floricoltura, Via Carducci 12, 18038 Sanremo (IM), Italy. [2]Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 40, 40127 Bologna, Italy. E-mail: umazzucc@agrsci.unibo.it*

In Liguria region (northern Italy), during 2007 pot-grown plants of *Phalaenopsis* hybrid, cv. Malibù Lagoon, with leaf spots symptoms were observed in a commercial nursery at Albenga (SV). In 2010, the same symptoms were observed on *Phalaenopsis* hybrid, cv. Musashino Moon, in a commercial glasshouse near Sanremo (IM). The leaves of both samples showed soft, water-soaked lesions which eventually turned brown or black. The older plants died. To isolate the causal agent, cut leaves tissue (1-3 mm tips) of each sample exhibiting bacterial streaming were disinfected for 1 min in 10% NaClO solution and rinsed for 1 min with SDW; then the tissues were macerated into mortars containing a sterile saline solution. Serial dilutions up to 1×10^{-4} were made in sterile water; 10 µl of each suspension was plated onto NA medium and incubated at 25°C for 3 days. Non-fluorescent, Gram-negative bacteria were constantly isolated from the lesions. For each sample, three pure cultures were identified phenotypically. Using Biolog GEN III Microplates and the Micrologg System (data base 5.1.1; Biolog, Hayward, CA) the bacteria showed 0.927-0.997 similarity with *Acidovorax avenae* subsp. *cattleyae.* The identity was confirmed by PCR using specific primers. Pathogenicity tests were carried out on *Phalaenopsis* hybrid plants. The isolates caused typical symptoms and reisolations were successful. This is the first report of *A. avenae* subsp. *cattleyae* on *Phalaenopsis* hybrids in Liguria, and the third in Italy.

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EVIDENCE AGAINST PATHOADAPTATION OF *ERWINIA AMYLOVORA* IN EMILIA-ROMAGNA AND VENETO INVOLVING AN *HRP*-LINKED PATHOGENICITY LOCUS, *DSP*EF. P. Minardi[1], S. Mucini[2] and U. Mazzucchi[2]. *[1]Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. [2] Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. E-mail: umazzucc@agrsci.unibo.it*

In Emilia-Romagna and Veneto (northern Italy) a clone of *Erwinia amylovora* (Ea1994) gradually spread mostly on pear trees, starting from the primary 1994 foci. In recent years an unusual increase of certified fire blight cases on apple trees was observed. The introduction of novel strains or the modification of virulence in the existing clonal populations were hypothesized. A pathoadaptation to the niche of a different natural host might involve the *dsp*EF locus of the pathogenicity island (PAI1) subjected to immune selective pressure of the host plants. Pathoadpatation hypothesis was tested by restriction fragment analysis of the *dsp*E gene, by the *dsp*F gene sequence analysis in strains with virulence higher than Ea1994 and by *dsp*E and *dsp*F genes sequence analysis of Ea1994. The restriction profiles were indistinguishable. Moreover, sequence analysis revealed that the sequences of the *dsp*E and the *dsp*F genes correlated 100% with those of Ea321, a wild-type North American strain, used for comparison. The results confirmed that the *dsp*EF locus is highly conserved in the genome of the species. Moreover, our results showed that in Emilia Romagna and Veneto *E. amylovora* strains more virulent than the one associated with the primary foci can be found on different host plants and locations, and that the increase of fire blight cases in apple do not seem attributable to pathoadaptation involving changes in the *dsp*E and *dsp*F genes, without ruling out possible modifications in their expression.

THE *DSP*F AMPLICON AS A RELIABLE DIAGNOSTIC TRAIT FOR THE FIRE BLIGHT PATHOGEN, *ERWINIA AMYLOVORA*. P. Minardi[1], S. Mucini[2], C. Lucchese[2] and U. Mazzucchi[2]. *[1]Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. [2]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. E-mail: umazzucc@agrsci.unibo.it*

Fire blight has gradually spread in Europe, becoming endemic in many areas. Indeed, in northern Italy, except for Valle d'Aosta, cases of fire blight have been ascertained in all regions. An accurate diagnosis of fire blight is essential to gather reliable evidence of its appearance in new areas. Moreover, an additional assay for the identification of *E. amylovora* (*Ea*) can be very useful in the analysis of asymptomatic nursery material during the quiescent period. In Italy, the currently used diagnostic protocol was developed about 15 years ago when the compulsory control decree was laid down (DM 356, 1999) and needs now to be updated. In the most recent diagnostic protocol proposed by EPPO (2004) the analysis includes the use of PCR primers designed to amplify sequences of the pEA29 plasmid that until a few years ago was

believed to be present in all strains of *Ea* and essential for virulence. Recent studies have drawn attention to the existence of virulent *Ea* strains free of pEA29. The risk of false negative reactions caused by the absence of this plasmid could not be excluded. In this study, the development is described of a PCR assay for *Ea* diagnosis based on the use of primers designed on the sequence of the *dsp*F gene which encodes the chaperon of the crucial effector DspE. Its reliability was tested in a collection of *Ea* strains, and in saprophytic and pathogenic bacteria isolated from *Ea* host plants. The results showed that the PCR primers based on the sequence of the *dsp*F gene produce an amplicon highly reliable for the diagnosis of fire blight.

STUDY OF THE *DSP*E GENE ESPRESSION IN *ERWINIA AMYLOVORA* USING THE GREEN FLUORESCENT PROTEIN. **P. Minardi[1], S. Mucini[2] and U. Mazzucchi[2].** *[1]Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. [2]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. E-mail: umazzucc@agrsci.unibo.it*

In phytopathogenic bacteria, the study of gene expression is of great value to understand the mechanisms involved in pathogenicity. The use of fluorescent markers, such as GFP, allow to observe the pathogen *in planta* and to better understand the host-pathogen interaction focusing on the temporal expression of the genes involved. In *E. amylovora* (*Ea*), the expression of the genes of the pathogenicity island (PAI1) play a crucial role in the early plant-pathogen interactions.With this preliminary study we aim at determining the *in vitro* and *in planta* conditions and timing under which the *dsp*E gene expression occurs. To do so, the reference *Ea*1994 strain, isolated from pear in 1994 and virulent to pear trees, and 7 northern Italian strains more virulent than *Ea*1994 were electro-transformed with the pDFl 124.B1 plasmid containing the gene for the protein *gfp*III GFP under the control of the *Ea dspE* gene encoding the DspE effector essential for pathogenesis. As for the *dspE* gene expression in minimal medium, our preliminary data confirm that the *dsp*E gene is expressed only in conditions of nutrient deficiency. The absence of the expression in rich media is associated with several factors including pH and a high osmolarity, and the presence of carbon sources available for the pathogen. As for the *dspE* gene expression in immature pears, our results showed that *Ea* may need an initial period of adaptation to the host niche since the *dspE* gene expression was observed between 24 and 48 h after penetration. However, the *dspE* gene expression in immature pears, in the first 24 h, concurs with the hypothesis that the *dsp*E gene product is injected into the host cell 3-4 h after penetration when the bacterium has not yet multiplied or has just completed its first division.

EVIDENCE AGAINST THE PRESENCE OF *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* IN FRUITS OF ACTINIDIA ORCHARDS AFFECTED BY BACTERIAL CANKER IN EMILIA-ROMAGNA. **P. Minardi[1], C. Lucchese[2], S. Ardizzi[2] and U. Mazzucchi[2].** *[1]Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. [2]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. E-mail: umazzucc@agrsci.unibo.it*

In Emilia Romagna, kiwifruit is of great economic relevance for the horticultural industry and large investments have been made in production plants and commercial platforms. From October 2009, the sudden appearance of sporadic cases of bacterial canker and the high costs of diagnosis and control are threatening the prospects of the entire industry which plays a relevant role for the Italian agricultural export as a whole. Hence, to investigate whether the fruits themselves can harbor the causal agent of bacterial canker of kiwifruit, *Pseudomonas syringae* pv. *actinidiae* (*Psa*) acting as a possible source of dissemination of the pathogen, is essential. Samples of fruits of *Actinidia chinesis* cv. Jin Tao were harvested from symptomatic plants affected by bacterial canker and symptomless plants close to them before breaking down. Two samples were harvested directly from the plants and one sample from waste bins. Groups of 15 fruits were assayed for the presence of *Psa* on the surface and within the columellae. Isolation from these samples allowed to select 36 *Psa*-like cultures (19 epiphytes and 17 endophytes). PCR analysis using *Psa*-specific primers, HR on tobacco leaves and pathogenicity test on tobacco leaves and on *Actinidia deliciosa* seedlings excluded the presence of *Psa.* Our results show that in the fruits of plants affected by bacterial canker, or close to them, *Psa* is not associated with either the surface or inside the fruit. However it cannot be ruled out that *Psa* cells present on the fruits might be numerically below the sensitivity threshold of the detection technique.

DIGOXIGENIN-LABELED DNA PROBES APPLIED TO THE SIMULTANEOUS DETECTION OF TEN ARTICHOKE VIRUSES. **S.A. Minutillo[1], T. Mascia[2,3] and D. Gallitelli[2,3].** *[1]Agritest S.r.L. Strada Provinciale per Casamassima 3, 70010 Valenzano (BA), Italy. [2]Dipartimento di Biologia e Chimica Agroforestale ed Ambientale, Università degli Studi "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. [3]Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E. mail: serenaminutillo@hotmail.it*

Artichoke Italian latent virus (AILV), *Artichoke latent virus* (ArLV), *Artichoke mottled crinkle virus* (AMCV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Pelargonium zonate spot virus* (PZSV), *Tomato infectious chlorosis virus* (TICV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) and *Turnip mosaic virus* (TuMV) are pathogernic to artichoke, so new artichoke fields should be planted with certified virus-free germplasm. Accurate assessment of the sanitary condition of artichoke nursery productions can be carried out using nucleic acid-based tests, which overcome some of the drawbacks of serology-based virus detection for this crop. We have developed a protocol for the simultaneous detection of ten artichoke viruses by non-isotopic molecular hybridisation with a DNA probe. All DNA probes had approximately the same size and were labelled with DIG-UTP in a PCR reaction. The probe mix detected all viruses with a sensitivity similar to that obtained using individual probes. In addition, we evaluated the possible use of the tissue-printing as a sample preparation technique applied to routine diagnosis of artichoke viruses. DNA probes were also used to detect AILV and ArLV in artichoke seed coats and cotyledons and for early detection in young explants produced during sanitation of artichoke germplasm. The detection limit for each individual probe was determined by using the complementary unlabeled plus-strand viral RNA added to artichoke sap extracted from healthy plants. The color-based detection of hybrids overcomes the needs of expensive equipments and makes the technique affordable also for small diagnostic laboratories.

SOURCES OF RESISTANCE TO THE TUBER NECROTIC STRAIN OF *POTATO VIRUS Y* (PVY^{NTN}) IN WILD POTATO SPECIES. V. Miraglia[1], R. Garramone[1], C. Villano[1], D. Carputo[1] and D. Alioto[2]. *[1]Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università degli Studi di Napoli, Via Università 100, 80055 Portici (NA), Italy. [2]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli, Via Università 100, 80055 Portici (NA), Italy. E-mail: alioto@unina.it*

The tuber necrotic strain of *Potato virus Y* (PVY^{NTN}) can cause tuber necrotic ringspot, one of the most devastating diseases of cultivated potato. The use of resistant varieties is the best approach to control this virus in the absence of effective control measures. Source of resistance can be identified within the large genetic variability of wild potato germplasm. The objective of this work was to assess the response to artificial PVY^{NTN} inoculation of 21 genotypes belonging to 12 wild potato species. Plants of each genotype were grown under greenhouse conditions and were mechanically inoculated with PVY^{NTN}. Plants were visually inspected for symptom expression 15 and 30 days post inoculation. On the same day, tissue samples from upper non inoculated leaves were collected for serological analysis by ELISA. ELISA-negative plants were re-tested by RT-PCR. The screening test was replicated twice to rule out any potential escapes. Four genotypes belonging to *Solanum bulbocastanum, S. etuberosum, S. cardiophyllum* and *S. phureja* were extremely resistant (no disease symptoms and negative ELISA and RT-PCR reactions from all tested plants). Two genotypes showed partial resistance, in one the percentage of resistant plants decreased over time, in the second systemic infection was delayed of some days. Moreover, the similarity indexes obtained from SSR analysis highlighted differences among genotypes. Genotypes identified here will be used in breeding programs aimed at transferring the resistance into cultivated potato.

SELECTION AND *IN VITRO* CHARACTERIZATION OF POTENTIAL BIOCONTROL AGENTS TO PROTECT LAMB'S LETTUCE IN HYDROPONICS. S. Moruzzi, G. Firrao, and M. Martini. *Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. E-mail: marta.martini@uniud.it*

Fungi of the genera *Pythium* and *Rhizoctonia*, particularly adapted to wet environment, are among the most harmful pathogenic microorganisms in hydroponics. Biocontrol microorganisms can play an important role in suppressing these root pathogens in soilless systems. In the present work, bacterial microorganisms were isolated in order to select potential biocontrol agents. Fifty-one strains were isolated from 30 root samples, collected from healthy hydroponic lamb's lettuce plants grown in Friuli Venezia Giulia (FVG, north-eastern Italy). According to the results of preliminary antagonism tests 12 promising bacteria that underwent to further investigations, were selected. The isolates were characterized by RFLP and sequence analysis of a PCR amplified 16S rDNA region. The results showed that 3 isolates were closely related to *Pantoea agglomerans*, whereas 9 isolates belonged to the genus *Pseudomonas*. The selected strains were tested for their potential to inhibit i*n vitro* the growth of 2 strains of *Pythium aphanidermatum* (CBS 118745, CBS 116664) and 2 strains of *Rhizoctonia solani* isolated from symptomatic hydroponic plants in FVG. Fungal mycelium was centrally placed in Petri dishes that had been previously inoculated with bacteria and incubated 48 h. The plates were further incubated at 24°C for 9 days, and the fungal growth was measured daily. Among the 12 isolates, one strain of *Pseudomonas fluorescens* and one strain of *P. agglomerans* showed an average inhibition of mycelial growth of 89% and 70%, respectively. Trials to assess the potential of the selected bacteria as biocontrol agent against lamb's lettuce root pathogens are currently in progress.

MOLECULAR AND CHEMICAL CHARACTERIZATION OF THE PATHOSYSTEM *FUSARIUM VERTICILLIOIDES*-MAIZE. G. Mulè[2], I. Lazzaro[1], A. Susca[2], A. Ritieni[3], A. Lanubile[4], J. Bernardi[4], A. Marocco[4] and P. Battilani[1]. *[1]Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. [2]Istituto di Scienze delle Produzioni Alimentari, CNR, Via Amendola 122/O, 70126 Bari, Italy. [3]Dipartimento di Scienza degli Alimenti, Università degli Studi "Federico II", 80055 Napoli, Italy. [4]Istituto di Agronomia, Genetica e Coltivazioni Erbacee, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: paola.battilani@unicatt.it*

A two-year study on the pathosystem *F. verticillioides*-maize was conducted speculating on both *in vitro* and *in planta* perspectives. The former studied the effects of temperature (T) and water activity (a_w) on fumonisin B (FBs) production and expression of *FUM2* and *FUM21* genes in ITEM 10027 and 1744 *F. verticillioides* strains grown up to 21 days. The latter monitored which genes were differentially expressed in resistant and susceptible maize lines after 2-3 days infection by ITEM 1744. The *in vitro* study showed that ITEM 10027 was the highest FBs producer, with predominance of FB_1. The maximum level of FB was registered after 21 days in both strains. *FUM2* and *FUM21* were expressed in all studied conditions, with a 10X difference between the former and the latter. The peak of transcription level was reached after 14 days of incubation for both *FUM* genes. No data were collected on cultures grown at fixed a_w=0.900 because the fungus did not grow at all temperatures tested till 21 days of incubation. The *in planta* study showed that resistant lines tested were poorly infected by ITEM 1744. Genes differentially expressed were divided into 11 functional categories and nearly 10% was assigned to the class "cell rescue, defence and virulence". Most of the pathogenesis-related genes were differentially activated after fungal infection in relation to the resistance level of maize genotypes. In the resistant kernels, defence-related genes provided basal protection against the fungus, while in the susceptible kernels, the same genes were induced specifically after pathogen attack.

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STUDY OF *VENTURIA INAEQUALIS* SENSITIVITY TO STROBILURINS: CORRELATION BETWEEN MOLECULAR AND BIOLOGICAL APPROACHES. I. Nanni, C. Turan, R. Fiaccadori, G. Alberoni, M. Collina and A. Brunelli. *Centro di Fitofarmacia, Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale G. Fanin 46, 40127, Bologna, Italy. E-mail: marina.collina@unibo.it*

Scab caused by *Venturia inaequalis* (Cke.) Wint. is the most important disease of apple trees worldwide and requires a high number of fungicide applications. The introduction, in the late 1990s, of strobilurin fungicides led to improve disease control.

After a few years of excellent protection, several cases of reduced scab control caused by pathogen resistance to strobilurins were reported from many countries. Our previous studies pointed out the occurrence in Italy of *V. inaequalis* populations resistant to kresoxim-methyl and trifloxystrobin, with the presence of G143A substitution in cytochrome b, which is the most common mutation in strobilurin resistance in plant pathogenic fungi. The aim of this study was to compare the sensitivity of several *V. inaequalis* populations coming from distinct locations obtained by conidial germination tests ($DE_{50,}$ percentage of germination at the higher concentration) and molecular analysis detecting presence and frequency of G143A substitution (CAPS PCR and Real Time PCR). Preliminary results from samples collected in 2009 and 2010, generally demonstrate a good but not completely clear correlation between these different approaches.

BIOPROSPECTING FUNGAL ENDOPHYTES FROM FOREST PLANTS AT THE ASTRONI NATURE RESERVE FOR ANTITUMOUR EXTROLITES BASED ON THEIR FUNGITOXIC PROPERTIES. R. Nicoletti[1], A. Carella[1], A. De Filippis[2] and E. Buommino[2]. *[1]CRA, Unità di Ricerca per le Colture Alternative al Tabacco, Via Vitello 108, Scafati (SA), Italy. [2]Dipartimento di Medicina Sperimentale, Seconda Università degli Studi di Napoli, Via de Crecchio 7, Napoli, Italy. E-mail: rosario.nicoletti@entecra.it*

Fungal endophytes represent an effective component of biodiversity of natural ecosystems which is increasingly considered for its implications in plant protection and growth promotion. Interactions in the first field can be mediated by antibiotics and/or cytostatic compounds whose bioactivity is sometimes exploited in human medicine. Therefore the search for new pharmaceuticals can take advantage by the discovery of novel such products, and actually our previous experiences concerning soil-borne fungal antagonists showed that fungitoxic compounds may have interesting antiproliferative and/or pro-apoptotic properties on human tumour cells. On the occasion of the International Year of Biodiversity in 2010, an investigation concerning fungal endophytes of forest trees was carried out at the Astroni Nature Reserve near Naples, with the aim to identify a number of strains to be further exploited as a source of new antitumour compounds. One hundred and 7 isolates were recovered from 28 plant species, and screened for their antifungal properties by evaluating the interactions with a strain of *Rhizoctonia solani* AG-2-1/Nt in dual cultures. In addition to 11 isolates which were able to develop mycoparasitically, 37 isolates displayed some extent of inhibitory capacity. Liquid cultures of these isolates were prepared, and the resulting culture extracts assayed for inhibitory effects on hyphal growth on the same *R. solani* strain. Five isolates were able to completely inhibit *R. solani* over a week, while 28 isolates induced retarded growth and/or alterations of the hyphal structure. Effective extracts will be evaluated on human tumour cell cultures for their further characterization and the isolation of the bioactive compounds.

SILVER LEAF OF APRICOT: AN EMERGENT DISEASE IN EMILIA-ROMAGNA. P. Nipoti[1], A. Mirotti[2], S. Tonti[1,3], A.R. Babini[2], E. De Paoli[2], C. Montuschi[2] and A. Prodi[1]. *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Viale Fanin 44, 40127 Bologna, Italy. [2]Servizio Fitosanitario Regione Emilia-Romagna, Via di Saliceto 81, 40128 Bologna, Italy. [3]Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Via Ca' Nova Zampieri 37, 37057 S. Giovanni Lupatoto (VR), Italy. E-mail: paola.nipoti@unibo.it*

Apricot (*Prunus armeniaca*) is an expanding crop in eastern Emilia Romagna (northern Italy). Since last two years the characteristic symptoms of silver leaf were noticed on 2- to 3-year-old apricot orchards planted with new cultivars (Faralia, Farbaly, Kioto). The main symptom is a partial or total silvery sheen on the foliage. The pathogen penetrates through wounds, the branches may gradually die back and, sometimes, the affected plant dies. Infected branches appear brown stained in transverse section, whereas the fruits remain smaller and fewer in number. A similar disease caused by *Chondrostereum purpureum* (Pers.) Pouzar was described on peach 20 year ago in many Emilia Romagna orchards. The fungus can colonize different host plants like apples, pears, cherries, plums, peaches, apricots, nashi, berryfruit, poplars, willows, silver birches, eucalypts and roses. During summer 2010, many samples were collected from symptomatic apricot plants. Wood disks were cut from the samples and cultured on a semi-selective medium, malt extract agar amended with benomyl, neomycin and streptomycin sulphate (BNS). The colonies showed early aerial mycelium, white turning to cream, woolly to floccose, granular to felty at 6 week, azonate and not fruiting. The colonies analyzed by a specific PCR primer pair, were molecularly identified as *C. purpureum*. This disease appears as a limiting factor in apricot fruit production particularly for the new varieties. So, appropriate investigations are desirable for reducing the impact of this pathogen.

PHYSIOLOGICAL AND MOLECULAR ALTERATIONS INDUCED BY *ALTERNARIA ALTERNATA* IN TWO VARIETIES OF APPLE. C. Nobili[1], D. Carbone[2], A.A. Fabbri[2], M. Scarpari[2], M. Punelli[2], A. Ricelli[3], C. Fanelli[2] and M. Reverberi[2]. *[1]Unità Tecnica Innovazione Biotecnologie Agroindustriali ENEA C.R., Casaccia, Roma, Italy. [2]Dipartimento di Biologia Ambientale, Università "Sapienza", Roma, Italy. [3]ICB-CNR, P.le Aldo Moro 5, 00185 Roma, Italy. E-mail: chiara.nobili@enea.it*

Alternaria alternata pv. *mali* causes a foliar and fruit disease to susceptible varieties of apple known as *Alternaria* blotch disease (ABD). In Italy, a new disease of apple always related to an *Alternaria* infection, showing symptoms very similar to those described for ABD, was recently observed. The appearance of necrotic lesions on leaves and fruits suggests that also these new Italian isolates of *A. alternata* (B19, T25) may produce as the pv. *mali* (T65) virulence factors like AM toxin. The aim of this study is to evaluate the pathogenicity of two Italian isolates of *Alternaria* in comparison with *A. alternata* pv. *mali*, during the experimental infections of detached fruits and leaves of the apple cvs Golden and Gala. In particular, we have evaluated in fruits and leaves the effects of this disease on the induction of an hyper-oxidant status through the evaluation of the main reactive species formation and degradation in the response to fungal infection. Reactive species (RS) can prime cell death, consequently the activation of PCD, particularly in the leaves, was monitored at different time intervals, 0, 6, 24, 48 and 96 h post inoculation. These results were matched, with determination by citofluorimeter of the apoptotic process in infected leaves. The results indicate that as far as the evidences obtained, it could be hypothesised that cell death induction, probably also consequent to the release of a not yet identified virulence factor, may play an important part in the establishing this new disease of apples in Italy.

ANTIMICROBIAL ACTIVITY OF CEREAL BIOACTIVE COMPOUNDS. F. Nocente[1,2], S. Bellato[1], K. Carbone[1], E. Galassi[1], L. Gazza[3], L. Sereni[1], F. Taddei[1], M. Pasquini[1] and D.

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The antimicrobial properties of natural compounds or synthetic peptides against economically important plant pathogenic fungi and bacteria have been studied for their utilization in crop protection. Vromindolines, starch-bound proteins involved in the extra-softness of the oat endosperm, are structurally similar to puroindolines PIN-A and PIN-B and as PINs are characterized by a triptophan-rich domain likely responsible for their antimicrobial activity. Alkylresorcinols (ARs) are a group of phenolic lipids involved in a multitude of interactions with biological membranes; their amphiphilic and phenolic nature has been associated with their biological activity, but the mechanism of action is not yet explained. Cereal alkylresorcinols are found to be mixtures of saturated, monoenoic and dienoic homologues with mainly saturated alkyl chains in the range of 15-27 carbon atoms. Analyses have been carried out to test the antibacterial and antifungal activity of the vromindolines and a synthetic peptide (TRP), resembling their specific tryptophan-rich domain. When tested *in vitro* these compounds showed a 70% inhibition of the growth of *Escherichia coli* and a minor effect on a DON (deoxynivalenol) producing strain of *Fusarium graminearum*. The antifungal activity of AR extracts, isolated from wheat intact grain with two different solvents (ethyl acetate and acetone) was tested against *F. graminearum*. The bioassays showed that the fungal growth was distinctly reduced by addition of 400 µg of AR extracts to nutrient agar, the various extracts exhibiting a different inhibitory ability. The results suggest the availability of novel compounds that could be considered alternative natural fungicides.

CHARACTERIZATION OF THE HYPERSENSITIVE RESPONSE INDUCED BY THE C2 PROTEIN OF TOMATO YELLOW LWAF CURL SADINIA VIRUS IN *NICOTIANA* spp.

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The begomovirus *Tomato yellow leaf curl Sardinia virus* (TYLCSV) causes a devastating disease of tomato plants all over the Mediterranean basin. Several functions have been attributed to the C2 protein of different begomoviruses. A functional analysis of the TYLCSV C2 protein has been undertaken. *Agrobacterium*-mediated transient expression of C2 resulted in a local necrotic hypersensitive response (HR) in *Nicotiana benthamiana* and *N. tabacum*, as confirmed with trypan blue and 3,3'-diaminobenzidine staining, thus indicating that C2 plays a key role in pathogenicity.

We analyzed the dependence of C2-induced HR by utilizing VIGS vectors to silence some of the HR-related genes, such as SGT1, RAR1, and MEK2; HR was influenced upon silencing of all of them. We also observed that the severity of HR was temperature dependant, so that HR was more pronounced at 25°C than at 20°C. Using NahG transgenic *N. benthamiana* lines which fail to accumulate salicylic acid (SA), a key potentiator of HR, we observed a different HR in C2-infiltrated tissue at both temperatures, indicating that SA participates to regulate the intensity of C2-dependent HR. However, TYLCSV natural infection is not normally associated with HR, suggesting that the virus encodes factors that counter this response. Upon co-agroinfiltration with other viral non-structural proteins, C2-induced HR could be partially counteracted.

SEED HEALTH IN ORGANIC FARMING SYSTEM.

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Seed is the basic unit of crop production, and seed health plays a key role for successful production especially in organic farming systems, where less efficient seed-treatments are available for managing seed-borne disease. Phytosanitary seed analysis were carried out to verify the actual impact of seed-borne diseases in some organic farms distributed throughout Italy and to evaluate the health of seeds used in Italian organic agriculture. Samples of durum and common wheat, carrot, bean and chickpea seeds of different typologies (organic commercial seeds, self-produced organic seeds and conventional not treated seeds allowed) were analysed. In addition, seeds and production of some durum and common wheat varieties were analysed for two years, to verify a possible relationship between the health status of seeds sown and production under organic farming systems. Seed health testing was performed using the standard blotter method described by the International Rules for Seed Testing, and a list of the fungal species found and identified on seeds was drawn.

AN INTEGRATED STUDY ON THE EFFECTS OF *GRAPEVINE LEAFROLL-ASSCOCIATED VIRUS 1, GRAPEVINE VIRUS A* AND *GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS* ON FIELD PERFORMANCE AND BERRY QUALITY OF *VITIS VINIFERA* cv. NEBBIOLO.

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Viruses can affect grapevine yield and field performance, but the impact of infection on the quality and safety of final products has been scarcely investigated. This study reports the first analysis of agronomic performance, fruit texture and composition, and proteomic changes occurring in berries of virus-infected *Vitis vinifera* cv. Nebbiolo, grown in field conditions. The cv. Nebbiolo clone 308 infected by *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine virus A* (GVA), and *Grapevine rupestris stem pitting-associated virus* (GRSPaV), were compared to healthy vines obtained following heat treatment of the original infected mother plant. The phytosanitary status of each plant was assessed by RT-PCR, and GLRaV-1 and GVA concentration determined by quantitative reverse transcription-Real time PCR (qRT-PCR). Mean loads of GLRaV-1 and GVA in the infected plants were 2.5 (SD=0.8) and 0.4 (SD=0.2) viral genomes/100 GAPDH transcript copies, respectively. The distribution of the viral loads was uniform among the infected plants. The comparison of field performance and fruit quality of healthy and infected plants revealed similar agronomical behaviour, with significant

differences in titratable acidity, resveratrol content, and in some texture parameters. The proteomic analysis of skin and pulp from berries of healthy and virus-infected grapes disclosed significant differences for 12 pulp spots and 7 skin spots. Virus infection altered proteins involved in cell structure metabolism in the pulp, and proteins involved in the response to oxidative stress in the berry skin.

POTENTIAL ROLE OF ORGANIC CARBON FRACTIONS FROM VEGETABLE COMPOSTS, IN THE BIOCONTROL OF *RHIZOCTONIA SOLANI*. **C. Pane[1], R. Spaccini[3], D. Villecco[1], G. Celano[2], A. Piccolo[3] and M. Zaccardelli[1].** *[1]CRA, Centro di Ricerca per l'Orticoltura di Pontecagnano, Azienda Sperimentale di Battipaglia. SS 18 n. 204, 84091 Battipaglia (SA), Italy. [2]Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano n. 10, 85100 Potenza, Italy. [3]Centro Interdipartimentale di Ricerca sulla Risonanza Magnetica Nucleare per l'Ambiente, l'Agro-Alimentare ed i Nuovi Materiali, Via Università 100, 80055 Portici (NA), Italy. E-mail: catello.pane@entecra.it*

Composts-mediated soil-borne plant pathogen suppression can be affected by the organic matter structure that influence availability of carbon nutrients for microbes associated with biological control. In this work, four vegetable composts (VCs) obtained from tomato+endive (VC-TE), tomato (VC-T), artichoke (VC-A) and artichoke+fennel (VC-AF) residues, were assessed for their ability to reduce damping-off caused by *Rhizoctonia solani* (Kühn) on cress. A municipal waste compost (MWC) was used as reference. Active biotic component of composts relieved by CO_2-release, FDA-hydrolysis, Biolog analyses and microbial counts, was indispensable for *in vitro* and *in vivo* pathogen control, as demonstrated by loss of suppressiveness after sterilization. Cross-polarization magic angle spinning ^{13}C-nuclear magnetic resonance (CP-MAS-^{13} C-NMR) was used to analyze the molecular distribution of organic C in composts. Strong absorbance around peaks at 175, 150 and 130 ppm of resonance spectra suggest that the main components of the final mature composted vegetable residues are lignocellulosic materials and hydrophobic alkyl moieties, that are commonly found in well-humified organic matter (humic C). Rhizoctonia disease suppression levels, resulted in the following order: VC-TE, VC-T, VC-A, VC-AF and MWC They were associated directly to the relative abundance of carboxylic C (region 195-165), phenolic C (region 165-145 ppm) and aromatic C (region 145-110 ppm) and indirectly to alkyl C (region 45-0 ppm), but not with carbohydrate structures (O-alkyl C, 110-60 ppm). The described humic C fractions appear to be potentially responsible for suppressiveness by interaction with compost-niche inhabiting microbes.

CONTROL OF EARLY BLIGHT OF CARROT CAUSED BY *ALTERNARIA DAUCI* AND *A. RADICINA* USING PHYTOCHEMICALS. **C. Pane, D. Villecco, F. Campanile and M. Zaccardelli.** *CRA, Centro di Ricerca per l'Orticoltura di Pontecagnano, Azienda Sperimentale, SS 18 n. 204, 84091 Battipaglia (SA), Italy. E-mail: catello.pane@entecra.it*

Alternaria dauci (J.G. Kühn) J.W. Groves & Skolko and *Alternaria radicina* Meier, Drechsler & E.D. Eddy, are related pathogens causing severe blights on leaves, petioles and stems of carrot (*Daucus carota*). *A. radicina* can cause a distinctive black rot symptom, consisting in damping-off and rotting of roots, crowns and seedlings of carrots that become unmarketable. Control of these pathogens is currently entrusted to chemical fungicides, but the preference of the consumers for safe agricultural products has stimulated the search of sustainable alternatives. The goal of the present study was to investigate the *in vitro* antifungal activity of plant-derived compounds against Alternaria disease agents of carrot. *In vitro* assays indicate that *A. radicina* was more sensitive to essential oils and raw saponins than *A. dauci*. In fact, a large spectrum of plant essential oils (hyssop, oregano, basil, caraway, sage, marjoram and lemon balm) was able to suppress black rot agent, whereas *A. dauci* was inhibited only by oregano and thyme oils. No effects were observed with rosemary and verbena oils in both cases. In agreement, *A. radicina* showed increased sensitivity to treatments with saponin-enriched tissue meals derived from *Solanum chilense* and *Aster sedifolius*, and by treatments with *Brassica carinata* seed meal containing glucosynolates.

USE OF PLANT-DERIVED COMPOUNDS TO INHIBIT CAUSAL AGENTS OF BEAN ANTHRACNOSE AND CHICKPEA ASCOCHYTA BLIGTH. **C. Pane, D. Villecco, F. Campanile and M. Zaccardelli.** *CRA, Centro di Ricerca per l'Orticoltura di Pontecagnano, Azienda Sperimentale, SS 18 n. 204, 84091 Battipaglia (SA), Italy. E-mail: catello.pane@entecra.it*

Colletotrichum lindemuthianum (Sacc. & Magnus) Lams.-Scrib. and *Ascochyta rabiei* (Pass.) Labr. are the causal agents of anthracnose of common bean (*Phaseolus vulgaris*) and leaf blight of chickpea (*Cicer arietinum*), respectively. Management of these seed-borne pathogens requires an integrated approach, which often includes the use of certified disease-free seed, fungicide seed treatments, cultivar resistance and timely application of foliar fungicides. Protectants that provide a barrier against pathogens propagules could be useful since foliar fungicide applications are not cost effective when disease severity is low and are incompatible with organic farming protocols. Moreover, bean and chickpea resistance against these pathogens is very erratic. In this work, we have tested several phytochemicals, such as essential oils, vegetable colorants, raw saponins and meals from *Brassica carinata* containing glucosinolates, for *in vitro* antifungal activity against *C. lindemithianum* and *A. rabiei*. Results indicate that hyssop and oregano-derived essential oils inhibit the two pathogens at 1% concentration. Moreover, oils distilled from basil, caraway, sage and thyme, block mycelial growth of *C. lindemuthianum*, while essential oils from lemon balm and verbena block mycelial growth of *A. rabiei*. No effects were observed with rosemary and marjoram oils. Among over thirteen plant-extracted colorants tested, only chestnut cortex colorant showed partial inhibition of the radial growth of these fungi. In plate assays, *Solanum chilense* and *Aster sedifolius* tissue meals, containing raw saponins, exhibited no fungitoxic effect. Finally, volatile compounds released by *B. carinata* seed meals were effective in the *in vitro* control of both pathogens.

NEW STRAINS OF *BACILLUS SUBTILIS* (17S AND 08C) INVOLVED IN BIOCONTROL OF LETTUCE DROP CAUSED BY *SCLEROTINIA MINOR*. **C. Pane, D. Villecco, F. Campanile and M. Zaccardelli.** *CRA, Centro di Ricerca per l'Orticoltura di Pontecagnano, Azienda Sperimental , SS 18 n. 204, 84091 Battipaglia (SA), Italy. E-mail: catello.pane@entecra.it*

Introduction and establishment of living natural enemies of pathogens is viewed as a powerful alternative to chemical fungicides, meeting the consensus of consumers for sustainable prod-

ucts with few or no chemical residues. In this work, we have selected new thermophilic bacterial strains from suppressive soils, on the basis of their *in vitro* antifungal activity against *Sclerotinia minor*. A culture collection was obtained by selection, from countable plates, of over 250 colonies. Results of qualitative plate challenge experiments indicated that 39% of the isolates produced an inhibition zone between individual colonies and pathogen. On the basis of these data, quantitative plate challenge experiments were performed with selected potential antagonistic bacteria. Successively, these bacteria were separated in genetically distinct groups by DNA polymorphic analyses (M13 sequenced-based PCR fingerprinting). Crossing DNA fingerprints with the quantitative data of *in-vitro* assays, two strains named 17S and 08C, genetically different and with the highest *in vitro* antibiosis activity against *S. minor*, were identified by biochemical (Biolog™) and molecular analyses (16S rRNA gene sequencing) as *Bacillus subtilis*. Application of *B. subtilis* 17S and O8C strains to *S. minor*/*Lactuca sativa* pathosystem, showed a biocontrol efficacy of disease severity, respect to untreated control, of about 70 and 56%, respectively. Results suggest that these two new *B. subtilis* strains are biopesticides effective in the reduction of lettuce drop symptoms.

DETECTION OF *ALFALFA MOSAIC VIRUS* BY IC-RT-PCR IN *VIBURNUM OPULUS*. **G. Parrella[1], L. Cavicchi[2], S. Rosati[2] and M.G. Bellardi[3].** *[1]Istituto per la Protezione delle Piante del CNR, Via Università 13, 80055 Portici (NA), Italy. [2]Plesso Didattico "G. Scarabelli", Università degli Studi di Bologna, Viale G. Ascari 17, 40026 Imola (BO), Italy. [3]Dipartimento di Scienze e Tecnologie Agroalimentari, Patologia Vegetale, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. E-mail: parrella@ipp.cnr.it*

Viburnum opulus L. ("snowball", *Caprifoliaceae*) is a deciduous shrubs used as decorative plants for its fragrant white flowers that bloom in spring. In May 2011, snowball plants showing a virus-like disease were observed in two ornamental public gardens at Imola (Bologna, Emilia Romagna). Symptoms were distributed only on part of the foliage, and consisted of chlorotic rings and spots, line-patterns and, in some cases, vein clearing on younger leaves. Since only *Alfafa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) have been reported to naturally infect this *Viburnum* species, and considering that snowball could be an important source of virus infection, symptomatic plants were examined b IC-RT-PCR. Tests were performed on leaf extracts using two polyclonal antisera against an AMV isolate from lettuce and a CMV isolate from tomato, respectively. Only AMV was found in symptomatic *V. opulus.* The CPAMV1/CPAMV2 specific AMV primer pairs were used in RT-PCR reactions. A DNA fragment of *ca.* 750 bp, covering the entire AMV coat protein gene (CP), was obtained after IC-RT-PCR and the product was gel purified and cloned in pGEMT Easy for sequencing. Comparison of the CP sequence of AMV-*V. opulus* with the CP reference sequences of some AMV isolates, revealed the maximum nucleotide identities with subgroup I isolates. AMV has been previously found infecting *V. opulus* in Italy in 1995, but the leaf symptomatology observed was vein yellowing alone.

FUNCTIONAL CHARACTERIZATION OF CERATO-PLATANIN BY GENE DELETION AND PROTEOMIC APPROACH. **L. Pazzagli[1], S. Luti[1], F. Martellini[1], C. Comparini[2], P. Bettini[3], I. Baccelli[2], B. Pantera[4], G. Cappugi[1] and A. Scala[2].** *[1]Dipartimento di Scienze Biochimiche, Università degli Studi, Viale Morgagni 50, 50134 Firenze, Italy. [2]Dipartimento di Biotecnologie Agrarie, Università degli Studi, Via della Lastruccia 10, 50019 Sesto Fiorentino (FI), Italy. [3]Dipartimento di Biologia Evoluzionistica, Università degli Studi, Via Romana 17, 50125 Firenze, Italy. [4]Entomon s.a.s., Via di Ripoli 25, 50126 Firenze, Italy. E-mail: luigia.pazzagli@unifi.it*

Cerato-platanin (CP) is a protein abundantly secreted and localized in the cell wall of *Ceratocystis platani*. CP induces production of H_2O_2 and NO, programmed plant cell death, overexpression of defence genes, phytoalexin synthesis and restriction of conidia growth. Therefore, CP appears to act as PAMP able to activate primary defence mechanisms. CP is the founder of the "Cerato-platanin family", whose members are proteins involved in the microbe-host interaction. In the present study the physiological role of CP in the life style of *C. platani* and its role as PAMP in inducing defence responses are investigated by: (i) gene deletion experiments. A deletion cassette for the *cp* gene was obtained using the fusion PCR technique. To obtain the cassette, the determination of a 1,368 bp region upstream of the ATG codon was performed by genome walking. The construct for disruption of the *cp* gene will be further used to transform conidia and/or protoplasts which will be characterized for their ability to induce defences in host and non host plants. (ii) Proteomic approach. Conidia of *C. platani* are used to treat plane leaves for 48 h in a moist chamber. Proteins were extracted by phenol/chloroform treatment. 2D-electrophoresis was used to visualize proteins extracted from the treated leaves vs. proteins from control leaves. Other leaves were treated with purified CP for 15 and 30 min and for 1, 6, 12, 24 and 48 h and protein were extracted. SDS-PAGE and Western blot were performed to detect protein kinase activated upon CP interaction.

FIRST REPORT OF *PHYTOPHTHORA CRYPTOGEA* ON COMMON CYPRESS. **A. Pecchioli[1], R. Danti[1], A.M. Vettraino[2] and G. Della Rocca[1].** *[1]Istituto per la Protezione delle Piante del CNR, Via Madonna del Piano 10/I, 50019 Sesto Fiorentino (FI), Italy. [2]Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: g.dellarocca@ipp.cnr.it*

The common cypress (*Cupressus sempervirens*) is widespread throughout the Mediterranean area where it plays an essential role in the characterization of the landscape, and is widely used for its aesthetic value and timber production, for protection of soils from erosion and for windbreaks. In 2009 and 2010 in two young ornamental plantations in Tuscany and one nursery in Umbria (central Italy), some specimens of *C. sempervirens* cv. Bolgheri showed symptoms of sudden yellowing of the crown starting from the basal portion, followed by death of the plants. The incidence of diseased trees was around 10-15% at all sites. A *Phytophthora* species was isolated from the rhizosphere and from roots of symptomatic and dead plants on selective medium PARPH. Morphological characteristics were registered on 5-day-old cultures grown on carrot agar. Ribosomal DNA regions (ITS1, 5.8S rDNA and ITS2) were amplified, sequenced and compared to sequences of known *Phytophthora* species from GenBank. Morphological and molecular results identified this species as *Phytophthora cryptogea.* A pathogenicity test was conducted using 3-years-old ramets of several clones of *C. sempervirens* grown in pots containing soil inoculated with sterile millet seeds colonised separately by two isolates of *P. crytpogea*. For each *P. cryptogea* isolate twenty plants were inoculated and 10 untreated plants were used as controls. Reduced growth of the roots and crown wilting followed by death of the plants were observed, thus confirming the pathogenicity of *P. cryptogea* on *C. sempervirens*. This is the first report of *P. cryptogea* on *C. sempervirens*.

TESTING THE BENEFICIAL EFFECTS OF PLANT GROWTH PROMOTING RIZOBACTERIA ON PLANT FITNESS AND RESISTANCE TO BIOTIC STRESSES IN THE CONTEXT OF THE ORGANIC FARMING SYSTEM. **O. Petruzzelli[1], M. Ferrara[2], F. Cillo[1], F. Nigro[2] and L. Stavolone[1].** *[1]Istituto di Virologia Vegetaledel CNR, via Amendola 165/A, 70126 Bari, Italy. [2]Dipartimento di Biologia e Chimica Agro-forestale ed Ambientale, Università degli Studi "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. E-mail: l.stavolone@ba.ivv.cnr.it; nigrof@agr.uniba.it*

The relationships between plants and beneficial microorganisms in the rhizosphere (e.g. mycorrhizas, fungi and bacteria) are essential for root development, plant nutrition, and growth enhancement. The decline of agricultural soils fertility is due not only to the agricultural practices but also to the reduction of organic matter content, as well as to the depletion of the edaphic microflora. Plant growth promoting rhizobacteria (PGPR) werer shown to stimulate plant growth and natural resistance towards diseases. Some bacterial bioformulations are available on the market for the use in agriculture. In this study, the effects of a new PGPR mixture (based on *Pseudomonas* spp. and *Bacillus* spp. strains) on plant growth, yield and tolerance to pathogens of several horticultural crops, were evaluated. The experiments were conducted in a commercial field cultivated according to the organic farming system. Plants treated with water and the bioformulate Sublic (Elep Biotechnologies) were used as controls. The incidence of naturally occurring diseases caused by fungi and viruses was monitored. Moreover, PGPR-treated plants were also monitored in a greenhouse and challenged for resistance/tolerance to selected pathogen infections by means of mechanical inoculations of pathogenic viruses and fungi. Treatments with the new developed PGPR mixture greatly enhanced plant growth and significantly antedated harvesting for some horticultural crops. The effectiveness of the new PGPR mixture on plant fitness and tolerance to viral and fungal diseases, as well as the differences with the commercial bioformulation, are discussed.

EFFECTS OF COPPER ON *T. MELANOSPORUM*-INFECTED PLANTS. **F. Piattoni, M. Iotti, S. Boutahir and A. Zambonelli.** *Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale G. Fanin 46, 40127 Bologna, Italy. E-mail: zambonel@agrsci.unibo.it*

The positive results obtained by truffle cultivation in Europe and Oceania has determined the extension of this activity also to agricultural areas subjected to intensive crop production systems for a long time. It is known that copper accumulation in agricultural soils has a long-term impact on a wide range of soil biota. In this work copper effects on *Q. pubescens* seedlings infected with *T. melanosporum* were investigated for the first time. Cupper was added as $CuSO_4 \cdot 5H_2O$ chelated with ETDA. After repeated treatments the Cu accumulation obtained in soil was 145 ppm of total Cu and 89.4 ppm of assimilable Cu. *T. melanosporum*-infected plants showed reduced symptoms of copper toxicity in comparison with the plants without mycorrhizae. The quantity of Cu accumulated in roots and leaves was lower in *T. melanosporum*-infected plants. *T. melanosporum* mycorrhizae produced oxalates which might play a fundamental role in Cu soil detoxification. *T. melanosporum* mycorrhizae at high Cu concentrations became dark and the formation of new mycorrhizae was inhibited. In Cu-treated plants the formation of *T. melanosporum* stroma along *Q. pubescens* roots was detected. These structures could be interpreted as a quiescent, resistance phase of the fungus.

THE MELANIN BIOSYNTHESIS INHIBITOR TRICYCLAZOLE REDUCES SECONDARY INFECTION OF *MAGNAPORTHE ORYZAE* IN RICE LEAVES. **C. Pizzatti, J. Gómez-Ariza, A. Kunova and P. Cortesi.** *Dipartimento di Protezione dei Sistemi Agroalimentare e Urbano e Valorizzazione delle Biodiversità, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: paolo.cortesi@unimi.it*

Melanin biosynthesis inhibitors are used as fungicides in agriculture. Among them, tricyclazole (TCZ) has demonstrated to play an important role against rice blast, one of the most destructive diseases of rice worldwide, caused by *Magnaporthe oryzae*. One discussed point regards the capability of the fungus to complete its life cycle after being exposed to TCZ. The aim of this work was to analyze the capability of conidia originated from mycelium that had been exposed to TCZ to infect rice. TCZ was compared with azoxystrobin, a strobilurin fungicide. Strains of *M. oryzae* were grown on medium supplemented with different TCZ or azoxystrobin concentrations in order to harvest conidia for downstream microscopic and pathogenicity analyses. Conidia amount, appressoria size and the number of infection points in rice leaves revealed that the exposure of *M. oryzae* mycelium to TCZ negatively affects conidia production and appresoria formation and function, thus resulting in a reduction of the fungus virulence. Azoxystrobin was less effective than TCZ. These new results highlight the potential of TCZ to reduce secondary infections lowering conidia formation on diseased treated areas and conidia virulence. These properties will reduce the need of repeated treatments benefiting both the rice ecosystems and farmer's economy.

***TRICHODERMA* spp. IN INNOVATIVE SUBSTRATES FOR ORNAMENTAL PLANTS.** **D. Prisa[1], S. Sarrocco[2], M. Forti[1], C. Piaggeschi[2], G. Burchi[1] and G. Vannacci[2].** *[1]CRA, Unità di Ricerca per il Vivaismo e la Gestione del Verde Ambientale ed Ornamentale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [2]Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sezione Patologia Vegetale, Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: domenico.prisa@entecra.it*

Trichoderma spp. are free-living fungi commonly widespread in soil and root ecosystems. Recent findings show them as opportunistic, avirulent plant symbionts as well as parasites of other fungi. Some strains establish robust and long-lasting colonization of roots by entering the first epidermal layers. Root colonization frequently results in the enhancement of growth and development, crop productivity or induction of resistance to abiotic and biotic factors. Due to the reduction of peat in ornamental substrates, great attention is focuses on setting up new and innovative substrates for this market. In this work, *Trichoderma* spp. isolates were selected for endophytism in *Limonium sinuatum*, *Cupressus sempervirens* and *Camelia* sp. Fungal solates (162) were used as inoculants of soil employed for germination and growing of both *Limonium* and *Cupressus* whereas 202 isolates were inoculated in soil used for transplanting *Camelia* cuttings. Germination rate and germination time, growth parameters and % of root colonization (endophytism) in inoculated thesis were submitted to ANOVA and compared to uninoculated controls (natural pot mix). Ten isolates for *Limonium,* 9 for *Cupressus* and 8 for *Camelia* improved one or more parameters and were chosen for further analysis. All selected isolates were identified by a molecular approach. Selected strains are actually under evaluation in order to confirm their endophytic fitness on all the three ornamental species. In addition, the ability to induce resistance against *Botrytis cinerea* in *Limonium* will be investigated.

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FIRST REPORT OF GRAPEVINE BLACK ROT IN SARDINIA. V.A. Prota, S. Serra, L. Cogotzi, G. Serra and R. Garau. *Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: vprota@uniss.it*

Grapevine black rot disease is caused by *Guignardia bidwellii.* It originated from North America but is now widespread all over the world. In Italy, it was reported only in northern regions like Friuli Venezia Giulia, Veneto, Liguria and Piedmont where infections are variable and rarely reach a high level of severity. In 2010 in northern Sardinia (Oschiri) symptoms resembling black rot were observed on young cv. Sultanina vines. The basal leaves of some shoots showed necrotic reddish brown spots with dark red margins and small black dots which were sometimes concentric. The canes were asymptomatic and the affected plants were few and contiguous. In spring 2011, the same symptoms were observed on cv. Cannonau in central Sardinia (Oliena). Infections were more severe and did not affect only the basal leaves but were also present in the axis of green shoots, petioles and rachis of several plants in the same vineyard. Some spots on the leaves were observed also in other vineyards in the same area. Microscopical observations revealed the presence of asexual fruiting structures, i.e. pycnidia containing one-celled, hyaline ovoid conidia with an apical hyaline appendage that measured 5.4±0.66 x 9.42±0.82 µm. All parameters were in accordance with those of *Phyllosticta ampelicida*, anamorph of *G. bidwellii*. According to typical symptoms and microscopic characters the disease was identified as black rot. Further studies are in progress to characterize the fungus and to assess its pathogenicity.

SUPPRESSIVE EFFECT OF COMPOST AGAINST *PHYTOPHTHORA CINNAMOMI* ON ORNAMENTALS. M. Pugliese, M.L. Gullino and A. Garibaldi. *AGROINNOVA, Università degli Studi di Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: massimo.pugliese@unito.it*

Suppression of soil-borne plant pathogens with composts has been widely studied. Composts have been found to be suppressive against several soil-borne pathogens in various cropping systems. Ornamental plants are generally cultivated in pots, allowing the use of suppressive substrates to control zoospore-producing pathogens, like *Phytophthora* spp. The efficacy of compost against basal and root rot in *Skimmia japonica* and *Rhododendron azalea* caused by *Phytophthora cinnamomi* was evaluated under greenhouse conditions. A compost (ACM) produced through a forced type of composting for 3 months from municipal organic wastes and a compost (ACV) produced from green wastes were mixed with peat substrate to obtain application rates of 10, 20 and 40% (v/v). Substrates were inoculated at 1g/l dosage of wheat kernels infected by *P. cinnamomi* and, after one week, 15-20 plants were transplanted for each treatment in 2 liter volume pots and placed in greenhouse at 20°C. A chemical control (Metalaxil-M) was also used. Diseased plants were assessed weekly after transplanting and above-ground biomass of plants was assessed at the end of the trials. Results showed a significant disease control when compost was used at 20-40% on *S. japonica*, without showing any phytotoxic effect. Disease suppression was shown at 20% on *R. azalea*, while higher concentrations of compost where slightly phytotoxic to plants. The use of compost substrates can be a suitable strategy for controlling soil-borne diseases on ornamentals, but results depend also on alkalinity tolerance of different species.

EFFECT OF CLIMATE CHANGE ON GRAPEVINE INFECTION BY DOWNY MILDEW UNDER CONTROLLED ENVIRONMENT. M. Pugliese, M.L. Gullino and A. Garibaldi. *AGROINNOVA, Università degli Studi di Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: massimo.pugliese@unito.it*

Plant responses to elevated CO_2 and temperature have been much studied in recent years, but effects of climate change on pathological responses are largerly unknown. The pathosystems grapevine (*Vitis vinifera*)-downy mildew (*Plasmopara viticola*) was chosen as model to assess the potential impact of increased CO_2 and temperature on disease incidence and severity under controlled environment. Potted grapevine plants were grown in phytotrons under 4 different simulated climatic conditions: (i) standard temperature (ranging from 18 to 22°C) and standard CO_2 concentration (450 ppm); (ii) standard temperature and elevated CO_2 concentration (800 ppm); (iii) elevated temperature (ranging from 22 to 26°C, i.e. 4°C higher than standard) and standard CO_2 concentration; (iv) elevated temperature and CO_2 concentration. Each plant was inoculated with a spore suspension containing $5x10^5$ CFU/ml. Disease index and physiological parameters (chlorophyll content, fluorescence, assimilation rate) were assessed. Results showed an increase of the chlorophyll content with higher temperatures and CO_2 concentration, to which corresponded a higher fluorescence index. Disease incidence of downy mildew increased when both CO_2 and temperatures were higher. Considering that the rising concentrations of CO_2 and other greenhouse gases will lead to an increase in global temperature and longer seasons, we can assume that this will allow more time for pathogens evolution and could increase pathogen survival, indirectly affecting downy mildew of grapevine.

A LIPIDOMIC APPROACH FOR UNCOVERING THE OXYLIPIN DETERMINANTS WHICH DRIVE *ASPERGILLUS FLAVUS-ZEA MAYS* INTERACTION. M. Punelli[1], M. Reverberi[1], M. Scarpari[1], E. Di Fabio[1], E. Camera[2], A.A. Fabbri[1] and C. Fanelli[1]. *[1]Dipartimento di Biologia Ambientale, Università "Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. [2]Laboratorio di Fisiopatologia Cutanea e Centro Integrato di Metabolomica, Istituto Dermatologico San Gallicano IRCCS, Roma, Italy. E-mail: marta.punelli@uniroma1.it*

Aflatoxins are carcinogenic secondary metabolites produced by *Aspergillus flavus* and other closely related species. Many internal and external factors, such as nutrition, environment and interaction with the host (*Zea mays*) can affect aflatoxin biosynthesis. Host defence-related compounds, like oxylipins, may induce sporulation and modulate mycotoxins biosynthesis in several pathogenic fungi, apparently by replacing fungal endogenous oxylipins, produced by lipoperoxidative processes of lipoxygenase (LOX) and dioxygenase enzymes. In *A. flavus*, these oxylipins are crucial signals for the regulation of mycotoxins biosynthesis, conidiogenesis and sclerotia formation. To study how inhibition of oxylipins formation may affect the *A. flavus* metabolism and its relation with the host (maize kernels), we obtained a mutant (AfΔ*lox* strain) presenting a knock-out of the *lox1* gene, encoding a homologue of the human arachidonate 15-lipoxygenase. Onto liquid and solid media, the mutant displayed a different

colony morphology in comparison with 3357 wild-type strain, i.e. absence of conidia, lack of aflatoxins biosynthesis and a lower basal activity of LOX enzyme. Interestingly, its conidiation and aflatoxins production was recovered by growing the strain onto maize seeds. To better understand the involvement of the cross-talk *Z. mays-A. flavus*, we performed an oxylipin profile of the two strains (WT and Af**Δ***lox*) and of the maize seeds after host colonization using an LC-TOF based lipidomic approach followed by a PCA statistical analysis. The results will contribute to the current knowledge on the role of lipid peroxidation governed by the *lox1* gene in the morphogenesis, aflatoxins biosynthesis, and in host-pathogen interaction.

AN OVERVIEW OF THE GENOME OF THE BIOCONTROL AGENT *LYSOBACTER CAPSICI* STRAIN PG4. G. Puopolo[1,2] and A. Zoina[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Fondazione Edmund Mach IASMA-Research and Innovation Centre, Via Mach 1, 38010 S. Michele all'Adige, Italy. E-mail: puopolo@unina.it*

The genus *Lysobacter* encompasses nonfruiting gliding bacteria that exhibit remarkable lytic activity exploitable for the biological control of phytopathogenic fungi. Recently, *L. capsici* strain PG4 was proven to efficiently control *in vivo* tomato crown and root rot caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. The bacterial strain showed proteolytic and chitinolytic activity and was able to produce antibiotic substances active against Gram-positive bacteria exclusively. In order to unravel the mechanisms responsible for the biocontrol aptitudes of strain PG4, the genome of this bacterium was sequenced through Illumina GAIIX platform. The genome of strain PG4 consists of almost 7 Mbp with a 63.96% GC content. Once assembled and annotated, the genome has been analyzed for the presence of genes coding for lytic enzymes and for the synthesis of antibiotic compounds. Interestingly, strain PG4 genome possesses a high amount of genes (sixty nine) coding for proteases, eight and four genes respectively coding for glucanases and chitinases. The presence of all these lytic enzymes is coupled with the production of antibiotics. A 9 kb region showing 98% homology with the operon responsible for the biosynthesis of HSAF, an antibiotic compound produced by *L. enzymogenes* strain C3, has been found in the PG4 genome. The availability of the whole genome of strain PG4 represents a sound basis for the development of new studies aimed at better understanding how PG4 accomplishes the biological control of plant pathogenic fungi.

SELECTION OF NEW FUNGAL STRAINS FOR THE BIOLOGICAL CONTROL OF *OROBANCHE RAMOSA* ON TOBACCO. G. Puopolo[1,2] and A. Zoina[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Fondazione Edmund Mach IASMA-Research and Innovation Centre, via Mach 1, 38010 S. Michele all'Adige, Italy. E-mail: puopolo @unina.it*

The species *Orobanche ramosa* (broomrape) regroups obligate root parasite plants causing severe losses to tobacco. As these weeds attack crop roots they cannot be easily controlled mechanically or by using synthetic herbicides. The exploitation of biological control agents able to parasitize broomrapes offers a promising solution to this problem. At the moment many *O. ramosa* pathogenic organisms have been isolated to potentially control this holoparasitic weed. During this work several naturally diseased broomrape plants were collected in tobacco fields surveyed in Campania (southern Italy). Fifty five fungal isolates recovered from broomrape-diseased tissues were identified and evaluated for their capacity to control *O. ramosa*. Culture filtrates of all the fungal isolates were evaluated for their aptitude to inhibit the germination of broomrape seeds *in vitro*. Nine isolates, that drastically reduced the percentage of seed germination, were further evaluated *in vivo* to verify their ability to attack broomrape tubercules developing on the roots of parasitized plants. Although able to inhibit seed germination, strains belonging to genus *Ulocladium* and *Chalara* were not able to attack the tubercules. Only four fungal strains of *Fusarium solani* and one strain of *Fusarium oxysporum* actively attacked broomrape tubercules causing their death. These fungal strains might represent good candidates for the formulation of new products suitable for the biological control of *O. ramosa* on tobacco.

ALTERNATIVE CONTROL APPROACHES AGAINST GRAY MOLD OF *PELARGONIUM ZONALE*. M. Quaglia, C. Moretti, G. Canala and R. Buonaurio. *Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, Perugia, Italy. E-mail: mara.quaglia@unipg.it*

In collaboration with a nursery located near Aprilia (central Italy), we started to investigate the use of antagonistic fungi of the genus *Trichoderma* (*T. atroviridae* P1, *T. harzianum* T22 and T67, *T. reseii* T34, *Trichoderma* spp. 8009, *T. hamatum* T382 and a commercial product containing the isolates ICC012 of *T. harzianum* and ICC080 of *T. viride*) and resistance inducers in the control of pelargonium gray mold. In dual-culture tests, each *Trichoderma* isolate caused a significant reduction in the growth of an isolate of *Botrytis cinerea* collected in the nursery, which was iprodione-resistant as confirmed by *in vitro* assays. The effect was significantly higher when *Trichoderma* isolates were inoculated two days before the pathogen rather than simultaneously. Moreover, the isolates P1, T22, T34 and ICC012+ICC080 caused an abnormal hyphal branching of the pathogen, while T67 formed coiling around them. Preliminary *in vivo* assays seemed to confirm the effectiveness of all the antagonists with the exception of T22, as they reduced significantly the severity of gray mold. Among the resistance inducers, Biochikol, which contains chitosan, reduced the disease severity, while treatments with pure chitosan dissolved in 1% acetic acid (0.04%, w/v) or 0.3 mM acibenzolar-S-methyl were ineffective and phytotoxic. As expected, pelargonium plants treated with iprodione (34 ppm) were not completely protected from gray mold, confirming the necessity for alternative control approaches.

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THE LACK OF INHIBITION OF *CLAVICEPS PURPUREA* POLYGALACTURONASES BY PvPGIP2 MAY EXPLAIN THE FAILURE OF TRANSGENIC WHEAT PLANTS TO RESIST TO THE ERGOT PATHOGEN. A. Raiola[1], C. Volpi[2], M. Janni[2], D.M. O'Sullivan[3], C. Castiglioni[1], F. Favaron[1] and R. D'Ovidio[2]. *[1]Dipartimento Territorio e Sistemi Agro-forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. [2]Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Via San Camillo de Lellis, s.n.c., Viterbo, Italy. [3]National Institute of Agricultural Botany (NIAB), Cambridge, UK. E-mail: alessandro.raiola@unipd.it*

Claviceps purpurea is a biotrophic fungal pathogen of cereals and grasses, attacking exclusively young ovaries. *C. purpurea* grows intercellularly in rye (*Secale cereale*) ovaries by degrading the pectic polymers and the fungal polygalacturonase (PG) has been shown to be a pathogenicity factor. Two *pg* genes of *C. purpurea*, *cppg1* and *cppg2*, are responsible for this activity. Transgenic plants expressing a bean polygalacturonase-inhibiting protein (PvPGIP2) proved to be a valuable tool to increase resistance against PG-producing fungi. However, a stable transgenic PvPGIP2 wheat line, previously shown to be more resistant to the fungal pathogens *Bipolaris sorokiniana* and *Fusarium graminearum*, exhibited only a very low reduction of symptoms after infection with *C. purpurea*. To understand whether this reduced protection of PvPGIP2 in wheat transgenic plants was ascribable to a lack of inhibition against the fungal PGs, we tried to perform inhibition experiments against the *C. purpurea* PG activity. Unfortunately, this fungus does not produce any PG activity in culture, thus it was necessary to express this activity in a *Pichia pastoris* heterologous system. The two heterologous expressed PGs, when assayed against the PvPGIP2, were poorly affected by this inhibitor, indicating that the lack of resistance in transformed wheat line may be due to the lack of recognition of the PGs of *C. purpurea* by PvPGIP2. This finding supports a role of PGIP in plant defence only when PG-PGIP interaction occurs. The expressed PGs may be useful to identify more effective PGIPs by a broad screening of plant PGIPs.

NEW INSIGHTS INTO KIWIFRUIT-INFECTING VIRUSES IN ITALY. C. Ratti[1], A.R. Babini[2], C. Lanzoni[1], C. Poggi Pollini[1], R. Credi[1], A. Pisi[1], G. Filippini[1] and C. Rubies Autonell[1]. *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 40, 40127 Bologna, Italy. [2]Servizio Fitosanitario Regionale, Regione Emilia Romagna, Via di Saliceto 81, 40128 Bologna, Italy. E-mail: concepcion.rubies@unibo.it*

In Italy, for protecting kiwifruit (*Actinidia* spp.) production by restraining the spread of new and harmful diseases on both nurseries and orchards, a specific Decree has been issued by the Italian Ministry for Agriculture, Food and Forest Policies (Decree 7th February 2011 – published in the Official Journal of the Italian Republic n. 69 25th March 2011). According to this Decree, primary source material must be free, at least, from 6 different virus species: *Actinidia virus A* (AVA), *Alfaalfa mosaic virus* (AMV), *Apple stem grooving virus* (ASGV), *Citrus leaf blotch virus* (CLBV), *Cucumber mosaic virus* (CMV), *Ribgrass mosaic virus* (RMV) and also from the Stolbur phytoplasma (*Candidatus* Phytoplasma solani). Deeper information on biological and molecular proprieties of kiwifruit viruses is therefore necessary to ensure the development of diagnostic tools for a rapid, sensitive and reliable analysis of kiwifruit vegetative propagation material. A CMV isolate (K35) has been recently identified in kiwifruit plants collected in Emilia Romagna (northern Italy). A deep molecular characterization of this isolate has been done to establish the virus origin. Moreover, agroinfectious K35 clones have been produced with the purpose to infect new kiwifruit plants on which to perform studies on natural transmission of the virus and evaluate performances of developed detection methodologies. New viral isolates have been identified during survey carried out in 2011. Molecular characterization is in progress, but these new reports suggest that viral infections occur more extensively than expected on Italian kiwifruit nurseries and orchards.

DETECTION OF *PRUNUS NECROTIC RINGSPOT VIRUS* IN ALMOND NATIONAL GERMPLASM OF AFGHANISTAN. S. Rehman[1], J. Ahmad[1], C. Lanzoni[2], C. Rubies Autonell[2] and C. Ratti[2]. *[1]Plant Biotechnology Laboratory, Aga Khan Foundation-Afghanistan, Wazir Akbar Khan, Rd 13, H43, Main Road, Kabul, Afghanistan. [2]Dipartimento di Scienze e Tecnologie Agroambientali Patologia Vegetale, Università degli Studi, Viale G. Fanin, 40, 40127 Bologna, Italy. E-mail: shams-urahman.shams@akdn.org*

About 70% of Afghanistan's population is dependent directly or indirectly on agriculture and livestock. Horticulture is one of the most important sectors in Afghan agriculture that can play a vital role in the overall recovery of Afghan economy. To strengthen the horticulture sector of Afghanistan, EC supporting PHDP (Perennial Horticulture Development Project), the main objective of the project is to provide true to type/ecotype and healthy planting materials, of local fruit varieties, to nursery growers and ultimately for the rehabilitation and establishment of new orchards. The plant biotechnology laboratory, located in Kabul, is providing services to PHDP by analyzing each year the national collections and motherstock nurseries of national germplasm. The national collection of almond had 102 variety plots (6 plants/plot). To know the sanitary status of this germplasm, samples (young leaves in spring season) were collected from PHDP research station of Mazar Sharif (northern Afghanistan). DAS-ELISA was used to assay for the presence of PPV *(Plum pox virus)*, PDV *(Prune dwarf virus)* and PNRSV *(Prunus necrotic ringspot virus)* in the collected samples. PNRSV was detected in three accessions: 4016 Kaghazi, 6309 Lauranne and 6041 Nonpareil. Molecular analysis is in progress in order to characterize PNRSV isolate detected. To our knowledge these results identified PNRSV in almond plants for the first time in Afghanistan. Moreover phytosanitary certification scheme is needed in Afghanistan for the production of quality planting materials to define strategies able to control viral and other pathogens.

***GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS* IN DECLINING SYRAH GRAPEVINES IN ITALY. S. Rizza, C. Oliveti and M. Tessitori.** *Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Sezione Fitopatologia e Genetica Vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: mtessitori@unict.it*

A "decline" of Syrah grapevines was first observed as an emerging disease in France and later it was reported from California. Recently a similar disease was observed in a viticulture area of Tuscany but no data on presence of *Grapevine rupestris stem pitting-associated virus* (GRSPaV) were reported. Recently a four year old Syrah vineyard (rootstock 1103 Paulsen) of 3344 plants in the Ragusa province (Sicily, insular Italy) were signaled for the presence of severe syndrome ascribable to virus infection. Symptoms observed during field inspection were: cler-cut leaf reddening, swelling and cracking at the graft level, stem pitting of the wood, decline and death of plants. To ascertain the presence of the main grapevine viruses, symptomatic samples were collected and were tested for the presence of GRSPaV, *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine leafroll-associated virus -1, -2, -3* (GLRaV-1, -2, -3*), Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV), *Arabis mosaic virus* (ArMV). Total RNA, extracted from leaf stems using the RNeasy method, was analyzed by RT-PCR assay by using primers designed in high conserved regions of the genome for each virus. GRSPaV was detected in the symptomatic samples whereas none of the viruses were found in synptomless vines. Deeper investigation will include

virus genome sequencing in order to define the nature of the isolate and the identification of other possible viruses associated to the syndrome. In our knowledge this is the first report of the association of the GRSPaV to Syrah decline in Italy.

SYMPTOMS EXPRESSION AND GROWTH OF CITRUS VARIETIES ON CITRANGE ROOTSTOCKS INOCULATED WITH DIFFERENT CITRUS VIROIDS. S. Rizza[1], R. La Rosa[1], M. Tessitori[1], G. Albanese[3] and A. Catara[1,2]. *Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Sezione Fitopatologia e Genetica Vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [2]Laboratorio di Diagnosi e Biotecnologie Fitosanitarie, Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Blocco Palma I, 95121 Catania, Italy. [3]Dipartimento di Gestione dei Sistemi Agrari e Forestali,Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. E-mail: srizza@unict.it*

The *Citrus tristeza virus* (CTV) epidemics in Italy require the substitution of sour orange, a rootstock highly susceptible to CTV but tolerant to citrus viroids (CVds), with citranges that are tolerant to CTV. Among the CVds just two are responsible of citrus diseases: *Citrus exocortis viroid* (CEVd) for exocortis and *Hop stunt viroid* (HSVd) for cachexia, respectively. CEVd causes bark scaling of trifoliate orange and its hybrids, whereas its performance on citranges and other hybrids has been questioned several times and it is still unclear. The behaviour of CEVd and/or different citrus viroids was evaluated on cv. Tarocco sweet orange and cv. Clementine comune grafted on Troyer and Carrizo citranges, respectively inoculated 28 and 15 years ago. Trees were monitored every year by field inspections for symptom observations, assays on Etrog citron, RT-PCR and real time RT-PCR analysis. CEVd-inoculated clementine trees (2×3 m density planting) showed no bark scaling but only size reduction, whereas those inoculated with a mixture of *Citrus dwarfing viroid* (CDVd), HSVd and CEVd showed reduced size, bud union line indentation and bark gumming. Tarocco sweet orange performed very well in size and yield despite CEVd infection similarly to healthy trees.

SURVEY OF VIRUSES INFECTING A LILIUM VARIETAL COLLECTION IN TUSCANY. D. Rizzo[1], B. Nesi[2], L. Stefani[1], S. Lazzereschi[2], S. Pecchioli[2], M. Della Bartola[3], A. Materazzi[3] and A. Grassotti[2]. *[1]Regione Toscana, Laboratorio Servizio Fitosanitario Regionale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [2]CRA-VIV, Unità di Ricerca per il Vivaismo e la Gestione del Verde Ambientale ed Ornamentale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [3]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: domenico.rizzo@regione.toscana.it*

In the frame of a phytosanitary survey of a *Lilium* varietal collection, carried out by CRA-VIV at Pescia (PT), leaf samples were collected from 60 plants belonging to 20 varieties and tested by RT-PCR for the presence of *Cucumber mosaic virus* (CMV), *Lilium symptomless virus* (LSV) and *Lilium mottle virus* (LMoV). Three plants of each variety were sampled in late spring-early summer 2010. Total RNA was extracted with a modified protocol using the "RNeasy plant mini kit" (Qiagen, USA) and retro-transcribed into cDNA. Diagnosis of CMV, LSV and LMoV was at first performed with specific protocols described in the literature.These protocols were then uniformed to unique amplification conditions to allow for a faster singleplex detection of the three viruses. Results obtained with the new amplification conditions perfectly matched the results of the standard protocols, confirming the specificity and sensitivity of the tests. Of 60 samples, 40 (66.7%), belonging to 17 varieties, were infected with CMV, 56 (93.3%), belonging to 20 varieties, resulted positive to LSV and 4 (6.7%), belonging to 2 varieties, were infected with LMoV. The same 60 samples were successively tested by RT-PCR for the tospoviruses *Impatiens necrotic spot virus* (INSV) and *Tomato spotted wilt virus* (TSWV), with negative results.

PHYTOSANITARY SURVEY ON POME FRUIT AUTOCHTHONOUS GERMPLASM IN TUSCANY. D. Rizzo[1], L. Stefani[1], M. Paoli[1], M. Della Bartola[2] and A. Materazzi[2]. *[1]Regione Toscana, Laboratorio Servizio Fitosanitario Regionale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [2]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: domenico.rizzo@regione.toscana.it*

During 2010, a survey was conducted for the prsence of some of the main virus, viroid and phytoplasma pathogens affecting the autochthonous germplasm of *Malus* and *Pyrus* from Tuscany. Samples, consisting of leaves, shoots and phloem tissues, were collected during spring 2010. In detail, a total of 97 plants, 73 of which belonging to 29 *Malus* varieties and 24 belonging to 13 *Pyrus* varieties, were tested by RT-PCR and nested PCR for the following pathogens: *Candidatus* Phytoplasma mali (*Ca.* P. mali), *Candidatus* Phytoplasma pyri (*Ca.* P. pyri), Apple *chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Apple mosaic virus* (ApMV), *Apple dimple fruit viroid* (ADFVd) and *Apple scar skin viroid* (ASSVd). Of the eight pathogens investigated, ACLSV and ASPV proved to be the most widespread (both present in 67.0% of the tested samples), while ASGV was present in 14.4% of the surveyed plants. In detail, ACLSV was detected on 59 *Malus* and 6 *Pyrus* plants, while ASPV was found on 54 and 13 accessions of *Malus* and *Pyrus*, respectively. As to ASGV, infections were detected in 13 *Malus* and 1 *Pyrus* plants. The remaining pathogens (ApMV, ADFVd, ASSVd, *Ca.* P. mali e *Ca.* P. pyri) were not found in any of the surveyed plants.

FURTHER DATA ON THE DISTRBUTION OF GRAPEVINE YELLOWS IN TUSCANY. D. Rizzo[1], H. Bouyahia[2], M. Della Bartola[2], P. Braccini[1], L. Stefani[1], M. Carli[1] and A. Materazzi[2]. *[1]Regione Toscana, Laboratorio Servizio Fitosanitario Regionale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [2]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: domenico.rizzo@regione.toscana.it*

Grapevine yellows (GY) are an important disease of *Vitis vinifera* caused by phytoplasmas. In Italy, GY are mostly represented by Flavescence dorée (FD) and Bois noir (BN) associated, respectively, with infection by 16SrV and 16SrXII phytoplasma ribosomal groups. In the frame of a regional strategy aimed at monitoring GY presence, an extensive survey was carried out in late summer 2010, collecting 629 leaf samples from commercial vineyards. Samples were preferably collected from plants showing symptoms of GY diseases. Each sampled plant was marked and localised by global positioning system (GPS) to allow tracking of FD-positive samples for uprooting. DNA extracts were tested by Real-time PCR for detection of BN and FD phytoplasmas. The results confirmed that BN is by far the most important GY in Tuscany, with an ubiquitous distribution. In fact, it was

present in almost every surveyed vineyard with an overall incidence of 53,6% (337 out of 629 plants tested). The presence of FD was recorded from 39 samples distributed in 4 provinces including Massa-Carrara, Florence, Pistoia and Lucca. An alarming situation was observed in this latter province, where 23 out of 74 plants (31,1%) were infected by FD. On the basis of these results, further investigations on insect vectors, natural reservoirs and molecular characterization of grapevine infecting phytoplasmas are needed for a better understanding of GY epidemiology in Tuscany.

OCCURRENCE OF *HYDRANGEA RINGSPOT VIRUS* IN A *HYDRANGEA*. VARIETAL COLLECTION. **D. Rizzo[1], B. Nesi[2], L. Stefani[1], M. Antonetti[2], S. Lazzereschi[2], S. Pecchioli[2], M. Della Bartola[3], A. Materazzi[3] and A. Grassotti[2].** *[1]Regione Toscana, Laboratorio Servizio Fitosanitario Regionale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [2]CRA-VIV Unità di Ricerca per il Vivaismo e la Gestione del Verde Ambientale ed Ornamentale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [3]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: domenico.rizzo@regione.toscana.it*

In the frame of a survey on 21 species/varieties of *Hydrangea* spp. and on 2 species of the close genus *Schizophragma* carried our by CRA-VIV in Pescia (PT), 44 plants were tested by RT-PCR for the presence of possible infections by *Hydrangea ringspot virus* (HdRSV). Leaf samples were collected in late spring-early summer 2010 from plants showing crinkling, rolling and asymmetry of the leaves, decreased number of florets per inflorescence, severe dwarfing and 'running out'. Total RNA was extracted with a modified protocol using the "RNeasy plant mini kit" (Qiagen, USA) and retro-transcribed into cDNA. RT-PCR analysis was done according to protocols already described in the literature. Results showed that 15 out of 44 plants (34.1%), belonging to 11 cultivars of 5 different species of *Hydrangea* were infected with HdRSV. The amplicon obtained was then sequenced and proved to share a high similarity with the HdRSV sequences present in GenBank. All plants of the genus *Schizophragma* gave negative responses.

MOLECULAR ANALYSIS OF ROOT ROT PATHOGENS IN ORNAMENTAL PLANTS. **D. Rizzo[1], L. Stefani[1], M. Paoli[1], A. Grassotti[2] and A. Haegi[3].** *[1]Regione Toscana, Laboratorio Servizio Fitosanitario Regionale, Via dei Fiori 8, 51012, Pescia (PT), Italy, [2]CRA-VIV Unità di Ricerca per il Vivaismo e la Gestione del Verde Ambientale ed Ornamentale, Via dei Fiori 8, 51012 Pescia (PT), Italy, [3]CRA, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: anita.haegi@entecra.it*

The sanitary situation of the ornamental plant industry is very dynamic and changing due to globalized market, introduction of new plant genera, species or varieties and new cultural techniques (which can favour or repress different pathogens). Monitoring this kind of phytosanitary situation prove somehow difficult, so, with the ultimate aim of developing new diagnostic technologies based on molecular tools, molecular analysis of pathogens were performed in ornamental plant industries of Tuscany. We focused on woody plants, either conifers (e.g. *Cupressus* sp., *Pinus* sp. or *Taxus* sp.) or not (*Acer* sp., *Viburnum* sp. or *Pittosporum*) collected in 2010 as affected by root rot and/or tracheomycoses. A total of 85 symptomatic samples were collected and analysed: methods were set up to extract DNA from woody material and perform efficient PCR with various primers on material of different plant origin. PCR assays with ITS5/ITS4 primers were performed to amplify fungi and with ITS6/ITS4 primers to amplify oomycetes. The first couple of primer amplified 33 samples of 85, whereas the second set amplified 41 samples of 85. Some samples yielded multiple bands (two or more) indicating that there was more than one fungus either pathogenic or saprophytic. All samples were also amplified with primers specific for the most common fungi, namely *Phytophthora* sp., *Cylindrocarpon* sp. and *Phomopsis* sp. Some samples were also sequenced to confirm PCR results or to unravel other fungi.

EFFECT OF GREENHOUSE DEHUMIDIFICATION ON DAMAGES CAUSED BY *BOTRYTIS CINEREA* TO *POINSETTIA*. **D. Rizzo[1], G. Burchi[2], L. Stefani[1], M. Antonetti[2], A. Haegi[3], A. Teani[2] and A. Grassotti[2].** *[1]Regione Toscana, Laboratorio Servizio Fitosanitario Regionale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [2]CRA-VIV Unità di Ricerca per il Vivaismo e la Gestione del Verde Ambientale ed Ornamentale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [3]CRA, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: domenico.rizzo@regione.toscana.it*

This work was carried out in the framework of a research project, funded by Mi.P.A.A.F., that studies application of air dehumidification by means of hygroscopic solutions for humidity and climate control and energetic management in greenhouse floriculture. Phytosanitary surveys were performed in two greenhouses at Verdello (Bergamo, northern Italy), one containing the dehumidification system and the other used as a control, with the aim to evaluate the beneficial effects of dehumidification on phytosanitary control. Analyses were performed on poinsettia (*Euphorbia pulcherrima)* pot-grown plants cultivated in both greenhouses from October to December 2010. Damages on several plants, with symptoms of wilting that eventually led to plant death, were recorded. Eighty percent of symptomatic plants belonged to the 'control' greenhouse (without dehumidification system). Symptomatic samples (27) were collected to study the etiology of the disease: visual observation and mycological isolations revealed the presence of *Botrytis cinerea* and other saprophytic fungi. To confirm these results, molecular protocols were optimized to screen for ITS4/6 (PCR end point), *Thielaviopsis basicola (*PCR Real Time Taqman*), Phytophthora* spp (PCR end point) and *B. cinerea (*PCR Real Time Sybr Green*)*. Results on 27 symptomatic samples showed that 100% (27/27) were positive for *B. cinerea*, 92.6% (25/27) were amplified with ITS4/6 (that amplify oomycetes), 7.4% (2/27) were positive for *Phytophthora* spp. and no one was positive for *T. basicola.* Results show that in these environmental conditions (greenhouse) the main pathogen of *Poinsettia* seedlings is *B. cinerea* and that dehumidification of the environment can better control the pathogen.

A SURVEY FOR THE MAIN DISEASES OF *LIMONIUM spp.* **D. Rizzo[1], G. Burchi[2], L. Stefani[1], M. Paoli[1], M. Antonetti[2], A. Teani[2], A. Haegi[3] and A. Grassotti[2].** *[1]Regione Toscana, Laboratorio Servizio Fitosanitario Regionale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [2]CRA-VIV Unità di Ricerca per il Vivaismo e la Gestione del Verde Ambientale ed Ornamentale, Via dei Fiori 8, 51012 Pescia (PT), Italy. [3]CRA, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: domenico.rizzo@regione.toscana.it*

Main fungal diseases that affect *Limonium spp.* are induced by *Peronospora statices, Botrytis cinerea* and *Colletotrichum gloeosporioides*. In the frame of a survey of a *Limonium* collection carried out by CRA-VIV at Pescia (PT), a screening was performed in May 2010 to verify the presence/susceptibility to the above-mentioned fungi. Seventyfour samples of symptomatic and asymptomatic material (leaves and stems) were collected from 20 different clones of *Limonium*, belonging to 5 different species and hybrids, including 6 commercial cultivars. DNA was extracted from plant material through a modified protocol based on CTAB 3%. Diagnosis was done with molecular biology protocols optimized in our laboratory for *Colletotrichum gloeosporioides* (PCR end point), *Botrytis cinerea (*PCR Real Time SybrGreen*)* and with primers ITS4/6 that amplify oomycetes, to check for the presence of *Peronospora statices.* This latter assay was positive for 91.9% of the samples, whereas *Botrytis cinerea* was detected in 60.8%, and *Colletotrichum gloeosporioides* in 38.2% of the samples. These results were confirmed by wet room tests. As to the visible effects of pathogenic fungi, none of the tested varieties of *Limonium* showed *Peronospora statices* symptoms; 12 clones were completely asymptomatic for *Botrytis cinerea* and *Colletotrichum gloeosporioides* (*L. gmelinii*, *L. altaica L. serotinum* and hybrids between them); 10 clones showed symptoms due to *Botrytis cinerea* (mostly *L. latifolium, L. altaica*, and only one clone of *L. gmelinii*); and 4 clones showed strong symptoms of both *Botrytis cinerea* and *Colletotrichum gloeosporioides* (*L. latifolium* and *L. latifolium x otolepis*).

POSSIBLE INDUCTION OF SYSTEMIC RESISTANCE IN ZUCCHINI AGAINST *POWDERY MILDEW* FOLLOWING TREATMENT WITH *CYANOBACTERIA* EXTRACTS. R. Roberti[1], H. Righini[1], P.L. Burzi[2], C. Perez Reyes[3], S. Galletti[2], B. Garcia and G. Reina[3]. *[1]Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale Fanin 40, 40127 Bologna, Italy. [2]CRA, Centro di Ricerca per le Colture Industriali, Bologna, Italy. [3]Centro De Biotecnología Marina, Universidad De Las Palmas De Gran Canaria, Islas Canarias, Spain. E-mail: roberta.roberti@unibo.it*

A sustainable agriculture that prevents environmental pollution needs alternative control means. Cyanobacteria are components of fertilizer compounds which may interfere with plant physiology, making plants less susceptible to biotic and abiotic stresses. The aim of this research was to study the possibility to induce systemic resistance in zucchini plants by foliar application of cyanobacterium *Nostoc* sp. extracts. Two trials were carried out under controlled conditions: the first one consisted in a biological assay of *Nostoc* extract against *Podosphaera leucotricha* and the second one in biochemical assays in order to test the involvement of induced resistance. Chitosan was used as positive control, since it is a well-known resistance inducer. *Nostoc* extract (5 g l^{-1}) was applied by spraying one of the two cotiledonar leaves in both experiments, while the pathogen was inoculated on the non-treated leaves (5×10^4 conidia per ml of water) in the biological assay only. Disease symptoms were recorded as percentage of infected area on non-treated leaves 7-9 days post inoculation. For biochemical assays, total proteins were extracted following a non-denaturing method and the expression of some PR-proteins (PR-3, PR-5 and PR-9) was tested by spectrophotometric and by isoelectrofocusing methods. The biological assay revealed that the *Nostoc* extract reduced the disease by 20%, similarly to chitosan, with respect to the inoculated control. Both spectrophotometric and isolectrofocusing methods showed differences of the enzymatic activities tested between treated and non-treated plants, suggesting the ability of the *Nostoc* extract tested to induce systemic resistance in zucchini.

ACTIVITY OF BIOSTIMULANT PRODUCTS INTEGRATED WITH SULFUR FOR THE CONTROL OF ZUCCHINI POWDERY MILDEW. R. Roberti, M. Fabbri, A. Veronesi and A. Brunelli. *Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale G. Fanin, 46, 40127 Bologna, Italy. E-mail: roberta.roberti@unibo.it*

The biostimulants, Biosaral, Vitaflow and Chelal Alga (mainly based on seaweed extracts) and Salavida (*Pseudomonas trivialis*) were applied with preventative or post-inoculation timing, in combination with sulphur against *Podosphaera xanthii* on pot-grown zucchini under greenhouse conditions. The pathogen was inoculated on cotyledonar leaves on which sulphur was sprayed at a low dose (56 g/100 l). In the preventative timing, products were applied two days before inoculation, by spraying leaves, by drenching the potting mixture or by both, depending on the product: Biosaral was applied on the leaves, on potting mixture or on both with and without sulphur; Vitaflow was applied on the leaves only with and without sulphur; Salavida and Chelal Alga were applied singly with and without sulphur. In the post-inoculation timing Biosaral, Salavida and sulphur were considered: Biosaral was applied on the leaves seven and nine days post-inoculation (10-15% and 40-50% leaf area with disease symptoms respectively) with single or combined treatments with or without sulphur; Salavida was applied in the potting mixture seven days post-inoculation with or without sulphur. Both in the preventative and in post-inoculation timing all products, depending on their application (on leaves, potting mixture or both) showed effectiveness against the disease and their integration with sulfur enhanced powdery mildew control. Generally, Biosaral and Salavida were the more active products. In conclusion, biostimulants, integrated with a low dose of sulphur, provide a good opportunity to reduce powdery mildew of zucchini.

CURRENT STATUS OF RESISTANCE TO FUNGICIDES IN *BOTRYOTINIA FUCKELIANA* (*BOTRYTIS CINEREA*) ON STRAWBERRY IN BASILICATA. C. Rotolo[1], M. Masiello[1], D. Gerin[1], S. Pollastro[1], R.M. De Miccolis Angelini[1], A. Caponero[2] and F. Faretra[1]. *[1]Dipartimento di Biologia e Chimica Agro-Forestale ed Ambientale, Università degli Studi "Aldo Moro", Via G. Amendola 165/A, 70126 Bari, Italy. [2]A.L.S.I.A., Agenzia Lucana di Sviluppo ed Innovazione in Agricoltura, via C. Levi 6/I, 75100 Matera, Italy. E-mail: faretra@agr.uniba.it*

Botryotinia fuckeliana (*Botrytis cinerea*), is the causal agent of grey mould on a wide range of crops affecting quality and quantity of the produce. The disease management frequently requires numerous seasonal fungicide sprays increasing the risk of resistance development in the pathogen's populations. A two-year monitoring of *B. fuckeliana* response to anilinopyrimidines (AP), phenylpyrroles, hydroxyanilides and pyridine-carboxamides (SDHI) was carried out during 2009 and 2011 through a germination test on conidia sampled from naturally-infected strawberry fruits in a total of 25 farms located in the Metaponto area (southern Italy). Resistant conidia were distinguished from sensitive conidia by their ability to yield colonies on media amended with single discriminatory concentrations of each fungicide: pyrimethanil (1 mg l^{-1}), fludioxonil (0.3 mg l^{-1}), fenhexamid (0.4 and 4 mg l^{-1}) or boscalid (10 mg l^{-1}). AP-resistant isolates were detected in all monitored fields, frequently at high frequency (13-96%). A slight increase in the frequency of conidia resistant to phenylpyrrroles was detected in 2011 (up to 17%) as compared to that recorded in 2009 (0-7%). Resistance to fenhexamid was detected with a very low frequency (3×10^{-5} to 13×10^{-2}) in 5 of the 8 fields monitored in 2009 and in 16 of the 17 fields monitored in 2011. Resis-

tance to boscalid, which was applied in mixture with the QoI fungicide pyraclostrobin, was detected in all fields at variable frequency (3-73%). The results disclose a widespread incorrect use of fungicides against gray mould and stress out the importance of appropriate anti-resistance strategies.

VARIABILITY AMONG *PECTOBACTERIUM CAROTOVORUM* STRAINS ISOLATED FROM ARTICHOKE IN SOUTHERN ITALY. M. Russo[1], G. Ialacci[2], S. Loreti[3], V. Catara[2] and P. Bella[2]. *[1]Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Via V. Lancia, 95030 Catania, Italy. [2]Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. [3]CRA, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: patrizia.bella@unict.it*

Pectobacterium carotovorum subsp. c*arotovorum*, the causal agent of soft rot, has a broad host range and is distributed worldwide. In 2010, a severe outbreak of artichoke soft rot occurred in Sicily. The characteristic symptoms were leaf wilting and internal decay at the crown often accompanied by vascular discolouration. Eventually, the plants wilted completely and died. Bacteria were consistently recovered from the diseased plants on nutrient dextrose agar and identified as *P. carotovorum* (*Pc*) by biochemical tests, Biolog identification system, PCR using specie-specific primers Y1/Y2 and pathogenicity on potato tubers. Sicilian strains were genotyped with other representative *P. carotovorum* strains isolated from artichoke in different locations in southern Italy. Twelve *Pc* from artichoke and reference strains of *P.c.* subsp. *carotovorum* (*Pcc*), *P.c.* subsps. *odoriferum* (*Pco*), *P. wasabiae* (*Pw*) and *P. atrosepticum* (*Pa*) were analyzed by BOX-PCR and fluorescent amplified fragment length polymorphism (fAFLP). By BOX-PCR, a common core profile was identified in all *Pc* strains from artichoke, however different haplotypes were detected even in the same production site. Adequate numbers of fragments for fAFLP analysis were selected by *in silico* AFLP-PCR experiments on *P. c.* subsp. *carotovorum* PC1 genome (http:/www.in-silico.com). Four primer combinations were screened for their reproducibility and number of polymorphic bands on four representative *Pc* strains. The dendrogram of distances obtained by analysis of fAFLP data highlights that *Pc* strains from artichoke form two distinct clusters including also *Pcc* and *Pco* reference strains, but clearly separated from *Pw* and *Pa* strains.

***CYLINDROCARPON* SPECIES ASSOCIATED TO KIWIFRUIT AFFECTED BY ELEPHANTIASIS. A. Rustignoli, A. Prodi, M.A. Mir, S. Borsari, P. Nipoti and A. Pisi.** *Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. E-mail: antonio.prodi@unibo.it*

Kiwifruit is an important fruit crop of temperate regions and Italy is the leader in production. An unusual disease, named elephantiasis, in kiwifruit (*Actinidia deliciosa*) cv Hayward orchards of northern Italy, was observed since 2001. Trunk hypertrophy is the most typical symptom of elephantiasis. A complex mycoflora belonging to different genera, mainly *Phialophora*-like taxa, *Fusarium* (mostly *F. solani*) and *Cylindrocarpon* spp., was isolated from necrotic wood of symptomatic trunks. The causal agent/s is/are unknown because pathogenicity field assays are still in progress. The aim of this study was to identify at the species level isolates of *Cylindrocarpon,* which is the genus until now less investigated. *Cylindrocarpon* isolates were identified morphologically, assayed by a multiplex PCR protocol for the identification of *C. liriodendri*, *C. pauciseptatum* and *C. macrodidymum* - species usually associated to a severe grapevine disease (black foot) and further investigated using sequencing and phylogenetic analyses of the partial ß tubulin gene. *C. macrodidymum* and *C. destructans* var. *crassum* were identified. To our knowledge, this is the first report of these species in *Actinidia deliciosa*. The role of *Cylindrocarpon* spp. in elephantiasis needs more investigations in order to understand the synergy with the other fungi involved in this complex disease.

EFFICACY OF ZATARIA OIL ON POSTHARVEST CONTROL OF RHIZOPUS ROT OF PEACHES. S. Alizadeh-Salteh[1], K. Arzani[1], R. Omidbeigi[1], N. Safaie[2] and P. Mohammadi[3]. *[1]Department of Horticultural Science, Tarbiat Modares University (TMU), Iran. [2]Department of Plant Pathology, Tarbiat Modares University (TMU), Iran. [3]Department of Plant Pathology, Tehran University, Iran. E-mail: kazem.arzani@gmail.com*

The advantage of essential oils is their bioactivity in the vapour phase, a characteristic that makes them attractive as possible fumigants for stored product protection. The aim of this investigation was to evaluate the antifungal effects of the herbal essential oil of Shiraz Thyme (*Zataria multiflora* Boiss) against *Rhizopus stolonifer*, causal agent of Rhizopus rot of peach. In *in vitro* trials, the activity of Zataria oil was tested on the growth of the pathogen's mycelium. Essential oil was added to the medium (ranging from 120 to 360 µl l^{-1}), or to a filter paper (ranging from 1 to 48 µl l^{-1}) inserted on the lid of Petri dish tobe assayed as biofumigant. In *in vivo* experiments, four concentrations of Zataria oil (0, 120, 240 and 360 µl l^{-1}) were tested by dipping and seven doses (0, 50, 100, 200, 400, 800 and 1600 µl l^{-1}) were applied as vapour phase on peaches cv J.H. Hale artificially inoculated with the pathogen (10^6 conidia ml^{-1}). Fruits were stored at 25°C or 1°C for 20 and 45 days respectively. In *in vitro* results the concentrations of 360 µl l^{-1} used in the medium and that of 24 µl l^{-1} used as volatile showed the highest activity against *Rhizopus* growth. In *in vivo* trials, the most effective concentration of essential oil was 360 µl l^{-1} used by dipping and 24 µl l^{-1} used as biofumigant. More investigation are in progress to evaluate the practical application of Zataria oil against Rhizopus rot.

BIOFUMIGATION FOR THE CONTROL OF FUSARIUM ROOT ROT IN ITALY. A. Santori[1], S. Mocali[2], S. Landi[2] and A. Infantino[1]. *[1]CRA-PAV Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]CRA, Centro di Ricerca per l'Agrobiologia e la Pedologia, Piazza D'Azeglio 30, 50121 Firenze, Italy. E-mail: alessandro.infantino@entecra.it*

Crown and root-rot (CRR) is a disease of complex aetiology of wheat in which several *Fusarium* species and *Microdochium nivale* are involved. Attacked plants show browning and rotting of the crown and roots and whitening of the heads containing shrivelled kernels of no commercial value. A field experiment for the control of the disease by using green manure with *Brassica juncea* cv. Scala (SIS, Italy) was conducted at the CRA-PAV farm (Rome, Italy) during the 2010-2011 season. Four sub-parcels, each representing a different treatment, were considered: (i) green manure for two years in a row; (ii) green manure for one year; (iii) effect of green manure of previous year; (iv) plot without green manure. *B. juncea* was grown for three months in sub-parcel 1 and 2, then mulched and incorporated into the soil. After ten days, durum wheat cv. Claudio was sown in all sub-parcels. For microbiological analyses, soil samples were collected in two different periods,

before sowing *B. juncea* and wheat, respectively. Incidence of white heads was assessed at the hard dough growth stage by visual evaluation of 200 heads for each treatment. Bio-fumigation with *B. juncea* for two following years (treatment 1) determined the lowest incidence (9.5%) when compared to that of the control (32%). Intermediate results were obtained for treatment 2 (15.9%) and treatment 3 (23.5%). Microbiological analyses of soil samples and of wheat stem and crowns are in progress. The preliminary results obtained confirm that green manure is promising for the control of CRR of wheat.

PHYTOPHTHORA SPECIES ASSOCIATED WITH SUDDEN DEATH OF YOUNG OLIVE TREES IN SARDINIA. B. Scanu, B.T. Linaldeddu, S. Seddaiu, L. Maddau and A. Franceschini. *Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: bscanu@uniss.it*

Since 2007, about 7.000 ha of agricultural land has been planted with olive (*Olea europea*) in Sardinia (insular Italy). Following several reports of sudden death of young olive trees, a survey was carried out in 2010 in three 1- to 4-year-old groves and in a commercial nursery, one of the sources of olive saplings. Symptoms observed in the field were typical of *Phytophthora* infection, such as wilting and dieback of the crown, often associated with the presence of necrotic lesions at the collar level and root rot. The aim of the present study was to isolate and identify the species of *Phytophthora* associated with symptomatic olive trees. Fine roots, bark tissues and soil samples were collected and isolations were made using the selective medium SMA. *Phytophthora* colonies were then sub-cultured onto carrot agar to study their morphological and physiological properties. Identification of the isolates were confirmed by analysis of the ITS region (ITS1-5.8S-ITS2) of the rDNA. Sequences were compared with those present in GenBank database. Three different species of *Phytophthora* were identified. *P. palmivora* was the most frequent species. It was isolated from all orchards and from nursery stock material. With less frequency, *P. nicotianae* and another non-identified *Phytophthora* sp. were isolated from soil samples collected around infected olive trees at one site. Pathogenicity of two *P. palmivora* isolates obtained from different olive cultivars was verified by inoculating 2-year-old olive seedlings. Further tests are underway to assess the aggressiveness of the other *Phytophthora* species.

CHARACTERIZATION OF A _FUSARIUM GRAMINEARUM_ XYLANASE EXPRESSED DURING WHEAT INFECTION AND KNOCK-OUT OF ITS ENCODING GENE. L. Sella[1], K. Gazzetti[1,3], F. Faoro[2], A. Raiola[1], R. D'Ovidio[3], W. Schäfer[4] and F. Favaron[1]. *[1]Dipartimento del Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. [2]Dipartimento di Produzione Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. [3]Dipartimento 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. [4]Biocenter Klein Flottbek, Molecular Phytopathology and Genetics, University of Hamburg, Hamburg, Germany. E-mail: luca.sella@unipd.it*

Fusarium graminearum is the causal agent of Fusarium head blight (FHB) of wheat, barley and other cereal grains. During the infection process, this fungus secretes a large number of hydrolytic enzymes acting on the plant cell wall. In particular, *F. graminearum* is able to degrade xylans, the main constituent in the cell walls of monocot plants like wheat, through the coordinate action of a group of extracellular enzymes; among them, endo-ß-1,4-xylanases hydrolyze the inner ß-1,4 glycosidic bond and could be important pathogenic weapons for (pathogens) attacking cereal plants. However, xylanases could also exert effects independent of their enzymatic activity: a *Botrytis cinerea* xylanase, named Xyn11A, was shown to contribute to virulence on tobacco and tomato leaves with its necrotizing activity and not with the xylan hydrolyzing activity. In the genome of *F. graminearum* there is a xylanase-encoding gene (named Xyl1) whose deduced amino acidic sequence has a 55% identity with the *B. cinerea* xylanase Xyn11A. Since the transcript of Xyl1 gene has been detected in wheat spikelets infected with *F. graminearum*, we cloned this gene for heterologous expression in *Pichia pastoris*. The purified xylanase was characterized by studying its enzymatic activity *in vitro* and its necrotizing activity on wheat tissue. Knock-out mutants of the corresponding encoding gene were also obtained by site-directed homologous recombination. Wheat infection experiments aimed at evaluating the virulence of these mutants are in progress.

DIAGNOSTIC ANALYSES ON FOURTH RANGE PRODUCTION IN THE PROVINCE OF SALERNO. L. Sigillo, V. Senape, G. Serratore, V. Spina and R. Bravi. *Ente Nazionale Sementi Elette, Sezione di Battipaglia SS 18, km 77.700, 84091 Battipaglia (SA), Italy. E-mail: l.sigillo@ense.it*

The production of species for fourth range market has strongly increased in the last years. Important economical losses are reported due to the widespread of bacterial and fungal diseases. The Laboratory for Phytopathological Analyses of ENSE (Battipaglia, Italy) is specialized on detection of the most important pathogens that infect these species. From 2005 to 2010, 73 samples of rocket (*Dyplotaxis tenuifolia*), 65 of lettuce (*Lactuca sativa*), 19 of corn salad (*Valerianella locusta*), 9 of spinach (*Spinacia oleracea*) and many other minor species were analysed. Among these, 108 and 68 seed and symptomatic plant samples were analysed for the detection of the most important pathogens. Contamination by *Xanthomonas campestris* and pathogenic strains of *Fusarium oxysporum* of rocket seed samples was 15% and 8% respectively. Instead, 50% and 20% of rocket plants were infected by *F. oxysporum* and *X. campestris*. *F. oxysporum*, *Verticillium* sp. and *Rhizoctonia* sp. occurred rarely on lettuce seeds or plant tissues (about 3%). Generally corn salad, beet and spinach seeds resulted healthy. *X. campestris* was detected in a corn salad sample. In conclusion, an increase of *F. oxysporum* and *X. campestris* incidence on rocket has been reported in the last years. This increase is probably due to the diffusion of monocropping system and the use of contaminated seeds in the fourth range production.

SEVERE OUTBREAKS OF LETTUCE BIG VEIN IN LATIUM AND CAMPANIA. R. Sorrentino[1], R. Carrieri[2], G. Cirillo[3], G. Parrella[4] and D. Alioto[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli, Via Università 100, 80055 Portici (NA), Italy. [2]Dipartimento di Biologia e Difesa delle Piante, CRA-CAT, Via P. Vitiello 108, 84018 Scafati (SA), Italy. [3]Enza Zaden Italia srl, Ss. Aurelia km 96+710, 01016 Tarquinia (VT), Italy. [4]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. E-mail: robby-1983@libero.it*

Big-vein is a widespread disease of lettuce, transmitted through the soil by *Olpidium brassicae*. Two viruses are associated with the disease, *Lettuce big-vein associated virus* (LBVaV; genus

Varicosavirus) and *Mirafiori lettuce big-vein virus* (MLBVV; genus *Ophiovirus*). The latter has been identified as the disease-causing agent also if the etiology of big vein is still confused, since LBVaV alone was found in some symptomatic lettuces. In winter 2011, in the vegetable growing areas of Fondi (Latium) and Angri (Campania), big-vein symptoms appeared in field-grown lettuce, causing losses from 15 to 100% according to the cultivar and cultivation area. Symptoms of the disease appeared as chlorotic regions surrounding the vascular tissues accompanied by plant size reduction and head abort or retard. Lettuces from Fondi showed also symptoms resembling those of *Lettuce ring necrosis virus* (LRNV). Five symptomatic plants for each variety (Iceberg, Canasta, Cassiopea, Azimut) from Fondi and two (Iceberg) from Angri, were analyzed for the presence of LBVaV and MLBVV. Results of mechanical transmission were negative for all tested samples, probably due to greenhouse temperatures above 18°C. Mixed infections with MLBVV and LBVaV were detected in all samples analysed by DAS-ELISA using commercial kits. LBVaV presence in all symptomatic lettuce plants was also confirmed by RT-PCR using LBVaV-specific primers. On the other hand, only few samples tested positive by RT-PCR with the universal primers for *Ophioviruses* and, in this case, the identity of MLBVV was verified sequencing amplicons which shared 97% similarity with MLBVV sequences deposited in GenBank.

USE OF LAMIACEAE ESSENTIAL OILS TO CONTROL POSTHARVEST ROTS OF STONE FRUITS. D. Spadaro, J.G. Lopez-Reyes, A. Garibaldi and M.L. Gullino. *AGROINNOVA, Centro di Competenza per l'Innovazione in Campo Agroambientale, Università degli Studi di Torino, Via L. da Vinci 44, Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it*

Essential oils can be used to control postharvest pathogens of stone fruits, such as *Monilinia laxa*, *M. fructigena* and *Botrytis cinerea*. The efficacy of essential oils treatments was assessed on apricots cvs. Kyoto and Tonda di Costigliole, nectarines cvs. Big Top and Nectaross and on plums cvs. Italia and TC Sun against *Botrytis cinerea*, *Monilinia fructigena* and *M. laxa*. The essential oils from basil (*Ocimum basilicum*), fennel (*Foeniculum sativum*), lavender (*Lavandula officinalis*), marjoram (*Origanum majorana*), oregano (*Origanum vulgare*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), savory (*Satureja montana*), thyme (*Thymus vulgaris*) and wild mint (*Mentha arvensis*) were tested at different concentrations. Fruits were artificially inoculated with a spore suspension of each pathogen and essential oil emulsions were applied after 12 h incubation time. Treatment with savory essential oil was statistically more efficient to control the tested pathogens than the other treatments. Treatments with essential oils from basil and oregano were also effective but mainly on apricots cv. Tonda di Costigliole. Essential oils treatments at elevated concentrations permitted a high level of disease control after 15 days of storage, but they could induce severe damages on the carposphere of apricots and subsequent disease development after 30 days of storage. The efficacy of essential oils and their potential phytotoxicity on stone fruits depends both on species and carposphere strength.

***IN VITRO* BIODEGRADATION OF OCHRATOXIN A BY YEAST ANTAGONISTS. D. Spadaro, S. Patharajan, A. Lorè, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, Centro di Competenza per l'Innovazione in Campo Agroambientale, Università degli Studi di Torino, Via L. da Vinci 44, Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it*

Yeast biocontrol agents, previously selected to control different fungal pathogens, were screened initially for their ability to degrade ochratoxin A (OTA) in liquid medium amended with OTA (7.5 mg ml^{-1}) and different concentrations of yeast cells at 30°C. The highest OTA degradation was observed when the yeast cell concentration used was 10^8 cells ml^{-1}. Out of six yeast strains, three strains [*Metschnikowia pulcherrima* (MACH1), *Pichia guilliemonodii* (M8) and *Rhodococcus erythropolis* (AR14)] were selected for further studies. Yeast strains were evaluated for their ability to degrade OTA at different temperatures (10, 15, 20, 25, 30 and 35°C) and 30°C resulted the optimal temperature for yeast growth and OTA degradation. The three strains were further evaluated to test their potential on OTA degradation using different concentrations of OTA (5 and 10 μg ml^{-1}) at 30°C. The three yeast antagonists were further tested to confirm either degradation or cell wall adsorption of OTA at different time intervals. Among the strains, MACH1 effectively degraded OTA (>80%) at 30°C after 15 days incubation compared to the other strains tested and a little amount of OTA adsorption was observed in the yeast cell wall. LC-MS studies revealed that no by-product like ochratoxin or phenylalanine were found during the degradation process. Therefore, further studies are needed to understand the mechanism of action of these yeast strains during OTA degradation.

USE OF A NOVEL STRAIN OF *METSCHNIKOWIA* sp. TO CONTROL *PENICILLIUM EXPANSUM* AND PATULIN CONTAMINATION ON APPLES IN POSTHARVEST. D. Spadaro, A. Lorè, A. Garibaldi and M.L. Gullino. *AGROINNOVA, Centro di Competenza per l'Innovazione in Campo Agroambientale, Università degli Studi di Torino, Via L. da Vinci 44, Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it*

Penicillium expansum is the agent of blue mould, the most common form of post-harvest rot of pome fruits, which causes considerable economic losses. The pathogen is able to produce patulin, a lacton that may cause acute and chronic toxicity. The biocontrol efficacy of antagonistic yeasts against the agent of blue mould and its patulin production was evaluated on apples cvs Golden Delicious, Granny Smith, Red Chief and Royal Gala stored at 21°C for 7 days and at 2°C for 42 and 56 days. The results showed that a new strain of *Metschnikowia* sp. presented the highest efficacy on the control of *P. expansum*, on apples stored both at room temperature and at refrigerated temperature. In some cases, its efficacy was higher than the chemical control. At room temperature, a higher patulin contamination was observed on fruits treated with the most effective biocontrol agents. On apples stored at refrigerated temperature, the patulin concentration on cv. Golden Delicious apples was very low, in cvs Granny Smith and Royal Gala the highest concentration was observed in fruits treated with *Metschnikowia* sp. Considering the total weight of the apples, patulin was detected in low concentrations and the lowest amount was found on apples treated with the new strain of *Metschnikowia* sp.

SPECIFIC PCR PRIMERS FOR THE DETECTION OF ISOLATES OF *ASPERGILLUS CARBONARIUS* PRODUCING OCHRATOXIN A IN GRAPEVINE. D. Spadaro, S. Patharajan, A. Lorè, A. Garibaldi and M. L. Gullino. *AGROINNOVA, Centro di Competenza per l'Innovazione in Campo Agroambientale, Università degli Studi di Torino, Via L. da Vinci 44, Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it*

Aspergillus carbonarius is involved in ochratoxin A (OTA) contamination of grapes and wine. Polyketide synthases are involved in OTA biosynthesis in *Aspergillus* species. The ketosynthase (KS) domain of an *A. carbonarius* gene encoding polyketide synthase (pks) was isolated, cloned and sequenced. The nucleotide sequence showed high similarity to KS domains isolated from other *Aspergillus* species. The sequence was used to design a new set of primers in order to identify potential producers of OTA from isolates of *A. carbonarius*. The primers amplified all the *A. carbonarius* strains tested specifically, and did not amplify any other species of *Aspergillus* or *Penicillium* normally found on grapes or involved in OTA biosynthesis. Further, gene expression was related to OTA production. Both gene transcription and levels of the mycotoxin were higher when *A. carbonarius* was grown on YES medium at 15°C and 30°C, whereas a low transcription and mycotoxin presence were observed when the fungus was grown on PDB medium at the same temperatures. No transcription or OTA production were observed when the fungus was grown on either YES or PDB at 10°C. The primers will be useful to detect ochratoxigenic strains of *A. carbonarius* both in vineyards and during wine production.

TRANSPOSITION OF THE MINIATURE INVERTED-REPEAT TRANSPOSABLE ELEMENT *MIMP1* IN THE WHEAT PATHOGEN *FUSARIUM CULMORUM*. **F. Spanu[1], B. Scherm[1], V. Balmas[1], A. Marcello[1], M. Pasquali[2], M. Dufresne[3], M.J. Daboussi[4] and Q. Migheli[1].** *[1]Dipartimento di Protezione delle Piante, Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. [2]CRP-Gabriel Lippmann, 41, rue du Brill, 4422 Belvaux, Luxembourg. [3]Institut de Biologie des Plantes, Bâtiment 630, Université Paris Sud 11, 91405 Orsay Cedex, France. [4]Institut de Génétique et Microbiologie, Bâtiment 400, Université Paris Sud 11, 91405 Orsay Cedex, France. E-mail: qmigheli@uniss.it*

The genome of *Fusarium culmorum*, incitant of crown and foot rot on wheat and type B trichothecene producer, is now being sequenced. The number of predicted genes is estimated to exceed 10,000 and for many of them the function is still unknown. Consequently, there is a strong need for a high-throughout method for functional genomic analysis. Our aim is to test the efficacy of a two double component system based on the ability of the *impala* transposase to transactivate the miniature inverted-repeat transposable element *mimp1* of *Fusarium oxysporum*. In this system, the tagging element *mimp1* is inserted into the first intron of the *A. nidulans niaD* gene, and a phenotypic assay for excision allows recovery of excision events on minimal medium containing nitrate as the sole nitrogen source. Since *mimp1* has no coding capacity, its mobilization requires a trans-activating *impala* transposase under the control of the constitutive *A. nidulans gpdA* promoter on a separate vector. Similarly to what was previously observed in *Fusarium graminearum*, *mimp1* was shown to transpose in *F. culmorum* by a cut-and-paste mechanism into TA dinucleotides, which are duplicated upon insertion. Our results also show that *mimp1* reinserts in genic regions for 28% (i.e., 16 over 58) of the flanking sequences analysed, spanning throughout the entire genome of *F. culmorum*. Evidence that the *mimp1/impala* double-component system is fully functional in the heterologous species *F. culmorum* makes it an efficient tool for gene tagging in this pathogen.

DETECTION OF *SCLEROTINIA* ROT DISEASE ON *CITRUS MEDICA* IN A SOUTH ITALY AREA. **M.C. Strano, M. Calandra, V. Aloisi, G. Cicciarello and A. Caruso.** *CRA, Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale (CT), Italy. E-mail: mariaconcetta.strano@entecra.it*

Sclerotinia rot (cottony rot) is a serious disease induced by different species of the ubiquitous phytopathogenic fungi *Sclerotinia*, which cause economically important losses to several species of plants. The disease is more common in temperate and subtropical regions characterized by cool and wet climate. *Sclerotinia* spp. rarely cause diseases to citron trees (*Citrus medica*), but under specific environmental conditions and plant susceptibility, diseases can develop with a high incidence, with serious yield reduction. Citron trees are specially cultivated in Calabria (southern Italy) for candied production. Frequent rainfall and low temperature occurred in this area during winter season of 2010-2011. This paper reports the isolation of *Sclerotinia sclerotiorum* from infected twigs of citron trees, grown under net shade, which showed brown lesion shortly followed by a characteristic cottony, white mycelium on infected tissue and superficial hard black structures (sclerotia). Koch's postulates were tested for the confirmation of the fungus as the cause of the disease, morphology characteristics *in vitro* were studies and artificial inoculation to citrus fruit were made.

DETECTION OF NEW SEQUENCE VARIANTS OF *GRAPEVINE RUPESTRIS STEM PITTING ASSOCIATED VIRUS*. **F. Terlizzi[1], C. Li[2], C. Ratti[1], W. Qiu[3], R. Credi[1] and B. Meng[2].** *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Sezione Patologia Vegetale, Università degli Studi, 40127 Bologna, Italy. [2]Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Ontario, N1G 2W1 Canada. [3]Department of Agriculture, Missouri State University, Mountain Grove, MO 65711, USA. E-mail: federica.terlizzi@unibo.it*

Grapevine rupestris stem pitting-associated virus (GRSPaV) is a member of the genus *Foveavirus* (*Betaflexiviridae*). It occurs worldwide and is considered the causal agent of Rupestris stem pitting, a component of the Rugose wood complex. GRSPaV is composed of a wide range of sequence variants and so far, the complete genomes of five sequence variants have been sequenced. Quick and reliable detection of different GRSPaV variants is a critical step in the control of GRSPaV. Previously, primers designed from various genomic regions have been used in RT-PCR for the detection of GRSPaV variants. The efficiency of RT-PCR varied widely, depending on the spectrum of the primers that were used. In this study, we designed a pair of degenerate primers based on the consensus sequence of the genomic region encoding the highly conserved RNA-dependent RNA polymerase domain from five reference isolates of GRSPaV for which the genomes are available. We demonstrated that this set of primers is comparable to the broad-spectrum primers RSP13&14 in detecting multiples GRSPaV variants and, by using these new degenerate primers, we identified two new and distinct sequence variants. The 3' terminal genomic region of one of the new variants, GRSPaV-ML, spanning the 3' part of ORF1, through the entire ORFs 2-4, and the 5' region of ORF5, was sequenced. Sequence comparison showed that GRSPaV-ML is distinct from each of the five reference isolates.

A MULTIPLEX REAL TIME TAQMAN RT-PCR ASSAY FOR THE DETECTION OF GRAPEVINE FLAVESCENCE DORÉE AND BOIS NOIR PHYTOPLASMAS USING CRUDE SAP EXTRACTS. F. Terlizzi, C. Ratti, C. Poggi Pollini and R. Credi. *Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 40, 40127 Bologna, Italy. E-mail: federica.terlizzi@unibo.it*

Flavescence dorée (FD) and Bois noir (BN) are the most important phytoplasma diseases of grapevine in Europe. Detection of FD and BN phytoplasmas (16SrV and 16SrXII-A groups, respectively) is particularly difficult, as they are generally present in low concentrations and unevenly distributed in the plant phloem. Many efforts recently focused on developing rapid and sensitive methods for their detection. In this study a triplex real time TaqMan® RT-PCR methodology has been set up to simultaneously, but specifically, amplify a 16SrDNA gene fragment of the two phytoplasmas and a 18SrDNA gene region of the host plant using a crude sap extract as template. The use of RNA as template, instead of DNA, takes advantage from the high ribosomal-RNA copy number present in pathogen cells and provides evidence of viability and metabolic activity of phytoplasma, which is not provided by DNA-based detection. During our study, an efficient and rapid method for FD and BN phytoplasmas detection in different host plants has been developed. Absence of nucleic acid extraction step allows to greatly decrease both costs and time necessary to perform the assay. Therefore the method presented is a rapid, specific and sensitive alternative to conventional nested-PCR. We successfully applied this technique to the detection of FD and BN phytoplasmas on more than 300 samples of grapevine, strawberry, tomato, wild plants and insect vectors.

GENETIC BASIS OF THE LEAF RUST RESISTANCE *Lr14* LOCUS IN DURUM WHEAT. I. Terracciano[1], M. Maccaferri[1], F. Bassi[1], P. Mantovani[2], M.C. Sanguineti[1], A. Massi[2], J. Kolmer[3], H. Simkova[4] and R. Tuberosa[1]. *[1]Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. [2]Società Produttori Sementi Bologna, Via Macero 1, 40050 Argelato, Bologna, Italy. [3]USDA-ARS, Cereal Disease Laboratory, 55108 St. Paul, MN, USA. [4]Institute of Experimental Botany, Sokolovska 6, 77200 Olomouc, Czech Republic. E-mail: marco.maccaferri@ unibo.it*

Leaf rust (*Puccinia triticina*) is a main disease affecting durum wheat production in the Mediterranean region. Improving the resistance to leaf rust can effectively be accomplished through mapping of the resistance loci from valuable sources. The leaf rust resistant allele *Lr14-Creso* from durum wheat cv. Creso is one of the most important leaf rust resistance sources present in the modern durum wheat germplasm. *Lr14* is located in the distal portion of chromosome 7BL. The Colosseo (a Creso derivative) x Lloyd recombinant inbred line population (176 lines) has been used to enrich the *Lr14* region with new molecular markers derived from wheat ESTs. Phenotypic data were collected in the field and also at the seedling stage. A high heritability of the disease response was observed in both cases ($h^2 > 0.80$). The population allowed for mapping the locus at a good resolution level (5 cM). New molecular markers were developed and mapped within an interval of 14 cM that includes the *Lr14*-QTL peak. In particular some markers were obtained by exploiting the conserved colinearity between the most distal portions of rice chromosone 6, *Brachypodium* chromosone 1 and wheat chromosone 7BL. The results are supported by an independent association mapping study carried out using a panel of 164 durum elite accessions. The ongoing studies will provide further insights on the genetic basis of *Lr14*. The newly developed markers tagging the *Lr14-Creso* allele are presently used in marker assisted selection activities.

MULTIPLEX REAL TIME RT-PCR FOR *TOMATO CHLOROSIS VIRUS* AND *TOMATO INFECTIOUS CHLOROSIS VIRUS* IN TOMATO PLANTS AND WHITEFLIES. A. Tiberini[1], A. Manglli[1], V. Cavalieri[2], C. Rapisarda[2] and L. Tomassoli[1]. *[1]CRA, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. E.mail: antonio.tiberini@entecra.it*

Tomato chlorosis virus (ToCV) and T*omato infectious chlorosis virus* (TICV), genus *Crinivirus*, family *Closteroviridae*, are plant viruses characterized by flexuous, filamentous virions inducing symptoms often confused with nutritional or physiological disorder: interveinal yellowing, red and/or brown necrotic flecking and brittleness of older leaves. Firstly identified in the USA, TICV and ToCV rapidly spread worldwide now being reported in the Mediterranean basin, North America, South Africa and Asia. Both viruses are transmitted in a semi-persistent manner by whitefly vectors belonging to Aleyrodidae (Homoptera). In particular, TICV is transmitted by *Trialeurodes vaporariorum* and ToCV by *Bemisia tabaci*, *T. vaporariorum* and *T. abutilonea*. Since similar symptoms are induced on plants by the two viruses, as well as by nutritional deficiencies, and different vectors are involved in their transmission, a sensitive diagnosis and virus discrimination is essential for disease control. For these reasons, a multiplex TaqMan RT-PCR assay was developed for a simultaneous detection of the two viruses. Primer and MGB probe sets were specifically designed in coat protein gene region according to reference nucleotide sequences of TICV and ToCV published in GenBank. Plant internal control, designed in the cytochrome c oxidase gene (COX), and vector control, designed in consensus region of whiteflies (rRNA 18S) were also included. During the last tomato season, leaf samples and whiteflies were collected in several commercial greenhouses in Sicily and Sardinia. The method proved successful in detecting the viruses simultaneously, both in plant and insect extracts, and demonstrated to be useful for wider future monitoring and epidemiological studies.

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IDENTIFICATION AND CHARACTERIZATION OF *CHRYSANTHEMUM STUNT VIROID* INFECTING *ARGYRANTHEMUM FRUTESCENS* IN ITALY. E.M. Torchetti[1], B. Navarro[1], V.N. Trisciuzzi[2], M.E. Silletti[2] and F. Di Serio[1]. *[1]Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy, [2]Centro di Ricerca e Sperimentazione in Agricoltura Basile Caramia, Via Cisternino 281, 70010 Locorotondo (BA), Italy. E-mail: f.diserio@ba.ivv.cnr.it*

Chrysanthemum stunt viroid (CSVd), a harmful pathogen affecting ornamentals, is listed in the II/AII Annex of the Plant Health Directive in the European Union (2000/29/EC). Therefore, introduction and distribution of this viroid in European Countries is banned in chrysanthemum (*Dendranthema* spp.) plants, which can develop stunting, leaf chlorosis and floral developmental disorders as a consequence of infection. Although in the past CSVd has been occasionally reported in chrysanthemum in Italy, its spread in this ornamental species has efficiently been restrained so far. We

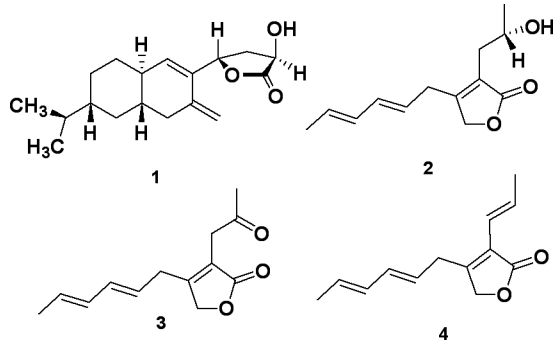
now report for the first time in Italy, the occurrence of several cultivars of *Argyranthemum frutescens*, an ornamental species, infected by CSVd. Infections were detected in symptomless plants by Northern–blot hybridization and by RT-PCR using specific primers, and viroid identity was conclusively ascertained by cloning and sequencing the cDNA amplicons. Molecular characterization of CSVd from different cultivars showed viroid populations consisting of RNA molecules with a uniform size of 354 nt and nucleotide sequences identical to CSVd variants AF394452 and X16408 previously reported from *D. grandiflora.* These results call for a prompt extension of surveys for assessing the presence of CSVd in *A. frutescens* and in other ornamentals, which could constitute hidden reservoirs of this pathogen. To this aim, a tissue-printing hybridization method for detecting CSVd in *A. frutescens* was tested and validated. The epidemiological risk of these findings for chrysanthemum crops will be discussed.

MIXED VIROID INFECTIONS OF *SOLANUM JASMINOIDES* IN ITALY. E.M. Torchetti[1], B. Navarro[1], V.N. Trisciuzzi[2], M.R. Silletti[2] and F. Di Serio[1]. *[1]Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. [2]Centro di Ricerca e Sperimentazione in Agricoltura "Basile Caramia", Via Cisternino 281, 70010 Locorotondo (BA), Italy. E-mail: f.diserio@ba.ivv.cnr.it*

Since 2006, *Potato spindle tuber viroid* (PSTVd), a quarantine pathogen, has been detected in symptomless *Solanum jasminoides* and other ornamental solanaceous species in Europe, causing general alert on the risk of outbreaks in susceptible horticultural crops, such as tomato and potato. This concern increased when natural transfer of PSTVd from *S. jasminoides* to tomato was documented in Italy. In the last few years, similar worries were raised in the EU by the discovery in several symptomless ornamental solanaceous hosts of other pospiviroid species, i.e. *Tomato apical stunt viroid* (TASVd) and *Citrus exocortis viroid* (CEVd), both of which are pathogenic to tomato and potato. We now document the first record of CEVd in *S. jasminoides* in Italy, highlighting its very high incidence (close to 100%) in this ornamental species. We also report the first identification of *S. jasminoides* plants concurrently infected by PSTVd and CEVd, and by TASVd and CEVd. These viroids were identified by RT-PCR using specific primers followed by sequencing and Northern blot hybridization. Identification of samples with mixed infections was not always successful using RT-PCR with generic pospiviroid primers followed by amplicon cloning and sequencing. This may depend on the lower concentration of both viroids in mix-infected samples, as confirmed by Northern-blot hybridization. Implications of these findings on detection and identification method efficiency are discussed. The molecular characterization of PSTVd from mix-infected samples revealed the presence of unreported strains largely differing from those so far isolated from *S. jasminoides.*

CERINOLACTONE, A HYDROXY-LACTONE DERIVATE FROM *TRICHODERMA CERINUM.* F. Vinale[1,4], I.A. Girona[2], M. Nigro[1,4], P. Mazzei[3], A. Piccolo[3], M. Ruocco[4], S. Woo[1,4], C.L. Herrera[3] and M. Lorito[1,4]. *[1]Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli 'Federico II', 80055 Portici (NA), Italy. [2]Instituto de Agricultura Sostenible C.S.I.C., 14080 Córdoba, Spain. [3]Centro Interdipartimentale di Spettroscopia di Risonanza Magnetica Nucleare, Università degli Studi di Napoli 'Federico II', 80055 Portici (NA), Italy. [4]Istituto per la Protezione delle Piante del CNR, 80055 Portici (NA), Italy.*

Trichoderma strains are among the most studied and applied fungal BCAs in industry and agriculture and secrete several secondary metabolites with different biological activity. The analysis of metabolic profiles (the "metabolome") of *Trichoderma* species is complex because of the wide range of compounds produced and the molecular activities identified, including the recently determined role in the activation of plant resistance and growth promotion. Metabolomic studies may provide new insights on the mechanisms that regulate the complex interactions between plants, fungal phyto-pathogens and microbial antagonists of the genus *Trichoderma*, thus improving the usefulness of these beneficial agents. A novel metabolite, 3-Hydroxy-5-(6-isopropyl-3-methylene - 3,4,4a,5, 6,7,8,8a-octahydro-naphthalen-2-yl)-dihydro-furan-2-one, named cerinolactone (i), has been isolated from culture filtrates of *Trichoderma cerinum* together with three known butenolides containing the 3,4-dialkylfuran-2 (5H)-one nucleus, harzianolide (ii), T39butenolide (iii) and dehydroharzianolide (iv). The structure of cerinolactone was determined by spectroscopic methods, including UV, MS, and 1D and 2D NMR analyses. *In vitro* tests with the purified compound exhibited a significant antifungal activity against the soil-borne fungal pathogen *Rhizoctonia solani.*



INVESTIGATIONS ON *PENICILLIUM* spp. POPULATION DYNAMIC IN CITRUS PACKINGHOUSE. K. Youssef[1,2], A. Ligorio[1], S.M. Sanzani[1], F. Nigro[1] and A. Ippolito[1]. *[1]Dipartimento di Biologia e Chimica Agroforestale ed Ambientale, Università degli Studi "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. [2]Agricultural Research Center, Plant Pathology Research Institute, 9 Gamaa St., 12619 Giza, Egypt. E-mail: ippolito@agr.uniba.it*

The most serious postharvest fungal diseases of citrus fruit are green and blue moulds, caused by *Penicillium digitatum* and *P. italicum*, respectively. Numerous factors related to the fruit itself, the pathogen, and the postharvest environment influence the incidence and severity of these diseases. Among these factors, the inoculum density of *Penicillium* spp. in the packinghouse environment and on citrus fruit can play a role in subsequent rot development during storage and shelf life. Population dynamics of *Penicillium* spp. on clementine fruit and in the packinghouse atmosphere were investigated considering five sampling zones along the packing line: fruit arrival, bins emptying, fruit washing, waxing, and calibration. Population of *Penicillium* spp. on the fruits fluctuated along the different zones, being significantly higher in "bin emptying" area as compared to the other control points, with a density exceeding 2.6 $\log_{10}$ CFU/g of fresh fruit. The environmental mycoflora was sampled according to the gravimetric method, using Petri dishes containing potato dextrose agar medium left open for 10 min. The highest inoculum of *Penicillium* spp. spores in the packinghouse atmosphere was detected in the area of "bins empting", with a density exceeding 66 CFU/plate. The incidence of *Penicillium* rot in clementines, tak-

en from the same sampling zones along the packing line, showed an increasing trend, with values ranging from 23% (bins emptying) to 40% (calibration). Results are discussed in relation to the possible epidemiological significance.

ATTEMPTS TO INCREASE THE ACTIVITY OF SALTS AGAINST POSTHARVEST CITRUS DECAY BY COMBINATION WITH NATURAL SUBSTANCES. K. Youssef[1,2], A. Ligorio[1], S.M. Sanzani[1], F. Nigro[1] and A. Ippolito[1]. *[1]Dipartimento di Biologia e Chimica Agroforestale ed Ambientale, Università degli Studi " Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. [2]Agricultural Research Center, Plant Pathology Research Institute, 9 Gamaa St., 12619 Giza, Egypt. E-mail: ippolito@agr.uniba.it*

The control of postharvest diseases is generally exerted by chemicals fungicides. However, the issues associated with their use as public increasing awareness about human health and environmental risks, pathogen resistance, costs for registration and re-registration of active ingredients, etc., have motivated the search for new and safer alternatives. Also the use of these control means have a limited practical application because of the inconsistent activity, lack of preventive effect, limited persistence, risk of fruit injury and disposal of exhausted solutions. To overcome these problems and to attain commercially acceptable performance, the integration of alternative means has been recommended. Chitosan and xanthan gum are widely used as thickening agents and have been found to be useful as film-forming polymers. The activity of several salts (Na_2CO_3, $NaHCO_3$, K_2CO_3, $KHCO_3$, $CaCl_2$, and NH4HCO_3, 2%), known for their effectiveness, and natural substances (chitosan, 1% and xanthan gum, 0.05%), alone or in combination, against *Penicillium* rots on cv. Comune clementine is reported. A significant ($P \leq 0.05$) reduction of the percentage of rotted fruits was achieved with all tested salts and chitosan when applied alone. Na_2CO_3 and chitosan were the most effective, with a performance statistically not different from the chemical control imazalil. The activity of salts was not improved when applied in combination with chitosan and xanthan gum. Results demonstrate that the combination of alternative control means are not always useful and worthy to pursue.

STUDY OF THE DEFENCE MECHANISMS OF MUSKMELON TO TRACHEOFUSARIOSIS. A. Zechini D'Aulerio, F. Piattoni, R. Roberti and G. Servidio. *Dipartimento di Produzione e Valorizzazione Agrolimentare, Università degli Studi, Viale Fanin 46, 40127 Bologna, Italy. E-mail: aldo.zechinidaulerio@unibo.it*

In Italy, tracheofusariosis caused by *Fusarium oxysporum* f.sp *melonis* (FOM) causes serious damages to muskmelon. Once assessed the reliability of certified cultivars (resistant or susceptible to FOM (race0–ISPAVE909-; race1–ISPAVE 1296-; race2-ISPAVE1411-) we focused on root exudates (RE), substances thought to be involved in plant-pathogen interactions, showing no inhibition on conidial germination, independently from plant age (14 and 21 days old), pathogen race, and cultivar. Subsequently we used RE from the most resistant (Bingo) and susceptible cultivars (Retato degli Ortolani) and the most pathogenic race (2) to further investigate mycelial growth, promycelium length and promycelia number per conidium. Mycelial growth, analyzed for the cultivar effect, increases at any incubation time (from 0 to 72 h) with RE addition; in case of cv. Retato the effect is higher. Considering the incubation time, independently from RE age, in the test mycelia growth enhances constantly whereas with Bingo RE it stops after 20 h; cv. Retato path is similar to the test. Promicelium length increases with RE from plants of both ages, evidently for cv. Bingo; no enhancement is observed for 14-day-old cv. Retato RE. In case of macroconidia, 14 and 21-day-old RE increase promycelia number per conidium whereas for microconidia such effect is present only with 21-day-old RE, although less clear-cut. We conclude that RE contain substances able to interfere with mycelial growth, but their role has to be assessed. Thus, further investigations on defence mechanisms like suberin and callose production in infected root tissues from susceptible *vs* resistant cultivars are necessary.