TRICHODERMA SPECIES WITH BIOCONTROL ACTIVITY AGAINST NURSERY SOIL-BORNE PATHOGENS. M.P. Aleandri, G. Chilos, N. Bruni, A. Tomassini, E. Lucchioli, A.M. Vettraino and A. Vannini. Dipartimento per l’Innovazione nei Sistemi Biologici, Agronomie e Forestali, Università degli Studi della Tuscia, Via S Camillo de Lellis 1100, Viterbo, Italy. E-mail: vannini@unitus.it

Soil-borne diseases are important limiting factors for the nursery production of woody, ornamental and official plants. The potential of fungal biocontrol agents such as Trichoderma spp. has raised considerable research interest stimulated by the concern for the environmental impact of fungicides. The present study was undertaken to isolate and characterise Trichoderma species associated with the rhizosphere of holm oak, olive and lavender with the aim to select effective isolates as biocontrol agents. Among Trichoderma species isolated, three were from olive (T. asperellum T2, T. hamatum T3), T. harzianum T6 and holm oak (T. hamatum T19, T. asperellum T20, T. viride T21), and two from lavender (T. asperellum T12 and T. harzianum T14). Trichoderma isolates were characterised by their direct antagonism against the soil-borne pathogens of nursery plants Sclerotinia sclerotiorum, Rhizoctonia solani, Verticillium dahliae, Phytophthora nicotianae and P. cinnamomi. Their total volatiles and non-volatile antibiotic compounds and lytic isoenzyme patterns (chitinase and ß-1,3-glucanase) were assessed. Mycelial growth of target pathogens was differently affected by the volatile and diffusible metabolites of Trichoderma isolates, suggesting different mechanisms involved in the antagonistic activity towards pathogens with different lifestyles. The analysis of expression of the transcription factor gene MYB72 in Arabidopsis in an in vitro system revealed that the fungal isolates display a different priming degree. Our data suggest that the Trichoderma species complex occupying the same niche is characterised by different or complementary modes of action, thus representing a promising approach to improve the biological control of plant diseases.

MYCOTOXIN PRODUCTION BY SYRIAN FUSARIUM SPECIES. D. Alkadri1, B. Amato1, K. Döll2, P. Karlovsky2, A. Pisi1 and A. Prodi1. 1Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. 2Molecular Phytopathology and Mycotoxin Research, University of Göttingen, Grisebachstrasse 6, 37077 Göttingen, Germany. E-mail: antonio.prodi@unito.it

Some Fusarium species produce mycotoxins, responsible of acute and chronic symptoms. Great attention has been paid to the analysis of wheat grains affected by Fusarium head blight (FHB) whose causal agents are deoxynivalenol (DON), acetyl-esters of DON (3Ac-DON and 15Ac-DON), nivalenol (NIV), fusarenon X (Fus X) and zearalenone (ZEN) producers. The knowledge of the occurrence of Fusarium species in growing areas helps to predict mycotoxin content in harvested grains. The limits allowed for mycotoxins have not been yet determined in Syria while they are well established in Europe (EU-regulation1881/2006). Strains of Fusarium species associated with FHB, F. culmorum, F. graminearum, F. pseudograminearum and F. equiseti, isolated from Syrian wheat kernel samples and previously identified morphologically and molecularly, were examined for the presence of the mycotoxin biosynthetic genes and for mycotoxin production and mycotoxin production. F. graminearum and F. culmorum strains were characterized in chemotypes and the presence of tri5 gene was tested in F. equiseti strains. Fusarium strains were cultured on rice medium for mycotoxin quantification using HPLC tandem mass spectrometry. The chemotyping analyses showed that 60% of F. culmorum were 3Ac-DON, while 40% NIV. All F. graminearum strains belonged to NIV chemotype. Some strains of F. equiseti have a potential ability of produc- trichothecene. The mycotoxin quantification revealed corre- spondence between F. culmorum and F. graminearum chemotypes and their real mycotoxin production. F. pseudograminearum strains produced DON, 3Ac-DON and 15Ac-DON. NIV pro- ducers were present among F. equiseti strains. This is a prelimi- nary picture of the possible risk of mycotoxin contamination in wheat kernels in Syria.

INFLUENCE OF THINNING TREATMENT ON THE OCCURRENCE OF TREE DECLINE IN DECIDUOUS OAK FORESTS. N. Anselmi and A. Saraceni. Dipartimento per l’Innovazione nei Sistemi Biologici, Agronomie e Forestali, Università degli Studi della Tuscia, Via S. Camillo de Lellis 1100, Viterbo, Italy. E-mail: anselmi@unitus.it

The decline of the deciduous oak forests, often encountered in Mediterranean regions, seems to be generally derived from prolonged and repeated severe droughts, often due to competition caused by the lack of thinning. A survey was undertaken in a declining Quercus cerris and Q. pubescens forest to evaluate the effects of thinning treatments on the occurrence of decay. Four plots of approximately one acre each were selected. Two of them were subjected to thinning in June 2007, leaving 26 trees in each plot. In the other two plots, with similar health conditions, no thinning treatments were made. Through specific monitoring sheets, all plants of the four plots were subjected to phytosanitary assessment just before and after thinning, repeated in 2009, 2010 and 2011. Stem diameter, number of dead plants and the incidence of decline symptoms, such as leaf discoloration, cortical necrosis and presence of Biscogniauxia mediterranea stroma on the stems were also recorded. While plant health conditions in the thinned plot were significantly improved over time, with diameter increment and significant decrease of canopy damage from 33.46 to 16.73%, the reference plants faced a substantial deterioration, six of which being dead, and the others exhibiting increased canopy damage from 30.76 to 44.03%. The decline has also strongly concerned the complex plant system without thinning, with ceasing of growth and develop- ment, strong progressive increase of dead plants, canopy dam- age (over 30%) and pathogen attacks. Thinning is therefore a vi- able sylviculture technique to counteract the oak decline phe- nomenon.

TOWARDS THE OPTIMIZATION OF STANDARDIZED ASSAYS FOR SAMPLING AND DETECTION OF CITRUS HUANGLONGBING-ASSOCIATED BACTERIA. P. Bella1, G. Liciardello2, S. Dai3, M. Daden4, C. Strano1, F. Li3, M. Tessitori1, A. Catara2, X. Deng3, Z. Deng3 R. La Rosa1 and V. Catara1. 1Dipartimento di Scienze delle Produzioni Agrarie e Alimen- tari, Sezione Fitopatologia e Genetica Vegetale, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. 2Parco Scientifico e Tecnologico della Sicilia, ZI, Via V. Lancia 57, 95121 Catania, Italy. 3National Center of Citrus Improvement, Hunan University of Agriculture, Changsha, People’s Republic of China. 4IPAD-LAB, International Plant Analysis and Diagnostics, Via Einstein, Località Cascina Codastra, 26900 Lodig, Italy. 1Laboratory of Citrus Huanglongbing Research, Department of Plant Pathology, South China Agricultural University, Guangzhou, Guangdong 510642, People’s Republic of China. E-mail: vcatara@unict.it
Huanglongbing, the most destructive citrus disease, is induced by three Gram-negative, phloem-limited bacteria, *Candidatus Liberibacter asiaticus*, *C. L. africanus* and *C. L. americanus*, which are spread by propagative material and the Asiatic and African citrus psyllids, and are listed as A1 quarantine pests by EPPO and in most of the NPPOs worldwide. Because of the potential risk of introduction of the pathogen in the Mediterranean area and in Sicily, where more than 90,000 ha of citrus are grown, the standardization of laboratory procedures to recognize symptoms, perform sampling and detection has been foreseen within the framework of a project on citrus harmful parasites supported by the Regione Sicilia. Thanks to an agreement with the National Center for Citrus Improvement, Hunan University of Agriculture, Changsha, the plant material for such study was sampled during a survey in Guangdong province (China). Young shoots of different trees showing HLB-like symptoms were partially processed in the farms. The leaf midribs of the young fully expanded leaves and the bark stripped from the shoots were comminuted and impressed both on FTA Plant Cards and on Whatman 3MM filter paper or were transferred to the Chinese laboratory and the DNAs extracted. Membranes and DNAs were divided among the three laboratories to detect the pathogen by PCR and/or real-time PCR. *Ca. L. asiaticus* was detected and identified in all samples showing leaf mottle and in some of those showing nutritional problems. Results of the three laboratories confirm that the standardization of procedures is a prerequisite for a proper detection of this harmful organism.

**SEPTORIA CITRI IS A COMMON PATHOGEN OF CITRUS IN SOUTHERN ITALY.** A. Biasi1, A. De Patrizio1, R. Faedda2, L. Schena1, A. Pane1, S.O. Cacciola2 and G. Magnano di San Lio2. 1Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89124 Reggio Calabria, Italy. 2Dipartimento di Gestione dei Sistemi Agroambientali e Ambientali, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. Email: gmagnano@unirc.it

During 2011 and 2012, following low temperatures and a prolonged wet period necrotic spots were observed on overripe lemon and mandarin fruits in citrus orchards in Sicily. On lemons, symptoms consisted of pin-point reddish spots, 1 to 2 mm in diameter, on the flavedo. On mandarins, symptoms consisted of pin-point reddish spots, 1 to 2 mm in diameter, on the flavedo. On mandarins, symptoms consisted of pin-point reddish spots, 1 to 2 mm in diameter, on the flavedo. Interestingly, the same species *Septoria citri* was detected and identified in all samples showing leaf mottle and in some of those showing nutritional problems. Results of the three laboratories confirm that the standardization of procedures is a prerequisite for a proper detection of this harmful organism.

**ANALYSIS OF KIWIFRUIT BLEEDING SAP: AN USEFUL METHOD FOR THE EARLY DETECTION OF PSEUDOMONAS SYRINGAE pv. ACTINIDIAE.** E. Biondi1, S. Ardizzi1, N. Kuzmanovic2, A. Galeone1 and A. Bertaccini1. 1Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. 2Department of Plant Pathology, Faculty of Agriculture, University of Belgrade, Serbia. E-mail: enrico.biondi3@unibo.it

The strain D747 of *Bacillus amyloliquefaciens* (Amylo-X) was assayed to evaluate its efficacy against *Pseudomonas syringae pv. actinidiae* (*Psa*), the causal agent of bacterial canker of kiwifruit, which acquired significant relevance after the heavy epidemics occurred in central-northern Italy since 2008. The ability of strain D747 to inhibit the growth of two different *Psa* strains in vitro, its ability to survive and reduce populations of a rifampicin-resistant pathogenic strain (*CRA-FRU 3.1 RifR*) on female flowers of *Actinidia delicosa* and *A. chinensis* was investigated. The microbial control agent was effective against *Psa* in vitro, and was able to survive on kiwifruit female flowers, reaching a population of ca. 10^6 CFU/flower after its application. Moreover, it reduced the *Psa* population on *A. chinensis* flowers by more than one order of magnitude 48 h after pathogen application. Furthermore, strain D747 of *B. amyloliquefaciens* survived on kiwifruit leaves for 14 days, reaching a population of ca. 10^6 CFU/ml. These results suggest that strain D747 might be a promising tool for the biological control of bacterial canker of kiwifruit.

**POTENTIAL OF BACILLUS AMYLOLIQUEFACIENS STRAIN D747 AS CONTROL AGENT AGAINST PSEUDOMONAS SYRINGAE pv. ACTINIDIAE.** E. Biondi1, N. Kuzmanovic2, A. Galeone1, E. Ladurner1, M. Benuzzi1, P. Minardi1 and A. Bertaccini1. 1Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. 2Department of Plant Pathology, Faculty of Agriculture, University of Belgrade, Serbia. 1Intrachem Bio Italia, Servizio Tecnico, Sviluppo e Ricerca, Via Calcinato 2085/int. 7, 47521 Cesena, Italy. 2Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy. Email: enrico.biondi3@unibo.it

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**BIOLOGICAL CONTROL OF CHESTNUT BLIGHT: UPDATES OF CRYPHONECTRIA PARASITICA VEGETATIVE**
COMPATIBILITY IN CAMPANIA. L. Bosso, M.R. Di Luca, A. Testa and G. Cristinzio. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. E-mail: luciano.bosso@unina.it

The damages caused by Cryphonectria parasitica (Murr.) Barr to European chestnut populations (Castanea sativa) have been and are still serious. Biological control, based on vegetative compatibility between virulent and hypovirulent strains of the fungus, is the most important strategy against the disease. The aim of this study was to investigate the suitability of biological control of chestnut blight, checking the vegetative compatibility within fungal populations from all the provinces of Campania region (southern Italy). For each province (Avellino, Benevento, Caserta, Naples and Salerno), 28 fungal strains (6 hypovirulent and 22 virulent) were isolated. Two vegetative compatibility experiments were carried out in Petri dishes on PDA. Initially, all virulent and hypovirulent strains of each province were crossed. Then, three hypovirulent strains from each province were crossed with all virulent strains. The highest ratios of vegetative compatibility among strains collected within the same province were 95% (Salerno), 80% (Caserta), 70% (Avellino), 60% (Naples), and 50% (Benevento). Crossing virulent and hypovirulent strains of different provinces compatibility values of 100% were obtained at least once for each province. According to these preliminary results, the biocontrol of chestnut blight by hypovirulent fungal strains seems to be feasible in all provinces of the Campania region. Further analyses are in progress for the characterization of the mycoviruses associated to virulent and hypovirulent strains of C. parasitica.

FURTHER STUDIES ON THE CONTROL OF “CARDONCELLO” MUSHROOM YELLOWING IN SOUTHERN ITALY. G. Bruno1, G.L. Rana2, L. Piscicelli3, F. Mannerucci4, P. De Luca4, E. Giccarese5, L. Scarola6 and C. Cariddi7. Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: gbruno@agr.uniba.it

Yellowing or bacteriosis is the most severe disease of Pleurotus eryngii (DC.: Fr.) Quéhl., the mushroom commonly called “cardoncello” that is particularly appreciated in Italy and in some foreign countries (Germany, Canada, etc.). Pseudomonas ‘reactans’ species complex, and P. tolaasii Paine, saprophytic bacteria inhabiting the mushroom myphosphere, are reported, though with different importance, as the causal agents of the disease that sometimes causes huge losses to “cardoncello” cultivations. Control of the bacteriosis using white vinegar (68‰ acidity, pH 3.2), ozone (12-15 ppm) or 95.2 mM acetic acid was attempted. Two different cultivation cycles were carried out with P. eryngii strains “51” and “65” provided by Micotec (Gravina di Puglia, Italy) after incubation in standard substrate contained in thermoresistant polypropylene bags. The casing soil of each bag was inoculated with 10 ml of a suspension containing 1.7x10^6 CFU ml^-1 of PR51 strain of P. ‘reactans’, previously isolated from “cardoncello” basidioma affected by yellowing. Antibacterial treatments were carried out 13 times during the 43 days of each productive cycle. Substrate bags used as control were sprayed with tap water. For each bag, appearance and development of disease symptoms, basidioma shape and size, marketable mushroom yield and number of total bacteria, fluorescent Pseudomonads and total fungi present in casing soil were recorded. Several basidioma produced by control bags showed severe yellowing symptoms. Vinegar, ozone and acetic acid significantly prevented bacteriosis appearance and development and reduced the number of total bacteria, fluorescent Pseudomonads and total fungi present in casing soil of compost bags.

MODE OF ACTION AND RAINFASTNESS OF COPPER-BASED PRODUCTS AGAINST GRAPEVINE DOWNY MILDEW. T. Caffi, S.E. Legler, M. Carini and V. Rossi. Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it

Control of grapevine downy mildew (caused by Plasmopara viticola) requires fungicide application. Although a Decision Support System (DSS) that considers interactions among host, pathogen, and environment has been developed for grapevine downy mildew control, the optimization of fungicide application requires knowledge of the physical mode of action (PMoA) of each fungicide. PMoA describes the effect of a fungicide with respect to the time of its application relative to the host-pathogen interaction (e.g., pre- or post-infection) and the duration and degree of fungicide activity. This work characterized the PMoAs of new, copper-based fungicides formulated as water dispersable granules which release copper ions gradually. One fungicide contained copper oxychloride and hydroxide (both at 14%), and the other contained only copper oxychloride (37.5%). Both were tested in a controlled environment (20°C) at 100, 75, and 50% of the label dose and were applied: (i) 1 to 20 days before inoculation with P. viticola sporangia; (ii) 1 to 24 h after inoculation; or (iii) 4 days after inoculation (to reduce sporulation). Both products provided 100% control of infection when applied between 14 days before and 6 h after inoculation, although efficacy differed depending on dose and timing. Overall, infection control was greater for the product containing both copper salts. Neither product efficiently reduced sporulation. Product rainfastness was measured on potted grapevines, and a model was developed to predict rainfastness based on rain events and plant growth. The results of this study were used to implement the web-based DSS “ViteBio.net™” for downy mildew control in organic viticulture.

AFLA-MAIZE, A MECHANISTIC MODEL TO PREDICT THE RISK OF AFLATOXIN PRODUCTION IN MAIZE. M. Camardo Leggieri, P. Giorni, V. Rossi 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

Maize is one of the principal crops destined to food and feed worldwide, after wheat and rice, with more than 600 million tons produced annually (FAO, 2003). Unfortunately, maize is susceptible to mycotoxin-producing fungi such as Aspergillus flavus, which can contaminate the ripening kernels with aflatoxins. Aflatoxin B1 is reported as the most toxic natural compound, causing chronic and acute toxicity to humans and animals. EU legal limits in raw maize are fixed at 5 µg/kg for products destined to humans and dairy animals and 20 µg/kg for other animal species (Commission Regulation 1181/2006, 165/2010 and Directive 100/2003). No mechanistic models for A. flavus-maize pathosystem are available in the literature. In this work, the relational diagram of A. flavus infection cycle was developed following the principles of “system analysis”; state variables, rates and driving variables were determined and linked in a coherent framework.
Quantitative data for each step of the cycle were collected from literature and mathematical equations were elaborated to connect driving variables to rates; an algorithm was also developed to finalize the model. The model predicts fungal development and toxin production (output) based on weather conditions (air temperature, relative humidity and rain). After a proper validation, the model may support stakeholders in order to: (i) describe the dynamic of the contamination risk during the maize-growing season and at harvest, to rationalise harvest and post-harvest logistic; (ii) draw different scenarios based on real and simulated (climate change) meteorological data.

VARIABILITY OF PSEUDOMONAS SAVASTANOI POPULATION ISOLATED FROM MYRTLE. T. Cinelli1, R. Marongiu2, D. Fernandez Neira2, G. Marchi1, L. Mugnai2 and M. Fiori2. 1Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. 2Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: fiorn@mss.it

Based on the pathogenic ability of strains isolated from different hosts, the populations of Pseudomonas savastanoi are subdivided in six pathovars: savastanoi, nerri, fraxini, retacarpa, glycinea and phaseolicola. The typical symptom caused by pvs savastanoi, nerri and retacarpa is an outgrowth of plant cell tissue called knot, to whose differentiation concur auxins and cytokinins synthesized by the bacterium. The ash strains P. s. pv fraxini induce wart-like excrescences, while the strains from bean and soybean P. s. pv glycinea and phaseolicola cause halo blight and blight on their respective hosts. Regarding phenotypic characters a great heterogeneity has been reported among the pvs savastanoi, nerri, fraxini and retacarpa as well as between strains belonging to the same pathovar. The presence of P. savastanoi on Myrtus communis has only recently been reported and a comparative analysis of the variability that exists within bacterial populations associated to this host has never been carried out. In this study we have characterized a set of strains originally isolated from myrtle in three different Mediterranean countries with a polyphasic approach, including multilocus sequence analysis, substrate metabolisation profiles, phytohormone production and cross inoculation tests. While MLSA showed a low degree of variability, we observed a great heterogeneity both on phenotypic and phytopathogenic characters, suggesting the existence of a very complex population structure of P. savastanoi associated with this host.

HINTS ON THE BIOLOGICAL FUNCTION OF A PUTATIVE NUCLEAR LOCALIZATION SIGNAL OF THE OURMIA MELON VIRUS COAT PROTEIN. M. Ciufo, M. Rossi, S. Abbà, M. Vallino and M. Turina. Istituto di Virologia Vegetale del CNR, Strada delle Casce 73, 10135 Torino, Italy. E-mail: m.ciufo@ivv.cnr.it

Ourmia melon virus (OuMV) is the type member of a recently characterized plant virus genus with a tri-segmented genome: RNA1 codes for the RdRp, RNA2 for a movement protein (MP) and RNA3 for the coat protein (CP). We have previously shown that an amino terminal fusion of GFP and CP locates in the nucleus, preferentially in the nucleolus. Preliminary analysis showed that the first 11 amino acids of the CP sequence are sufficient for nucleolar-specific targeting. We then proceeded to perform small deletions and alanine scanning mutagenesis on selected residues of the putative nucleolar localization signal in the CP sequence. At the same time, each mutant was studied for its biological properties in the context of virus infection. Our small deletion analysis showed that ability to efficiently infect systemically Nicotiana benthamiana is correlated to the ability of the same mutant to accumulate the GFP-CP fusion inside the nucleolus, irrespective of its ability to form virions. Alanine scanning mutagenesis of a number of charged amino acids inside the putative nucleolar localization signal showed that simultaneous presence of three basic residues changes is sufficient to abolish nucleolar targeting of the GFP-CP fusion. Such mutant, in the context of a viral infection, shows ability to form virions, but in N. benthamiana it is somewhat impaired in the ability to maintain the initial systemic infection, since the infected tissue recovers. In cucurbit hosts, the same mutant is not able to infect systemically both cucumber and melon plants.

DETECTION AND MOLECULAR CHARACTERIZATION OF PHYTOPLASMAS INFECTING ROSMARINUS OFFICINALIS. L.N. Contaldo1, A. Bertaccini2, G. Bozzano2, L. Cavichi2 and M.G. Bellardi. 1Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. 2Società Cooperativa L’Ortofrutticola, Via Dalmazia 169, 17031 Albenga, Italy. 3Pleso Didattico G. Scarabelli, Università degli Studi di Bologna, Via G. Ascarelli 17, 40026 Inola, Italy. E-mail: assunta.bertaccini@unibo.it

Rosemary (Rosmarinus officinalis) family Lamiaceae, is a common and attractive evergreen shrubby species growing wild in the Mediterranean area, but cultivated for aromatic, ornamental and medicinal purposes. The interest towards this species is strongly arising, due to the biological activity of essential oils extracted from leaves and flowers. In the spring 2011, symptoms of yellowing, necrotic spots, leaf rolling and stunting were observed on rosemary plants in three commercial aromatic plant-growing farms of Piana di Albenga (Liguria, northen Italy); in 2012 other plants showing witches’ broom symptoms were collected in the same area. To verify phytoplasma presence and to determine their identity, symptomless and symptomatic samples were tested by direct PCR with universal primers P1A/P7a, followed by nested-PCR using primers F1/B6 or R16F2n/R2. Only from symptomatic samples bands of the expected length were obtained and RFLP analyses using restriction enzyme TruII allowed the classification of phytoplasmas as members of ribosomal group 16SrXII-A (stolbur) in the 2011 samples and 16SrI-B (aster yellows) in the 2012 samples. The presence of phytoplasma in rosemary represents the first report for this species. Considering that Liguria is the most important Italian region for aromatic plants production, for a rapid and efficient eradication of this disease it is necessary to eliminate all symptomatic plants that could play a role in the epidemiology of stolbur and aster yellows, as well as vectors (leaffoppers) and weeds, using suitable mechanical methods. Specific phytoplasma control of mother-plants before their vegetative propagation is also a necessity to prevent economic losses in rosemary crops.

NEOTYPHODIUM sp., AN ENDOPHYTIC SYMBIONT OF SARDINIAN TALL FESCUE. B. Corsi, A. Haegi, M. Aragona and L. Riccioni. CRA, Centro di Ricerca in Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. Email: luca.riccioni@entecria.it

Fungi of the genus Neotyphodium are obligated mutualist sym-
INVESTIGATIONS ON THE PRESENCE OF FUSARIUM SPECIES, CHEMOTYPE CHARACTERIZATION AND TRICHOTHECENE MYCOTOXIN DETERMINATION IN SOFT AND DURUM WHEAT IN UMBRIA. L. Covarelli1, G. Becatti1, F. Etruschi1, S. Generotti1, E. Ferrer2, G. Meca2 and J. Mañés Vinuesa2.

A study was conducted on 162 grain samples of durum and soft wheat harvested in 2009 and in 2010 in different areas of Umbria (central Italy). Samples were used for fungal isolation on PDA and identification of the principal infecting fungi. Members of the genus Fusarium were identified by PCR. In addition, a representative sub-sample of F. graminearum and F. culmorum strains was examined for determining the presence of 15ADON, 3ADON and NIV chemotypes by multiplex-PCR. Furthermore, the main type A (T-2 and HT-2 toxins and DAS) and type B (DON and NIV) trichothecenes were analyzed by LC-MS/MS. Fungal isolation showed that Fusarium spp. were present in all samples, with incidence peaks of about 80%. PCR identification showed that, with the exception of soft wheat sampled in 2009, F. graminearum was the most frequent species. In 2009, a year characterized by unfavourable climatic conditions for the development of the main causal agents of Fusarium head blight, the occurrence of F. avenaceum and F. poae was higher than in 2010. On the contrary, the incidence of F. graminearum was higher in 2010 than in 2009. Multiplex-PCR analyses showed that all F. culmorum strains were 3ADON producers, while, among F. graminearum strains, the 15ADON chemotype was the most frequent (68%), followed by NIV (19%) and 3ADON (13%) chemotypes. Mycotoxin analyses showed a high incidence of type B trichothecenes, even if DON levels never exceeded the EU legal limits. Relationships between isolated Fusarium species and the analyzed mycotoxins are discussed.

PRELIMINARY STUDY OF INTERACTIONS BETWEEN THE GRAPEVINE AND PHOMOPSIS VITICOLA THROUGH WHOLE TRascriptOME ANALYSIS. R.M. De Miccolis Angelini, D. Abate, S. Pollastro and F. Faretra. Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 163/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it

Next generation sequencing (Illumina technology) has been used to study transcriptional responses of Vitis vinifera (cv. Victory) to infection by Phomopsis viticola, the causal agent of Phomopsis cane and leaf spot of grapevine. Total RNA from healthy or artificially infected leaves collected 72 h postinoculation was submitted to cDNA library construction using standard protocols. About 30 million short sequencing reads (50 bases) generated from each of the two libraries were aligned to the grape reference genome (12x; http://genomes.cribi.unipd.it/) and analyzed to measure transcript levels. Comparing the two samples of inoculated and non-inoculated leaves, differentially expressed genes were identified, assigned to functional categories, and examined for their potential involvement in plant response to fungal infection. The majority of modulated transcripts were upregulated in infected leaves. Transcripts showing the greatest induction in response to infection (fold change ≥ 20) included: (i) sequences encoding pathogenesis-related proteins (PR-1, PR-5, PR10 and PR-17) or other defence-related compounds (such as dirigent-like proteins, gamma-thionins and antimicrobial compounds); (ii) transcripts involved in the regulation of gene expression, signal transduction and hypersensitive reaction; (iii) sequences encoding several enzymes required for the biosynthesis of phenolic compounds, alkaloids and terpenes; (iv) enzymes implicated in catabolic processes. Results also suggest the activation of the ethylene/jasmonate-mediated signaling pathways in grapevine following P. viticola infection. A few assembled sequences putatively assigned to the fungal transcriptome were detected in inoculated sample. These may help deepening the knowledge on key genes of the pathogen involved in the interaction with the host.

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DEOXYVINALENOL CONTENT IN COMMON AND DURUM WHEAT KERNELS OF OLD AND MODERN CULTIVARS GROWN UNDER ORGANIC FARMING. R. Di Silvestro, I. Marotti, S. Bosi, V. Bregola, M. Accorsi, A. Di Loreto, A. Prodi, P. Pignatelli and G. Dinelli. Dipartimento di Scienze Agroambientali, Università degli Studi, Via Fanin 44, 40127 Bologna, Italy. E-mail: giovanni.dinelli@unibo.it

Mycotoxin contamination of flours is a major concern of organic farming, as no fungicides can be used. The most common toxin occurring in wheat flour is deoxynivalenol (DON), a trichothecene produced by several fungi of the genus Fusarium (mainly F. culmorum and F. graminearum). Two different field trials were set up for two consecutive growing seasons (2009/2010; 2010/2011) encompassing comparisons between: (i) one modern (Palesio) and five old (Inallettabile, Andriolo, Gentil Rosso, Verda, Frassino) common wheats varieties and (ii) one old durum type (Kamut Khorasan) and one modern durum wheat cultivar (Claudio) all grown under organic farming. Different DON accumulation in the grains was observed comparing the two cropping years as a result of changing weather conditions: wheat varieties grown during the first cropping season presented higher myco-
toxin levels (0.19 and 1.19 mg/kg for common and durum wheat, respectively) as compared to those analysed in the second year (0.02 and 0.33 mg/kg for common and durum wheat, respectively). The higher DON levels of Kamut and Claudio flours confirmed the higher susceptibility of durum-type wheat to Fusarium head blight, but no differences were observed among genotypes for each wheat species. Interestingly, notwithstanding the interdiction of fungicide treatments, in common wheat cultivars the mycotoxin content was lower than the thresholds set for all food categories in the current EU regulation (EC N. 1126/2007).

PHYLOGENETIC RELATIONSHIPS AND IDENTIFICATION OF DISCRIMINATING SINGLE NUCLEOTIDE POLYMORPHISMS AMONG 16SrV GROUP PHYTOPLASMAS. G. Durante1, P. Casati1, D. Clair2, F. Quaglini2, D. Bulgari2, E. Boudon-Padieu2 and P.A. Bianco1. 1Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. 2UMR Plante Microbe Environnement, INRA-CNRS-Université de Bourgogne, Dijon, France. E-mail: piero.bianco@unimi.it

Phytoplasmas are cell-wall less, uncultivable bacteria, belonging to the class Mollicutes. They parasitize both plant hosts and insect vectors, but knowledge of their biological and molecular properties is limited. Analyses of 16S rDNA nucleotide sequences revealed that 16SrV group phytoplasma strains identified in grapevine plants are closely related, but associated with distinct ecologies. In the present study, we investigated the evolutionary relationships of eight representative 16SrV phytoplasma strains (EY1, ULW, ALY, HD1, PGY-A, FD-C, FD92 and FD70) by sequencing 15 ribosomal protein (rp) genes positioned within the S10-rpc operon. Phylogenetic analyses of concatenated nucleotide and amino acid sequences underscored that 16SrV phytoplasma strains infecting grapevine cluster together in a distinct clade, supporting the hypothesis that they have a probable common origin. Furthermore, sequence typing revealed the presence of single nucleotide polymorphisms (SNPs) distinguishing the analyzed 16SrV phytoplasma strains. In detail, in silico restriction digest assays on rpIV-rpO nucleotide sequences showed that the DNA segment including the genes rpIV, rpE, rpmD, and rpO contained numerous SNPs positioned within restriction sites for AluI and HhaI endonucleases, revealing the presence of distinct strain-specific virtual RFLP patterns. Based on SNPs identified in this study, candidate rp-genes could be proposed for finer analyses. For example, in the case of Flavescence dorée (FD) disease, SNPs located within the gene rpE could be used for distinguishing FD phytoplasma strains. In-depth investigation will be performed for confirming and describing more accurately the complex population structure of FD phytoplasmas of 16SrV-C and -D subgroups.

ANALYSIS OF APPLE FRUIT PROTEOMES INDUCED BY ALTERNATIVE POST-HARVEST TREATMENTS. A.M.E. Eid1, M. Ruocco2, S.M. Sanzani3, S.L. Woo1,2, F. Vinalè2, S. Lanzuise1,2, M. Nigro1,2, R. Varlese1, R. Marra1, V. Matteoli1, G. Manganiello1, A. Pascale1, D. Stellitano2 and M. Lorito1,2. 1Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici, Italy. 2Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. 3Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126 Bari. E-mail: lorito@unina.it

Apple, the most cultivated and utilized fruit tree in temperate climate zones, represents an important and profitable source of food. During post-harvest storage, apples are susceptible to rot caused by latent infection of field pathogens, such as Pseudomonas vagabunda (lenticel rot), Botrytis cinerea, and Alternaria alternata, or by pathogens infecting wounds made during harvesting and postharvest handling, such as Penicillium expansum. The control of postharvest pathogens is usually achieved by using synthetic fungicides. However, the development of resistant strains, the difficulties in registering new active substances and the growing concern for the negative effect of chemicals on the environment and the consumer’s health have increased the interest for alternative disease control means. The exploitation of natural mechanisms of plants resistance to diseases induced by various factors (IR) has gained attention in many laboratories worldwide as part of an integrated pest management strategy. The objective of our project was to develop a new method for controlling microbial post-harvest pathogens by improving IR in apple fruit. Here we report the results from experiments in which the total proteome of apples subjected to different treatments, including antagonistic microbes, quercetin and essential oils, was obtained at different time points (0, 24 and 48 h post treatment). Several differentially accumulated proteins, separated by 2D-electrophoresis from various apple proteomes, have been isolated and are being identified and characterized by MALDI-TOF analysis.

CHARACTERIZATION OF PSEUDOMONAS CORRUGATA STRAINS ISOLATED FROM CHRYSANTHEUM PLANTS, ROOTS AND RHIZOSPHERE IN SARDINIA. M. Fiori, V. Ligiós, R. Marongiu, V.A. Prota and D. Fernandez Neira. Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola, 07100 Sassari, Italy. E-mail: fiorim@uniss.it

Pseudomonas corrugata, the causal agent of pith necrosis, is an ubiquitous bacterium initially found in tomato plants and, subsequently, in pepper, geranium and chrysanthemum. This bacterium has been isolated from roots and rhizosphere of symptomless plants and from soil, and has been used for the biocontrol of plant pathogenic bacteria and fungi. In Sardinia pith necrosis has been observed in tomato and chrysanthemum plants showing similar symptoms, i.e. adventitious roots, brown to black lesions and a whitish mucous exudate on stems; chlorosis and necrosis of the leaves; hyperhydrosis, necrosis and/or hollowing of the pith; loss of turgidity, rotting and blight of the plants. The observations were focused on chrysanthemums plants grown in greenhouses located in central and southern Sardinia. Isolations were made from plants, rhizosphere and soil. Based on morphology on nutrient dextrose agar and hypersensitivity on tobacco leaves, a total of 28 isolates were selected. These were tested for pathogenicity on chrysanthemum, pepper and tomato and were characterized by morphological and physiological tests, serological agglutination test, Biolog using microplates Gen III, and PCR using specific primers PC1/1, PC1/2 and PC5/1, PC5/2. All isolates were identified as Pseudomonas corrugata. Biolog results showed a variable utilization of organic compounds. Cluster analysis performed using Biolog results produced different groupings. Further genomic characterization is now in progress to evaluate the differences found in the chrysanthemum population of P. corrugata.

HORIZONTAL GENE TRANSFER OF THE CERATO-ULMIN GENE IS WIDESPREAD BETWEEN OPHIOSTOMA NOVO-ULMI AND GEOSMITHIA spp. A. Frascella1,2,
P.P. Bettini1, M. Kolarik1, C. Comparini2, L. Carresi2, L. Pazzaglia1, A.L. Pepori1, A. Santini2, F. Scala1 and A. Scala2. 1Dipartimento di Biologia Evoluzionistica Leo Pardi, Università degli Studi, Via Roma 17-19, 50123 Firenze, Italy. 2Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi di Firenze, Via della Lucestrica 10, 50019 Sett. Fiorentino (FI), Italy. 1Institute of Microbiology of the ASACR, Videnaska 1083, 142 20 Prague 4, Czech Republic, and Department of Botany, Faculty of Science, Charles University, Benátska 2, 120 01 Prague 2, Czech Republic. 4Dipartimento di Scienze Biochimiche, Botany, Faculty of Science, Charles University, Benátska 2, 120 01 Prague 2, Czech Republic. 5Dipartimento di Scienze Biochimiche, Botany, Faculty of Science, Charles University, Benátska 2, 120 01 Prague 2, Czech Republic. 6Department of Environmental Science, Policy and Management, University of California at Berkeley, 137 Mulford Hall, 94720 Berkeley CA, USA. 1Department of Environmental Science, Policy and Management, University of California at Berkeley, 137 Mulford Hall, 94720 Berkeley CA, USA. 2Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. 2Istituto per la Protezione delle Piante del CNR, Via Madonna del Piave 20, 33100 Udine, Italy. E-mail: p.bettini@unifi.it

In 2011 about 247,000 ha were cultivated in Italy with barley (13% of the total surface planted with cereals), mainly intended, in order of importance, for livestock feed, malt industry and human food. Fusarium head blight (FHB) is a disease of small-grain cereals which causes yield reduction and losses in grain quality and that may produce accumulation of secondary metabolites that pose a health risk to humans and animals. The species frequently associated with FHB of cereals in Europe are F. graminearum Schwabe, F. culmorum (W. G. Smith) Sacc. and F. avenaceum (Fr.) Sacc. but in the last years particular importance was given to species comprised in the Sporotrichiella Section as F. poae, F. langsethiae and F. sporotrichioides. Thirty kernel samples of distic and polistic barley, hulless and covered, were analyzed for fungal presence. The samples were collected during 2011 from different Italian regions, i.e. Emilia Romagna, Marche, Latium and Apulia. Morphological identification of different Fusarium species was done, species-specific PCR assay was used for confirmation of some species. F. poae and F. tricinctum were mainly detected, followed by F. langsethiae and F. avenaceum. The finding of a high incidence of species belonging to Sporotrichiella complex suggests the possible risk of the presence of both type A and B trichothecenes in barley grains.

PRELIMINARY RESULTS ON THE PRESENCE OF FUSARIAUM HEAD BLIGHT AGENTS IN BARLEY KERNELS IN ITALY. M. Giannini1,2, M. Dal Pra1, S. Tonti1,4, M. Montanari2, A. Prodi3, G. Innocenti1 and D. Pancaldi1. 1Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale Fanin 46, 40127 Bologna, Italy. 2Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Via Sicilia 2, 40060 Osteria Grande, Italy. 3Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Via Ca’ Nova Zampieri 37, 37037 S. Giovanni Lupatoto, Italy. 4Dipartimento di Scienze e Tecnologie Agroambientali Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. E-mail: davide.pancaldi@unibo.it

In 2011 about 247,000 ha were cultivated in Italy with barley (13% of the total surface planted with cereals), mainly intended, in order of importance, for livestock feed, malt industry and human food. Fusarium head blight (FHB) is a disease of small-grain cereals which causes yield reduction and losses in grain quality and that may produce accumulation of secondary metabolites that pose a health risk to humans and animals. The species frequently associated with FHB of cereals in Europe are F. graminearum Schwabe, F. culmorum (W. G. Smith) Sacc. and F. avenaceum (Fr.) Sacc. but in the last years particular importance was given to species comprised in the Sporotrichiella Section as F. poae, F. langsethiae and F. sporotrichioides. Thirty kernel samples of distic and polistic barley, hulless and covered, were analyzed for fungal presence. The samples were collected during 2011 from different Italian regions, i.e. Emilia Romagna, Marche, Latium and Apulia. Morphological identification of different Fusarium species was done, species-specific PCR assay was used for confirmation of some species. F. poae and F. tricinctum were mainly detected, followed by F. langsethiae and F. avenaceum. The finding of a high incidence of species belonging to Sporotrichiella complex suggests the possible risk of the presence of both type A and B trichothecenes in barley grains.

Comparative pine log inoculation experiments suggest that the saprobiic ability is a key factor driving the invasion of Heterobasidion irregularre in Italy. L. Giordano1, M. Garbelotto2, S. Michelotti3, G. Lione1, P. Capretti4 and P. Gonnelli3. 1Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. 2Department of Environmental Science, Policy and Management, University of California at Berkeley, 137 Mulford Hall, 94720 Berkeley CA, USA. 3Dipartimento di Biotecnologie Agrarie, Università degli Studi di Torino, Piazzale delle Cascine 28, 30144 Firenze, Italy. E-mail: paolo.gonnelli@unito.it

The North American forest pathogen Heterobasidion irregulare is invasive in coastal Pinus pinea stands of central Italy, where the closely related native species H. annosum is only marginally present. Comparative inoculation experiments using H. irregulare and H. annosum genotypes failed to detect a disproportionate pathogenicity of the exotic pathogen on native pine species. In this work we compared the saprobic ability of the two fungal species through two different experiments. In the first experiment, six genotypes per species were singly inoculated in the headpiece of freshly cut Pinus sylvestris logs (30 cm length, 20 cm diameter). The second experiment was designed to test the competitiveness of the two species in dual inoculations. Pine logs were inoculated at the two headpieces with combinations of H. irregulare and H. annosum genotypes displaying comparable in vitro growth. Six genotypes combinations were tested. In the first experiment, two months after inoculation, H. irregulare displayed...
The bacterial canker of kiwifruit is the most destructive disease of cultivated Actinidia spp. The causal agent is the Gram-negative bacterium *Pseudomonas syringae pv. actinidiae* (Psa). The two commonly grown host species, *Actinidia delicosa* (green fleshed) and *Actinidia chinensis* (yellow fleshed) show different susceptibility to the disease: the yellow kiwifruit is far more susceptible than the green one. The pathogen has significant epiphytic and endophytic phases: it penetrates the host through natural openings and thorough wounds. The control of the disease is difficult, due to the lack of effective chemicals that might be applied in the orchards. Copper compounds alone did not yield a satisfactory control of the disease. For endophyte and epiphyte isolation, orchards were chosen where the disease had the highest incidence and severity. Inside orchard plots, where most kiwi plants were dead, occasional symptomless plants were observed, cut and taken to the lab. Epiphytes were isolated and purified from washings of the leaves and endophytes were obtained from the washings of woody disks, taken at different levels along the trunk. A selection of ca. 60 isolates was chosen and subjected to preliminary genetic characterisation with rep-PCR, using the BOX primer. The selected endophytes and epiphytes were assayed *in vitro* for their ability to inhibit a set of phytopathogenic Gram-negative and Gram-positive bacteria, among them *Psa*. The results of the experiments showed that at least a dozen of endophytes and epiphytes were able to strongly inhibit both *Psa* and other important phytopathogenic bacteria.

**CHARACTERIZATION OF FUNGAL ENDOPHYTIC COMMUNITY OF THE GRAPEVINE BY CULTURE-DEPENDENT AND CULTURE-INDEPENDENT METHODS.**

S. Grisan, M. Martini, R. Musetti and R. Osler. Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. E-mail: simone.grisan@unisud.it

Endophytes are micro-organisms living inside host plants without causing disease symptoms or apparent injury. Reportedly, these organisms exert a positive effect on host plants improving tolerance to multiple stresses and protection from diseases and insects. In this work we used a combination of culture-dependent and culture-independent methods to describe the fungal endophytic community of grapevine plants grown in Friuli Venezia Giulia (north-east Italy). The combined use of the two methods allowed the identification of 56 different fungal endophytes grouped in OTUs on the bases of PCR/RFLP analyses of the ITS region. Overall, 27% of OTUs were obtained by the culture-dependent method, 48% by the culture-independent method, and 25% by both methods. Furthermore the collected data revealed that fungal endophytes belonging to genera *Alternaria*, *Phoma*, *Epicoccum*, *Aureobasidium*, *Cladosporium*, *Pestalotiopsis* and *Pestalota* constituted, respectively, about 89% of the total isolates obtained by the culture-dependent method, and 79% of total clones obtained from the culture-independent method. Sequence analysis of ITS region of fungal endophytes forming all the different OTUs revealed the presence of additional variability that could be associated to different ITS genotypes. Phylogenetic analysis indicated that *Ascomycota* was the most representative phylum with about 98% of isolation rate and 95% of total species. Conversely, *Basidiomycota* was the least represented with 2% of isolation rate and 5% in terms of species. Concluding, integrated use of different approaches led a better description of the biodiversity of fungal endophytes present in host plants, grapevine in our case.

**TRANSCRIPTIONAL RESPONSE OF SUSCEPTIBLE AND TOLERANT CITRUS COMBINATIONS TO INFECTION WITH CITRUS TRISTEZA VIRUS**

M. Guardo, M.P. Russo, G. Sorrentino, A. Caruso and G. Reforgiato Recupero. CRA, Centro di Ricerca per l’Agrumicoltura e le Colture Mediterranean, Corso Savoia 190, 95024 Acireale, Italy. E-mail: maria.guardo@en-teca.it

*Citrus tristeza virus* (CTV) is one of the most important pathogens of citrus. The majority of citrus species grafted on sour orange are susceptible to CTV infection. However, trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and its hybrids (cirtranges and citrumelos) are regarded as tolerant rootstocks. The aim of this work was to investigate the changes of gene expression on sweet orange cv. Tarocco Scirè grafted on sour orange (susceptible to CTV) and Carrizo citrange (tolerant to CTV) rootstocks after the inoculation with a severe isolate of CTV (AY263361). The analyses were carried out after nine months on the scion leaves, using a cDNA custom microarray containing about 32,300 probes belonging to 10,769 different citrus genes. The comparisons of microarray analysis, based on rootstocks (inoculated and not), were able to identify 154 genes (False Discovery Rates (FDR) ≤0.05). Some selected transcripts were also confirmed by quantitative real-time RT-PCR. Gene expression changes were grouped in eighth categories of different processes. Most of them were associated with metabolism and defence response. This is the first report on transcriptional changes in a commercial citrus variety grafted on CTV sensitive and tolerant rootstocks. The understanding of the molecular interactions between host/pathogen and the improvement of the knowledge on the influence of the rootstock on modulation of disease response may contribute to develop new tools in assisted selection of new rootstocks.

**FUNGICIDE RESISTANCE IN ITALIAN AND TUNISIAN POPULATIONS OF CALONECTRIA**

V. Guaraccia, A. Vitale, D. Aiello and G. Polizzi. Dipartimento di Gestione dei Sistemi Agronomici ed Ambientali, Università degli Studi, Corso Savoia 108, 95123 Catania, Italy. E-mail: gpolizzi@unict.it

*Calonecrtia* species are disease agents on a broad range of hosts worldwide. On horticultral crops, *Calonecrtia* species have been reported mostly from the northern hemisphere, especially in ornamental plants. Three new species (*C. polizzii*, *C. pseudomexi-
can and C. tunisiana) have recently been described. Benzimidazoles (MBCs) and prochloraz are the main fungicides for chemical management of Calonectria-induced diseases in nurseries. However, the use of MBCs has been seriously questioned because of the development of resistant C. paucivora and C. morganii populations in Italy. In this study, two assays were conducted to determine the prochloraz-sensitivity of an Italian population belonging to the C. scoparia complex, collected in two different periods (1993-1996 and 2005-2009). Additionally, two other assays were conducted to determine the MBCs-resistance and prochloraz-sensitivity in four Calonectria species collected in Tunisia and identified as C. polizzi, C. tunisiana, C. mexicana, and C. pseudomexicana. Sensitivity was expressed as the 50% effective dose (ED$_{50}$) and as minimum inhibitory concentration (MIC). High prochloraz ED$_{50}$ values (>100 g a.i./ml) were detected in the Italian population. However, the mean ED$_{50}$ values detected in C. scoparia complex population (2005-2009) were higher and could be related to fungicide exposure. Prochloraz ED$_{50}$ values were lower (<50 g a.i./ml) in all Tunisian populations. MIC values >100 g a.i./ml to MBCs were detected in all Tunisian species assayed. These are the first data on prochloraz-sensitivity of Calonectria species populations and is the first report of MBCs-resistant isolates of C. polizzi, C. tunisiana, C. mexicana and C. pseudomexicana.

**Development of a Transgenic Transformation Protocol to Study the Role of a Lectin Gene in the Susceptibility of Strawberry Fruits to Colletotrichum Acutatum**

M. Guidarelli, L. Zoli, A. Orlandini, P. Bertolini and E. Baraldi. Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale F. De Sanctis, 86100 Campobasso, Italy. E-mail: elena.baraldi@unimol.it

Colletotrichum acutatum is the primary causal agent of strawberry (Fragaria x ananassa) anthracnose causing severe economic losses to the crop. This fungal pathogen infects white fruits at pre-harvest unripe stage and remains quiescent during ripening causing anthracnose symptoms only on ripe fruits. In a previous microarray experiment a lectin gene was found overexpressed in white fruits inoculated with C. acutatum. For establishing whether this gene plays any role in the low susceptibility of white unripe fruits, an experiment was designed for silencing and overexpressing the lectin gene in white and red strawberry fruits, respectively, using Agrobacterium-mediated transient transformation. Exploiting the potential of plant species to silence genes when they recognize intron-containing constructs encoding self-complementary ‘hairpin’ RNA (ihpRNA), a construct containing the partial sense and corresponding antisense sequence of the lectin gene separated by an intron was generated to transform white fruits. On the other hand, to overexpress the gene on the red fruits, a vector containing the lectin gene under the control of 35S promoter was constructed. The timing for lectin gene silencing in white fruits was determined by qRT-PCR whereas, for monitoring transient lectin overexpression in red fruits, a DsRed fluorescent protein was used as reporter in microscopy analysis.

**Development of Molecular Tools for Gene Function Studies in the Biocontrol Agent Rhodotorulidum Kratochvilovae Strain LS11.**

G. Ianiri1,2, A. Idrnurn2 and R. Castoria1. 1Dipartimento di Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, Via F. De Sanctis, 86100 Campobasso, Italy. 2Division of Cell Biology and Biophysics, School of Biological Sciences, 5100 Rockhill Road, University of Missouri, Kansas City, MO 64110, USA. E-mail: castoria@unimol.it

Rhodotorulidum kratochvilovae strain LS11 is a Pucciniomycotina yeast with biocontrol activity against Penicillium expansum, the causal agent of blue mold. P. expansum is also the main producer of patulin, a genotoxic, mutagenic, teratogenic and immunotoxic mycotoxin. As a consequence of P. expansum infection, patulin is found at high levels in stored pome fruits and derived products. Strikingly, LS11 can also degrade patulin to different compounds, one of which was identified as desopsatulenic acid (DPA). Unlike patulin, DPA is not toxic to bacteria and eukaryotic cells, including human lymphocytes. In order to gain insights into the molecular mechanisms underlying the biocontrol and patulin-detoxifying activities of R. kratochvilovae LS11, random insertional mutagenesis approaches were developed. Initially, vectors containing marker genes placed under the control of promoters and terminators of the basidiomycete yeasts Cryptococcus neoformans and Sporobolomyces sp. IAM 13481 were tested, but no transformants were obtained. R. kratochvilovae is predicted to have a genome with a high GC content and is related to Rhodotorula graminis WP1, another Pucciniomycotina yeast whose entire genome has been sequenced (http://genome.jgi-psf.org/Rhoba1_1/Rhoba1_1.home.html). Thus, the promoter and terminator of the ß-tubulin gene from R. graminis WP1, identified by BLASTp analysis using the S. cerevisiae homolog TUB2, were fused to a high GC-containing nourseothricin acetyltransferase of Streptomyces noursei, and cloned into vectors for biolistic and A. tumefaciens-mediated transformation. The success of the transformation was confirmed by Southern blot. This is the first report on the development of molecular tools for gene function studies in R. kratochvilovae.

**Entoloma sp., a Parasite of Tuber Borchii Ascomata.**

E. Lancellotti1, A. Franceschini1, M. Iotti2 and A. Zambonelli2. 1Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. 2Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Via Fanin 46, 40127 Bologna, Italy. E-mail: lance@uniss.it

Tuber borchii Vittad. (the “bianchetto” truffle) is an ectomycorrhizal Ascomycetes which forms symbiosis with many shrubs and trees. It produces edible hypogeous fruit bodies from December until April. This truffle species has become increasingly popular commercially and, recently, it has also been cultivated in some European and extra-European countries. This study describes a new disease of T. borchii ascomata found in a pine wood of Sardinia (insular Italy) in February 2012. Peridium of the parasitized ascomata showed large patches of a whitish mycelial mat composed of parallel and clamped hyphae. In correspondence of this mycelial mat the peridium was thinner and did not adhere to the gleba. Under the peridium, a layer of amorphous material was present, which could derive from peridium degradation. The ITS1-5.8S-ITS2 region of the fungal parasite was amplified by direct PCR on mycelial felt, sequenced and identified as belonging to Entoloma sp. after BLAST-N search against GenBank database. The majority of Entoloma species have an ectomycorrhizal habit although some members of this genus are parasitic such as Entoloma saepium which attacks the roots of Rosa spp. and Prunus spp. Further investigations are needed to better understand this new disease of truffles.
PHYSICAL MODE OF ACTION OF MEPTYLDINOCAP AGAINST ERYSIPHE NECATOR, THE GRAPEVINE POWDER MILDEW FUNGUS. S.E. Legler, T. Caffi, G. Russo 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

In many grape-growing areas, the primary inoculum of powdery mildew caused by *Erysiphe necator* consists of ascospores produced in chasmothecia. Primary infections trigger epidemics, which are then driven by conidial infections. Although powdery mildew control has traditionally focused on preventing conidial infections, we recently developed a control strategy that combines sanitation of chasmothecia in autumn or winter and early fungicide applications against ascospores in the next spring; the fungicide applications are based on a weather-driven, mechanistic model. This “across-season” strategy requires knowledge the physical mode of action (PMoA) of each fungicide. PMoA describes the effect of a fungicide with respect to the time of application (relative to the host-pathogen interaction) and the duration and degree of its activity. This work investigated the PMoA of the recently available diminotrophen fungicide meptyldinocap against *E. necator*. In environmentally-controlled and semi-field experiments, meptyldinocap had high preventative and curative efficacy. When applied 1 to 6 days before inoculation, meptyldinocap prevented infection; when applied within 72 h after inoculation, it prevented colony formation. When applied on established colonies, it reduced conidia production by 55% and conidia vitality by 95%. Meptyldinocap also reduced chasmothecia formation and ascospore viability in mature chasmothecia. Scanning electron microscopy revealed that all fungal structures were collapsed. This work shows that powdery mildew control can be improved by combining weather-driven, mechanistic disease models and information on fungicide PMoA.

SCREENING OF WORLDWIDE ISOLATES OF CITRUS TRISTEZA VIRUS BY SAFE CE/SSCP AND MOLECULAR MARKER GENOTYPING. G. Licciardello1, M. Russo1, M. Daden2, M. Bar-Joseph4 and A. Catara1. 1Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Bivio Palma I, Stradale Lancia 57, 93121 Catania, Italy. 2IPADLAB, International Plant Analysis and Diagnostics, Via Einstein, Località Cascina Codazza, 26900 Lodi, Italy. 3National Center for Citrus Improvement, Hunan Agricultural University, 410128 Hunan, China. E-mail: glicciardello@pstsicilia.it

With more than 320,000 ha of orchards Hunan province contributes by 15% to the total production of citrus in China and is one of the most important in the world. The use of tolerant rootstocks has protected since long time the local citriculture from the devastating effects of seedling yellows and decline *Citrus tristeza virus* (CTV). For several years, however, stem pitting became a major issue causing stunning associated with specific wood symptoms on twigs on pummelos and sweet oranges. In this study we report the results of a preliminary screening performed on 23 isolates collected in three orchards located in the Yizhang and Yong Xing counties. In order to discriminate both multiple infections and recombinant genotypes, evaluation was performed by DT-BIA/CE-SSCP and multiple molecular marker. Samples were collected and spotted on nylon and hybrid membranes in the laboratories of Hunan and analyzed in the PSTS laboratories. Detection was carried out a week later using a commercial kit (PlantPrint Diagnostics, Spain) with a mixture of 3CA5 and 3DF1 CTV specific monoclonal antibodies. Five microliters of released virus were directly used as template for CE-SSCP and MAM analysis. CE-SSCP analysis was performed comparing the electrophoretic profiles of single strands generated after amplification of the coat protein and p23 genes. The analysis showed high genetic variation and the presence of divergent sequence variants within individual isolates. The MAM analysis revealed that VT-like and VT+T3-like are the most frequently occurred genotypes revealing the presence of severe isolates.

SUPPRESSIVE ACTIVITY OF ORGANIC AMENDMENTS ENRICHED WITH MICROBIAL ANTAGONISTS AGAINST FUSARIOSIS OF TOMATO AND CUCURBIT PLANTS. G. Lima1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1, D. Vitullo1

Cucurbits and solanaceous plants are widespread and economically important horticultural crops often attacked by different pathogens, among which soil-borne fungal pathogens are responsible for severe root and crown rots. Chemical control of soil-borne pathogens using synthetic fungicides is often uneconomic and involves technical and eco-toxicological negative side effects. Research on safer and environmentally-friendly alternative control means are then of particular interest. As emerging alternatives, new plant growth media enriched with microbial antagonists may...
display an interesting suppressive activity. In the present study, the suppressive activity of organic amendments, alone or enriched with microbial antagonists was tested against *Fusarium oxysporum* f. *lycopersici* and *F. oxysporum* f. *melonis*, the causal agents of tomato and cucurbit fusariosis, respectively. In blind trials experiments on tomato and melon plants, four organic amendments enriched with selected microbial antagonists and used in mixture (up to 10% v/v) with a standard plant-growth substrate were assayed. Trials were conducted on potted plants grown under controlled conditions and in commercial nurseries and the following parameters were periodically assessed: disease incidence and severity; pathogen isolation from plant tissues; survival of microbial antagonists in the rhizosphere; effect of the treatment on plant growth. On both tomato and melon, some enriched amendments induced a significant reduction of *Fusarium* disease incidence and severity as well as positive effects on plant growth. Furthermore, microbial antagonists added to the amendment survived in the rhizosphere at a high population level.

A NEW DISEASE OF *ERICA ARBOREA* IN ITALY CAUSED BY *NEOFUSICOCCUM L. TEYM.* B.T. Linaldeddu1, S. Seddaiu2, B. Scanzu1, A. Deidda1, L. Maddau1 and A. Franceschini1.

1Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. 2Dipartimento della Ricerca per il Sughero e la Silvicoltura, Agris Sardegna, Via Limbara 9, 07029 Tempio Pausania, Italy. E-mail: ben@uniss.it

Tree heath (*Erica arborea*) is a shrub or small evergreen tree typical of the Mediterranean scrub. The species is also cultivated as an ornamental plant. Since 2011, an unusual disease of tree heath has been observed in a natural area located on the Caprera Island, Sardinia (Italy). Typical symptoms of this disease appear in late spring with the sudden wilting of new shoots. The infection spreads within the young shoot tissues, rarely into older wood, causing the shoot tip to curl over and form a crook. A dense mass of new epicormic shoots grows under infected shoots, with the resulting structure resembling a witch’s broom. Fungal dense mass of new epicormic shoots grows under infected shoots, causing the shoot tip to curl over and form a crook. A dense mass of new epicormic shoots grows under infected shoots, with the resulting structure resembling a witch’s broom. Fungal isolates obtained from symptomatic shoots were identified as *Neofusicoccum luteum* on the basis of morphological features. On PDA at 23°C, all isolates developed a moderately aerial mycelium, with a yellow pigment diffusing into the medium that behaved as a weak pathogen on *Trichoderma* and *Arthroderma* spp. isolates were isolated from 17 soil samples collected in six organic tomato farms. The presence of *P. lycopersici* in the soil was evaluated using specific primers for this species. A total of 36 monosporic *Trichoderma* spp. isolates were obtained and identified by amplification of the ITS regions and of the gene *ef1* for translation elongation factor 1α (EF1α) and subsequent search for homologies on databases (NCBI, Trichoderma BLAST). *T. harzianum* was the most frequently isolated species, followed by *T. gamsii*. The isolates were evaluated *in vitro* for their potential antagonism towards *P. lycopersici* and by means of dual culture test and by measuring their ability to produce volatile and water-soluble inhibitors. Among the potential antagonists tested, two isolates of *T. gamsii*, one of *T. harzianum* and one of *T. brevicompactum* showed good promises in all tests. Their ability to produce water-soluble inhibitors was further evaluated against other pathogens of tomato (*Fusarium* sp. *F. oxysporum* f. *lycopersici*, *F. oxysporum* f. *radicis-lycopersici*, *Rhizoctonia solani*), melon (Monosporascus cannonballus, Acremonium cucurbitacearum and Rhizopycnis vagum) and walnut (Phytophthora cinnamomi and *P. cactorum*). Antagonistic activity against *P. lycopersici in vivo* is being evaluated by means of artificial inoculation in the greenhouse.

Research carried out within the framework of the Project BIOMED, funded by Mipaaf.

ISO-HARZIANIC ACID A NEW BIOACTIVE METABOLITE FROM *TRICHODERMA HARZIANUM*. G. Manganiel-lo1, F. Vinale2, M. Nigro1, P. Mazzei1, A. Piccolo1, A. Pascale1, M. Ruocco2, S.L. Woo1,2, S. Lanzuise1, R. Marras1, A. Eid1, R. Varlese1, V. Matteoli1, D. Stellitano2 and M. Lorito1,2. 1Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici, Italy. 2Istituto di Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. 1Centro Interdipartimentale di Spettroscopia di Risorsa Magnetica Nucleare, Università degli Studi di Napoli Federico II, 80055 Portici, Italy. E-mail: lorito@unina.it

*Trichoderma* are ubiquitous soil fungi of which many species have a potential as alternative to synthetic chemicals and fertilizers for diverse applications in a sustainable agricultural production. In addition to their biocontrol properties, some strains have been shown to enhance crop productivity by stimulating plant growth. These fungi secrete several secondary metabolites with different biological activity. Harzianic acid (HA) is one of these compounds that has been isolated from *Trichoderma* and already investigated for its ability to promote plant growth and strongly bind iron. In this work we have isolated a new metabolite named iso-harzianic acid (IsoHA), a stereoisomer of harzianic acid, from the culture filtrate of a *T. harzianum* strain isolated from decomposing hardwood bark. The structure and absolute configuration of this compound have been determined by spectroscopic methods, including UV-Vis, MS, 1D and 2D NMR analyses. Applications of IsoHA at concentrations of 10^-6 M and 10^-7 M increased the germination of tomato seeds by 10% and 15%, respectively, compared to the untreated control. Furthermore, treatments with IsoHA at concentrations of 10^-6 M and 10^-7 M increased the germination of tomato seeds by 10% and 15%, respectively, compared to the untreated control. This effect was compared to the control, whereas a concentration of 10^-5 M inhibited seed germination up to 46%. IsoHA may be useful for enhancing plant disease control and development, and deserves further investigation.

EVALUATION OF *TRICHODERMA* spp. ISOLATES FOR THE CONTROL OF CORKY ROOT OF TOMATO IN ITALY. L. Luongo, G. Di Giambattista, S. De Felice and A. Infantino. CRA, Centro di Ricerca in Patologia Vegetale Via C.G. Bertero 22, 00156 Rome, Italy. E-mail: alejandro.infantino@crera.it

Corky root caused by *Pyrenochaeta lycopersici* is a serious disease of tomato worldwide. Within the framework of the Project BIOMED, funded by the Italian Ministry of Agriculture, several isolates of *Trichoderma* spp. were isolated from 17 soil samples collected in six organic tomato farms. The presence of *P. lycopersici* in the soil was evaluated using specific primers for this species. A total of 36 monosporic *Trichoderma* spp. isolates were obtained and identified by amplification of the ITS regions and of the gene *ef1* for translation elongation factor 1α (EF1α) and subsequent search for homologies on databases (NCBI, Trichoderma BLAST). *T. harzianum* was the most frequently isolated species, followed by *T. gamsii*. The isolates were evaluated *in vitro* for their potential antagonism towards *P. lycopersici* and by means of dual culture test and by measuring their ability to produce volatile and water-soluble inhibitors. Among the potential antagonists tested, two isolates of *T. gamsii*, one of *T. harzianum* and one of *T. brevicompactum* showed good promises in all tests. Their ability to produce water-soluble inhibitors was further evaluated against other pathogens of tomato (*Fusarium* sp. *F. oxysporum* f. *lycopersici*, *F. oxysporum* f. *radicis-lycopersici*, *Rhizoctonia solani*), melon (Monosporascus cannonballus, Acremonium cucurbitacearum and Rhizopycnis vagum) and walnut (Phytophthora cinnamomi and *P. cactorum*). Antagonistic activity against *P. lycopersici in vivo* is being evaluated by means of artificial inoculation in the greenhouse.

Research carried out within the framework of the Project BIOMED, funded by Mipaaf.
EFFECTS OF FLAVESCENCE DORÉE PHYTOPLASMA ON THE PHENYL-PROPANOID PATHWAY OF INFECTED AND RECOVERED GRAPEVINES. P. Margaria,1, A. Ferrandino,2, A. Schubert1 and S. Palmano,1,2Istituto Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. 1Dipartimento Colture Arboree, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy. E-mail: s.palmano@ivv.cnr.it

Flavescence dorée (FD) is a quarantine disease of Vitis vinifera, caused by a phytoplasma (Candidatus Phytoplasma vitis), a phloem-limited pathogen in the class Mollicutes. Despite its economic relevance, the molecular basis of the disease and plant responses to the infection are little known. Moreover, an interesting though unknown phenomenon of spontaneous remission of symptoms (recovery) may occur in infected plants. The objective of this study was to study the biochemical and transcriptional changes in the phenyl-propanoid pathway in FD-infected and recovered grapevines. Two Italian cultivars, Nebbiolo and Barbera, were selected due to their different sensitivity to phytoplasma infection and recovery occurrence. Leaves of healthy, infected and recovered plants of the two cultivars were collected in the same vineyard at four time-points during the vegetative season (June, July, August and September). Biochemical and transcriptional analyses were carried out on preparations of the same starting material. Total anthocyanins and flavonoids were analyzed by spectrophotometry and flavonols profiles by high-performance liquid chromatography. The expression levels of several genes involved in the flavonoid biosynthetic pathway were monitored by quantitative reverse transcription PCR. The following genes were selected for transcriptional assays: chalcone synthase (CHS), flavanone-3-hydroxylase (F3H), leucoanthocyanidin dioxygenase (LDOX), UDP-glucose:flavonoid-3′-O-glucosyltransferase (UFGT), flavonol synthase (FLS), leucoanthocyanidin reductase (LAR) and MYB transcription factor gene. Variation in mRNA level of the selected target genes was evaluated and correlated to the plant phenological stage and to the phytosanitary status.

STUDY OF RESISTANCE TO TERUBCONAZOLE AND TIOPHANATE METHYL ON ITALIAN STRAINS OF MONILINIA FRUCTICOLA AND M. LAXA. C. Martini, M. Guidarelli and M. Mari. Dipartimento di Protezione e Valorizzazione Agricoltura, Università degli Studi, Via Fanei 46, 40127 Bologna, Italy. E-mail: camilla.martini@unibo.it

Brown rot caused by Monilinia laxa, M. fructicola and M. fructigena is the main disease of stone fruits in European countries. In the past M. laxa and M. fructigena were controlled by chemical treatments based on methyl benzimidazole carbamate (MBC). Three decades ago demethylation inhibitors (DMI) were introduced in substitution of MBC. The aims of this work were: (i) estimate the risk of resistance to these two classes of fungicide in Monilinia spp. population and (ii) detect mutations in the β-tubulin gene encoding the target protein of MBC fungicides in MBC-resistant strains. Symptomatic blossom, twigs and fruits were selected due to their different sensitivity to phytoplasma infection and recovery occurrence. Leaves of healthy, infected and recovered plants of the two SDHI-resistant strains. Symptomatic blossom, twigs and fruits were collected from orchards and over 100 single-spore isolates were generated: 61 belonged to M. laxa, 34 to M. fructicola and five to M. fructigena. The EC50 was calculated for both fungicides using the amended medium technique. Isolates showing EC50 values higher than 1 µg/ml and 0.1 µg/ml were considered resistant to MBC and DMI, respectively. Ten percent of M. laxa and 68% of M. fructicola strains resulted resistant to thiophanate methyl, while 7% of M. laxa and none of M. fructicola were resistant to tebuconazole. All M. fructigena strains showed resistance to tebuconazole while none of M. fructigena was resistant to thiophanate methyl. The mutation associated with MBC resistance was studied by amplification of the β-tubulin gene with forward primer TubA (AAATGGGTGAATGTTA) and reverse primer TubR1 (TGTTCATAAGCAAGAACCCT). The genotypic and phenotypic data obtained in vivo and in vitro are compared and the results discussed.

CHARACTERIZATION OF RESISTANCE TO SDHI FUNGICIDES IN BOTRYOTINIA FUCKELIANA (BOTRYTIS CINEREA). M. Masiello, C. Rotolo, R.M. De Miccoli Angelini, S. Pollastro and F. Faretra. Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via G. Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it

Botryotinia fuckeliana is a pathogen at high-risk of resistance to fungicides, as succinate dehydrogenase inhibitors (SDHIs), commonly used against gray mould. Isolates of the fungus carrying allelic variants of the SdhB gene associated with different phenotypes of sensitivity/resistance to boscalid were characterised for response to the new fungicide fluopyram. Cross-resistance between the two SDHIs appeared to be limited to specific boscalid-resistant genotypes. Substitution of proline with leucine or phenylalanine at position 225 (P225L/F), associated to a high level of resistance to boscalid (RF=10,000), conferred high (RF=1,000) or low (RF=10) level of resistance to fluopyram, respectively. The replacement of asparagine with isoleucine at position 230 (N230I) conferred moderate (RF=100) resistance to both SDHIs. Mutations at codon 272 resulting in histidine to tyrosine or arginine (H272Y/R) replacements, responsible for a high (RF=10,000) resistance to boscalid, confer sensitivity or hyper-sensitivity to fluopyram, respectively. PCR assays using allele-specific (AS)-primers were carried out on more than 180 resistant field isolates and confirmed a clear phenotype-to-genotype relationship in SDHI-resistant isolates. A real-time PCR assay using the same primers and SYBR-green was developed to quantify SDHI-resistant isolates in fungal population samples. A primer pair, able to amplify a region of SdhB gene common to wild-type and resistant isolates was used as internal calibrator. The sensitivity of the technique was as low as 1% of mutated alleles. This method should be useful to quantify the occurrence of SDHI-resistant isolates in B. fuckeliana field populations.

KIWIFRUIT BACTERIAL CANKER: BIOLOGY AND STRATEGIES OF INFECTION OF PSEUDOMONAS SYRINGAE pv. ACTINIDIAE. A. Mazzaglia1, A.R. Taddei2, P. Copini1, M. Renzi1, M.C. Taratufolo1, A. Anselmi1, A. Ercolani1, G. Covicchio1, S. Giarroni1, V. Tagliavento1 and G.M. Balestra1

1Dipartimento di Scienze e Tecnologie per l’Agricoltura, le Foreste, la Natura e l’Energia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. 2Centre Interdipartimento di Microscopia Elettronica, Università della Tuscia, 01100 Viterbo, Italy. 3Forest Ecology and Forest Management Group, Centre for Ecosystem Studies, Wageningen University, P.O. Box 47, 6700AA Wageningen, The Netherlands. E-mail: balestra@unitus.it

The bacterial canker of kiwifruit caused by Pseudomonas syringae pv. actinidiae (Psy) is a severe threat to kiwifruit production worldwide. After four years, the biology and strategies of infections of this microorganism begin to be understood. Psy causes damages mainly during spring and autumn. Typical symptoms are on aerial organs and along the trunk. Kiwi fruits are not directly affected but they collapse due to the occlusion of vessels
(xylem and phloem) by bacterial cells. This process was studied in naturally infected and artificially inoculated kiwifruit plants. The bacterium can infect host plants by entering natural openings and lesions. In naturally infected plants, *Psa* is present in the lenticels as well as in the dead phloem tissue beneath the lenticels, surrounded by a lesion in the periderm which indicates the importance of lenticels for kiwifruit infection. In advanced stages of *Psa* infection, necroses of the phloem occur, which are followed by cambial dieback and most likely by infection of the xylem. Anatomical changes in wood, such as reduced ring width, a drastic reduction in vessel size and the presence of thyloses were observed in several infected sites. In the field, these changes occur only one year after the appearance of leaf symptoms, suggesting a significant time lapse between primary and secondary symptoms. Cultural practices are highlighted as phytosanitary measures to prevent and contain kiwifruit bacterial canker disease.

**SUSCEPTIBILITY OF NEW GRAPEVINE GERMPLASM TO PLASMOPARA VITICOLA AND ERYSIPE NECTOR.**

M. Miazzi\(^1\), D. Digiaro\(^1\), C. Dongiovanni\(^2\), L. Susca\(^1\), P. La Notte\(^1\), C. Pirola\(^1\), S. Pollastro\(^3\) and F. Faretra\(^4\)

\(^1\)Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via G. Amendola 165/A, 70126 Bari, Italy.
\(^2\)Centro di Ricerca e Sperimentazione in Agricoltura Basile Caramia, Via Cisternino 281, 70010 Locorotondo, Italy.
\(^3\)Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it

The Directive 2009/128/EC on the sustainable use of pesticides aims at promoting the use of Integrated Pest Management and alternative approaches, such as the selection of new germplasm more tolerant or resistant to pests and diseases. In the frame of collaboration with Balkan breeders, two experimental fields were planted with 27 new cultivars obtained by crosses between *Vitis vinifera* (L.) and other species of the genus *Vitis*, and six reference cultivars commonly grown in Italy (Italy, Red Globe, Victoria, Michele Pallieri, Black Magic and Crimson Seedless). In particular, nine cultivars were supplied by the Research Institute for Viticulture and Enology, Pécs (H), seven by the National Institute of Winegrowing and Winemaking, Chisinau (MO), and 11 by the Experimental Station “Sremski Karlović” of the Institute for Pomology and Viticulture, University of Novi Sad (SE). Two years of field observations and *in vitro* assays were carried out to evaluate the adaptability of the new germplasm to the pedo-climatic conditions of Apulia (southern Italy). All the new cultivars showed an overall lower susceptibility to downy mildew as compared to the reference ones in the field as well as *in vitro*. Downy mildew infections were limited and sporification sparse and concentrated at the edges of the infected area. The most promising were the Hungarian cvs Ezat, Palatine, Bolgar, Rezy, Terez, and the Serbian cvs Poleskey Muskotyal, Venus, and Piroska. An *in vitro* assays showed that cvs Palatine, Poleskey and Muskotyal were also more tolerant to powdery mildew as compared with the other cultivars.

**PRELIMINARY OBSERVATIONS ON THE ACTIVITY OF CORDYCEPS EXTRACTS AGAINST PHYTOPATHOGENIC FUNGI.**

M. Miazzi\(^1\), C. Dongiovanni\(^2\), M. Xu\(^3\) and F. Faretra\(^1\)

\(^1\)Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via G. Amendola 165/A, 70126 Bari, Italy.
\(^2\)Centro di Ricerca e Sperimentazione in Agricoltura Basile Caramia, Via Cisternino 281, 70010 Locorotondo- do, Italy. \(^3\)AP Pre Ltd, 1 Science Park Drive, B-41 PSB Main Building, Singapore 118221. E-mail: faretra@agr.uniba.it

In recent years, interest has increased in developing measures for crop protection alternative to chemicals. Entomophagous species of the genus *Cordyceps* (Fr.) Link have been for centuries a cornerstone of Chinese medicine. A recent patented protocol allows to obtaining it at an economically affordable scale, opening to its use also in new areas such as plant protection. The potential of extracts from five *Cordyceps* species was assayed in controlling phytopathogenic fungi belonging to 12 genera: *Botrytis, Colletotrichum, Fusarium, Penicillium, Pyrenochaeta, Phaeoacremonium, Phytophthora, Rosellinia, Rhizoctonia, Sclerotinia, Stemphylium, Verticillium*, and the antagonistic fungus *Trichoderma* spp. Their colony growth was evaluated on PDA medium amended with different concentrations (w/v) of *Cordyceps* extract before autoclaving. Petri dishes (55 mm) were inoculated with 4 mm disks of actively growing mycelium of each fungus, and incubated in the dark at 21±1°C. Each combination fungus-extract was replicated three times. Mycelial growth inhibition (%) was measured three and eight days post inoculation. Experiments were repeated twice. All *Cordyceps* extracts at the concentration of 20% reduced fungal growth by 33% to 93%. The most inhibited fungal species were *Rootellinia* (92%), *Phytophthora* (71%), and *F. oxysporum* (69%), the less affected were *Colletotrichum* (20%) and *Penicillium* (21%). The three most effective extracts, CMM, CSX100M and CSX100S, assayed at lower concentrations (1, 5 and 10%, w/v) determined an average reduction of fungal growth respectively of 6%, 17% and 29%. These results are promising, and constitute premises for the development of possible alternative tools for controlling phytopathogenic fungi by using ecologically-sustainable techniques.

**INFLUENCE OF COPPER ON THE GROWTH OF PSEUDOMONAS SYRINGAE pv. ACTINIDIAE STRAINS ISOLATED FROM KIWIFRUIT ORCHARDS AFFECTED BY BACTERIAL CANKER.**

P. Minardi\(^1\), S. Arzidi\(^2\) and A. Bertacini\(^3\)

\(^1\)Dipartimento di Scienze Medie e Veterinarie Università degli Studi di Bologna, Via Tolaria di Sopra 50, 40064 Ozzano Emilia.
\(^2\)Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. E-mail: paola.minardi@unibo.it

The control of *Pseudomonas syringae* pv. *actinidiae* (*Psa*), the causal agent of the bacterial canker of kiwifruit, is currently based on the use of good agronomic practices and of copper compounds. In several phytopathogenic bacteria (*P. syringae*, *P. syringae* pvs. *tomato* and *syringae*, *X. campestris* pvs. *vesicatoria* and *juglandis*) copper resistance has developed, which could limit or impair the effectiveness of copper-based treatments. The molecular and genetic basis of copper resistance has been studied in detail in *P. syringae* pvs. *tomato*. Copper-resistance genes, located on plasmids (pPaCu), have a high degree of homology in different *Pseudomonas* species. The literature reports that copper-resistant strains of *Psa* bear one or two pPaCu plasmids, which carry genes for copper and streptomycin-resistance. In kiwifruit orchards frequent copper treatments may induce the selection of *Psa* populations resistant to copper with serious consequences for kiwifruit producers. In this study, the sensitivity to copper of *Psa* strains isolated in Emilia-Romagna (northern Italy) in 2009 in kiwifruit orchards affected by the bacterial canker was investigated. The growth of nine *Psa* strains in liquid culture media containing copper nitrate \([Cu(NO_3)_2]\) at concentrations ranging from 0 to 4.25 mM was followed over time and for each strain the MIC (minimum inhibitory concentration) was determined. Twenty-
four h after the treatment, the growth was inhibited by 3.5 mM Cu(NO$_3$)$_2$ in six strains and by 3.75 mM in one strain, while in five strains the antimicrobial effect was bacteriostatic.

**ABSENCE OF PSEUDOMONAS SYRINGAE pv. ACTINIDI-AE IN SYMPTOMLESS FRUITS AT HARVEST IN KI-WIFRUIT ORCHARDS OF EMILIA ROMAGNA SEVERELY AFFECTED BY BACTERIAL CANKER.** P. Minardi$^1$, S. Ardizzi$^2$ and C. Lucchese$^3$. $^1$Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy. $^2$Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Faini 44, 40127 Bologna, Italy. E-mail: paola.minardi@unibo.it

Italy is the first producer of kiwifruit in the northern hemisphere (excluding China) contributing to 65% of the overall output. Since 1999, in Italy the production capacity of kiwifruit exceeds 500,000 tonnes and, from `90 to date, export of kiwifruit has significantly increased to exceed 70% of the marketable production. To investigate thoroughly whether the fruits themselves, harboring *Pseudomonas syringae* pv. *actinidiae* (*Psa*) either epiphytically or endophytically, can be a means of disease spread is of great economic relevance for the production and commercial exchanges. In October 2011, in eight kiwifruit orchards (three *Actinidia delicosa* cv. Hayward; three *A. chinensis* cv. Hort16A; two *A. chinensis* cv. Jin Tao) severely affected by bacterial canker in Emilia Romagna eight samples of 100 symptomless fruits each were collected. In two kiwifruit orchards (*A. delicosa* cv. Hayward and *A. chinensis* cv. Hort16A), not affected by *Psa*, two samples of 100 fruits were collected to serve as controls. *Psa* presence on the fruit surface and columella was checked by microbiological and molecular analysis. In all fruits samples harvested in orchards with bacterial canker symptoms, the isolation of epiphytic/endophytic bacteria excluded the presence of *Psa*, and this was confirmed by PCR analysis. Since the fruits tested were collected directly from the plant in the field, it can be assumed that no endophytic fruit colonization occurred in the orchard. Our results indicate that *Psa* is not able to colonize the fruits at harvest. Therefore it is very likely that the fruits of *Actinidia* spp. do not represent a risk of pathogen dissemination.

**MOLECULAR CHARACTERIZATION OF A FUSARIUM OXYSPORUM f. sp. GLADIOLI POPULATION FROM SAFFRON.** C. Moretti, M. Quaglia, C. Cappelli and R. Buonarrio. Dipartimento di Scienze Agrarie e Ambientali, Via Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: chiara.uce.moretti@unipg.it

Fusarium corm rot, caused by *Fusarium oxysporum* f. sp. *gladioli* (fogl), is the most important disease of saffron, causing severe yield losses in Italy. Sixty-two isolates of fogl collected from saffron corms in central Italy (Abruzzo and Umbria) and five isolates obtained from Spain were characterized by rep-PCR, using the primers BOX and REP. Isolates of *F. oxysporum* f.sp. *cyclaminis*, *lactucae*, *lycopersici*, *melongenae*, *melonis* and *radicis-lycopersici* and isolates of *F.avenaceum*, *F. equiseti*, *F. graminearum*, *F. proliferatum*, *F. sporotrichioides*, *F. venenatum* and *F. solani* were included in the analyses for comparison. Primers REP discriminated fogl, from the other species or formae speciales better than BOX. Cluster analysis, performed with the Dice’s coefficient, revealed a big cluster which includes 50 fogl (75%) and one *F. solani* isolates. Within this cluster, Spanish isolates were clearly separated in a sub-cluster. Our results confirm the suitability of rep-PCR for *Fusarium* species and formae speciales char-
Fungi of the genus *Trichoderma* have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments due to their ability to protect plants by containing pathogen populations, as well as increase plant growth and development under different soil conditions. Some *Trichoderma* spp. are well known producers of secondary metabolites: a heterogeneous group of chemically different natural compounds potentially related to survival functions of the producing organism, such as competition against other micro- and macroorganisms, symbiosis, metal transport, growth differentiation. The involvement of secondary metabolites in the ability of *Trichoderma* to activate plant defence mechanisms and regulate plant growth has recently been investigated and demonstrated. In this study, we have examined the metabolic changes in *Arabidopsis thaliana* treated with 6-pentyl-±-pyrone (6PP), harzianic acid (HA) and hydrophobin 1 (HYTRA1), three *Trichoderma* metabolites that were found to be involved in the induction of disease resistance and growth promotion, by using LC-ESI-QToF MS. The effects of treatments with these compounds (concentration 10-7 M) on *A. thaliana* growth were observed. Applications of 6PP, HA and HYTRA1 increased plant root-length by 48%, 51% and 40%, respectively compared to the untreated control (water). The *Trichoderma* metabolite treatments produced significant modifications in the plant metabolome by acting on specific pathways involved in plant defence (i.e. phytoalexin production). Moreover, rapid changes in the production of major hormones were observed. The results obtained allow a better understanding of the role of some metabolites in the important beneficial interactions of *Trichoderma* with the plant.
Agricultural residue-based compost-teas exhibit a broad spectrum in in vitro control of phytopathogenic fungi. C. Pane1, D. Villecco2, G. Celano2 and M. Zaccardelli3, CRA, Centro di Ricerca per l'Orticoltura, Via dei Cavallaggi 25, 84098 Pontecagnano, Italy. E-mail: catello.pane@enteca.it

Compost-tea is a compost-derived liquid formulate that can provide protection from many plant diseases. In the current study, five agricultural residues-based compost-teas were assayed for their suppressive ability against a wide range of plant pathogens, chosen among the most feared in horticulture. Teas were produced by a compost water-phase fermentation (1:5 vol.) carried out for a week with active aeration. Used compost feedstock were derived from three-month on-farm composting of the following agricultural residues: artichoke (C1); artichoke-fennel (C2); cauliflower (C3); sweet corn+cauliflower+lettuce (C4); tomato+fresh cut salads (C5). In addition, an animal waste anaerobic digestate (C6) and a commercial municipal waste compost (C7) were used. All compost-teas inhibited in vitro growth of Verticillium dahliae, F. oxysporum f. sp. lycopersici, Rhizoctonia solani, Sclerotinia minor, Sclerotium rolfsii and Botrytis cinerea. Filter and thermal sterilization of teas completely eliminated their suppressive ability. The inhibition of mycelial growth observed in raw teas without physical interaction between pathogen and compost-tea microbiota reveal an antibiotic-like antagonistic effect due to active microbes. Future prospective aim at testing the best ACTs as potential alternatives to the use of synthetic chemical fungicides for disease control in open field.

Integrated use of Brassica carinata tissues containing glucosinolates, essential oils and Bacillus spp. in biocontrol applications. C. Pane, D. Villecco and M. Zaccardelli. CRA, Centro di Ricerca per l'Orticoltura, Via dei Cavallaggi 25, 84098 Pontecagnano, Italy. E-mail: catello.pane@enteca.it

Biodiesel chain based on Brassica carinata oil extraction produces green by-products, such as exhausted seeds, with a high potential as biofumigant. They contain glucosinolates that can be transformed, by myrosinase-mediated hydrolysis, in isothiocyanates, which are very fungitoxic volatile molecules. Within the current research, an in vitro strategy was developed to evaluate the use of B. carinata meals as biofungicide, used in combination with plant essential oils exhibiting antimicrobial activity and with antagonistic strains belonging to Bacillus genera. The study was focused on three soil-borne pathogens: Rhizoctonia solani, Sclerotinia minor and Fusarium oxysporum f. sp. lycopersici. B. carinata seed meals showed a significant antifungal effect, in a dose-dependent manner, towards all the three filamentous fungi. The glucosinolates-rich tissues, moreover, enhanced antiseptic activity of essential oils, but synergism between two combined molecules were detected in few cases. Some antagonistic Bacillus strains, very promising for tested soil-borne pathogens control and showing tolerance to B. carinata seed meals and to essential oils, were selected. Results of combined use of these three components indicate that B. carinata seed meals, together to essential oils, improved the control ability of antagonists. Results suggest that B. carinata seed meal can be used in the formulation of low-impact multicompound fungicides, able to replace banned or unsustainable chemical molecules.

Search for natural and natural-like inhibitors of trichothecene biosynthesis by Fusarium. G. Pani1, V. Balmas1, B. Scherm1, A. Marcello1, D. Fabbri2, M.A. Dettori2, E. Azara2, A. Dessì2, R. Dallocchio2, G. Delogu2 and Q. Migheli1, 1Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Unità di Ricerca Istituto Nazionale di Biostruttura e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. 2Istituto di Chimica Biomolecolare del CNR, Traversa La Crucca 3, 07100 Sassari, Italy. E-mail: qmigheli@uniss.it

Fusarium culmorum is a major fungal pathogen of wheat, causing crown and foot rot (CFR) and Fusarium head blight (FHB). Yield losses occur for the grain becomes contaminated by mycotoxins. Among the most bioactive compounds, trichothecenes are able to inhibit eukaryotic protein synthesis and cause toxicoses to humans or animals consuming contaminated food or feed. Tri- chothecenes induce apoptosis and play an important role in the aggressiveness of phytopathogenic Fusarium species towards plant hosts. The aim of this project was to design, prepare and study new natural and natural-like compounds to be applied in the control of F. culmorum mycotoxin production. Particular attention was paid to the selection and preparation of compounds with selective trichothecene B inhibitory activity compared to compounds showing both mycotoxin inhibitory and fungitoxic activities. The first inhibition experiments were performed using compounds belonging to the family of gallic acid, phenyl-
propanoids and cinnamic derived acids. In vivo and in vitro tests and molecular modeling with computational studies were carried out. A straightforward thin layer chromatography (TLC) method and a quantitative LC-MS analysis were used to identify the presence of B trichothecenes and to evaluate the influence of each compound on different F. culmorum culture extracts. Preliminary results indicated that several molecules are able to inhibit the severity of F. culmorum in planta and its growth as well as trichothecene production in vitro. The level of inhibition of 3Ac-DON range from 67 to 100% under inducing conditions. Fast and effective methodologies for seed dressing were developed using a natural matrix.

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MOLECULAR CHARACTERIZATION OF ITALIAN ISOLATES OF ALFALFA MOSAIC VIRUS INFECTION LIPPA CITRIOIDORA. G. Parrella1, B. Greco1, G. Bozzano2, A. Crotti2, L. Cavicchi3 and M.G. Bellardi4, 1Istituto per la Protezione delle Piante del CNR, Via Università, 133, 80055 Portici, Italy. 2Società Cooperativa L’Ortofrutticola, Via Dalmazia 169, 17031 Albenga, Italy. 3Plesso Didattico G. Scarabelli, Università degli Studi di Bologna, Via G. Ascarì 17, 40026 Imola, Italy. 4Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fannin 42, 40127 Bologna, Italy. E-mail: maria-grazia.bellardi@unibo.it

Lippia citriodora (family Verbenaceae) is an aromatic plant with a strong lemon scent, native to South America. This species is known to be susceptible to Alfalfa mosaic virus (AMV), responsible for a bright yellow mosaic of the leaves, necrotic spots and stunting. In the last decade, AMV has been frequently found infecting L. citriodora crops in the Herb Garden of Casola-Valseno (Emilia-Romagna, northern Italy). To confirm AMV presence, symptomatic leaves collected from two crops in 2006 and again in 2011, were tested by PAS-ELISA. To determine the specific subgroup, IC-RT-PCR was performed, using a polyclonal serum against an AMV isolate from lettuce combined with two AMV-group, IC-RT-PCR was performed, using a polyclonal serum symptomatic leaves collected from two crops in 2006 and again in (Emilia-Romagna, northern Italy). To confirm AMV presence, CROTON VEIN YELLOWING VIRUS IS AN ISOLATE OF L. citriodora at different time the crops of stunting. In the last decade, AMV has been frequently found infecting L. citriodora

SEROLOGICAL AND MOLECULAR EVIDENCE THAT CROTON VEIN YELLOWING VIRUS IS AN ISOLATE OF EGGPLANT MOTTLED DWARF VIRUS. G. Parrella1, B. Greco1, A. De Stradis2, L. Cavicchi3 and M.G. Bellardi4, 1Istituto per la Protezione delle Piante del CNR, Via Università, 133, 80055 Portici, Italy. 2Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. 3Plesso Didattico G. Scarabelli, Università degli Studi di Bologna, Via G. Ascarì 17, 40026 Imola, Italy. 4Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fannin 42, 40127 Bologna, Italy. E-mail: parrella@ipp.cnr.it

In spring 1990, Croton vein yellowing virus (CVYV) was found for the first time in some dwarfed plants of croton (Codiaeum variegatum L.) cv. Fred Sander showing malformation and yellow or pink veins. CVYV was mechanically transmitted to Nicotiana glutinosa and Chenopodium amaranticolor and, by leaf-graft, to healthy croton plants in which it induced the same disease. After about twenty years from the first finding, CVYV has been serologically identified and molecularly characterized as an isolate of Eggplant mottled dwarf virus (EMDV). In electron microscopy of negatively stained crude extracts from croton, fixed in gluteraldehyde, typical bacilliform virus particles were observed which were decorated in immunoelectron microscopy tests by an antiserum to an Iranian EMDV isolate from potato. EMDV was also detected in the original croton plant by DAST-ELISA (Bioreba, Switzerland) and by RT-PCR using a virus-specific primer pair designed to amplify a ca. 900 bp fragment of the P1 polymerase gene. The amplified was cloned and custom sequenced (MWG, Germany) showing 86.5% identity with an EMDV eggplant isolate from Greece. From this serological and molecular study, it appears that CVYV can be definitively considered an isolate of EMDV. In the last decade, EMDV has frequently been detected in two common ornamental species in the Mediterranean area, Hibiscus rosa-sinensis and Pittosporum tobira. The present results provide experimental evidence that croton is a new natural host of EMDV.

BENEFICIAL EFFECTS OF TRICHODERMA AND ITS SECONDARY METABOLITES ON VITIS VINIFERA. A. Pascale1, F. Vinale2, M. Nigro1, M. Ruocco2, S.L. Woo1, S. Lanzuise3, R. Marra1, A. Eid4, R. Varlese1, V. Matteoli1, G. Manganiello1, D. Stellitano2 and M. Lorito1,2, 1Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici, Italy. 2Istituto di Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. E mail: lorito@unina.it

Trichoderma strains are among the most studied fungal biocontrol agents and are successfully used as biopesticides and biofertilizers in agriculture. The beneficial effects of these microorganisms on the plant are strain dependent and include: suppression of pathogens by using a variety of mechanisms, such as plant growth promotion; enhanced nutrient availability and uptake, and induction of systemic resistance. The biological activity of Trichoderma is supported by the ability to produce a variety of metabolites. Some of these molecules directly inhibit the growth of the pathogens, increase disease resistance by triggering defence mechanisms and enhance plant development. In this work, we have examined the effect of some Trichoderma strains and their secondary metabolites on Vitis vinifera in terms of: (i) induction of disease resistance; (ii) increased plant size; (iii) higher levels of polyphenols and antioxidant activity in the fruits. Application in vivo of a spore suspension of T. harzianum M10 or T. atroviride P1, as well as their purified metabolites harzianic acid (HA) and 6-pentyl-2-pyrones (6PP), promoted plant development and suppressed powdery mildew caused by Uncinula necator. Treatments with T. harzianum M10 and 6PP in field experiments improved crop yield and increased the total amount of polyphenols and antioxidant activity in the harvested grapes, as demonstrated by substantial changes in the chromatographic profile of polyphenols.
**Fusarium culmorum** is one of the most harmful causal agents of crown and foot rot (CFR) and *Fusarium* head blight (FHB) diseases of durum wheat. Significant crop losses are caused by contamination of grain by mycotoxins, primarily due to type B trichothecenes. We have now identified and characterised the main function of *F. culmorum* gene, an APSES protein having 99% homology with the *F. graminearum* FgstuA protein, in the biological cycle of *F. culmorum*. Two wild-type strains, namely FCUK99 (a deoxynivalenol producer) and FC233B (unable to produce toxin in vitro) were used for *F. culmorum* deletion by homologous recombination. The functional analysis of deletion mutants obtained from both wild-type strains showed that *F. stu*A mutants had no monophialidic asexual conidia, and a decreased germination efficiency of conidia, stunted vegetative growth and altered pigmentaion on solid substrates, loss of hydrophobicity of the mycelium, higher sensitivity to oxidative stress, decreased production of pectolytic enzymes during infection and colonisation of the host. Fungicide sensitivity was unaltered compared with the respective ectopic transformants and wild-type strains. Toxin production by mutants derived from strain FCUK99 was significantly decreased in vitro (20 times factor). Furthermore, *F. stu*A was shown to play an important role in pathogenicity, for *F. stu*A mutants underwent complete loss of pathogenicity in both CFR and FHB pathosystems on durum wheat and were unable to colonise different plant tissues (apple, potato and tomato). It is argued that possible mechanisms determining *F. stu*A inability to cause crown rot in wheat include increased sensitivity to oxidative stress and delayed spore germination.

The first two authors have contributed equally to the present work. Work funded by Regione Autonoma della Sardegna (Legge Regionale 7 agosto 2007, n. 7 "Promozione della ricerca scientifica e dell’innovazione tecnologica in Sardegna").

**CERATO-PLATANIN AND CERATO-POLUPULIN INDUCE MAPK ACTIVATION IN PLANE AND ARABIDOPSIS LEAVES. L. Pazzaglì1, S. Lutti2, L. Lombardi3, I. Baccelli4, R. Bernardi2, P. Picciarelì2, F. Martellini1 and A. Scala1. 1Dipartimento di Scienze Biochimiche, Università degli Studi, Viale Mor- gagni 50, 50134 Firenze, Italy. 2Dipartimento di Biologia delle Piante Agrarie, Università degli Studi, Via della Piagge 23, 56124 Pisa, Italy. 3Dipartimento di Biotecnologie Agrarie, Università degli Studi di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino, Italy. E-mail: luiga.pazzaglì@unifi.it**

Cerato-platanin (CP) and cerato-polupulin (Pop1) are small proteins produced by the phytopathogenic fungi *Ceratocystis platani* and *C. populicola*, respectively. CP and Pop1 behave as PAMPs, since they elicit typical defense responses in various host and non-host plants such as the induction of synthesis of phytoalexins and of some defence related genes. CP structure has been solved by NMR and consists in a double Ψ-β-barrel protein. Pop1 has a similar fold but circular dichroism shows differences in the secondary structure between the two proteins. In order to better characterize the PAMP activity of these proteins we first assayed: (i) the production of hydrogen peroxide and nitric oxide; (ii) the viability of cells; (iii) the expression of the genes PKS (thiamatin), LTP (lipid transfer protein) and APX (ascorbate peroxidase). Results indicate that either CP and Pop1 induce synthesis of H$_2$O$_2$, NO and hypersensitive cell death with apoptotic features which are hallmark of successful recognition of infection and activation of plant defences. Recently, the activation of MAPKs has been detected in Arabidopsis and plane leaves treated with 1.5 nmol/10 µl of CP and Pop1. After 15 min and 1, 3, 6, 12, 24 and 48 h of incubation, leaves were subjected to protein extraction, and sample were analyzed by Western blot using anti-erk1/2 human antibodies. Preliminary results show the activation of MAPK 3/6 after 1-12 h of incubation. Therefore, another marker of defence response was shown to be activated by CP and Pop1 in specific and non-specific hosts.

**HIGH-THROUGHPUT SEQUENCING AND ANALYSIS OF THE TRANSCRIPTOME FROM DEFOLIATING AND NON-DEFOLIATING PATHOTYPES OF VERTICILLIUM DABLAEAE. I. Pentimone1, M. Ferrara1, J. Mercado-Blanco2 and F. Nigro1. 1Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. 2Departamento de Protección de Cultivos, Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas, Avenida. Menéndez Pidal s/n, Campus Alameda del Obispo, Apartado 4084, 14080 Córdoba, Spain. E-mail: franco.nigro@uniba.it**

A deep sequencing transcriptome analysis of *Verticillium dahliae* has been carried out by comparing the expression profile of two different isolates, previously characterized as defoliating (V9371) (D) and non-defoliating (V111) (ND) pathotypes in olive and cotton. Total RNA was extracted from mycelium grown in a synthetic simulated xylem medium and the corresponding expression libraries were constructed. The high-throughput sequencing of the libraries was performed on a HiScanSQ Illumina platform. Data were analyzed using CLC Genomics Workbench v5.1. A total of 68,152,130 reads were mapped on the reference genome of *V. dahliae* (http://www.broadinstitute.org/). Relying on the normalized gene signals, the average fold-change between the D and ND pathotype was assessed for 10,535 genes. A list of 502 genes differentially expressed was obtained by fixing a fold change (FC) cut-off at FC≥10 and ≤0.05. Among these, 48 genes were differentially expressed at a significant (p≤0.05) level between the two pathotypes. Up-regulated genes (47%) in the ND isolate referred to mitochondrial proteins, endonuclease/exonuclease/phosphatase family proteins, carboxypeptidase and permease, while the remaining 53% were identified as hypothetical proteins. By contrast, among the up-regulated genes of the D isolate, 18% were related to monoxygenase, kinase and transmembrane proteins, while 82% were identified as hypothetical proteins. Moreover, six and three genes were exclusively expressed in the ND and D isolate, respectively. Results confirmed the suitability of high-throughput sequencing for transcriptomics analysis of *V. dahliae* under axenic and nutritional-defined conditions. Further data will contribute to unravel the different lifestyle of the two *V. dahliae* pathotypes.

**Work carried out in the framework of the Project No. 14 “Laboratory network for the selection, characterization and conservation of germplasm and for preventing the spread of economically-relevant and quarantine pests (SELGE)”, founded by the Apulia Region.**
TEMPORAL VARIATIONS IN THE CUCURBIT POWDER MILDEW SPECIES COMPOSITION IN NORTHERN ITALY. A. Pirondi, I. Portillo, M. Collina and A. Brunelli. Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale Fanini 46, 40127 Bologna, Italy. E-mail: alessandro.pirondi@unibo.it

Cucurbit powdery mildew is a widespread disease that causes important losses to cucurbit crops. Observations on the occurrence of *Podosphaera fusca* and *Golovinomyces cichoracearum* were conducted in the years 2010 and 2011 in Bologna and Mantova provinces (northern Italy) to determine the epidemiology and etiology of powdery mildew in these important cucurbit-growing areas. Samples of infected leaves of zucchini, melon, cucumber, pumpkin and watermelon from field and greenhouse crops, were collected every two weeks from May to October. To distinguish the two fungal species, morphological observations of conidia germ tubes, presence/absence of fibrosin bodies in 3% KOH and observation of chlamydothecia were conducted. Molecular identification of both pathogens was carried out by multiplex PCR, using species-specific primers designed on the ITS region of both species and performed on a DNA pool isolated from infected leaves. The preliminary results show that the earlier infections are by *G. cichoracearum* that seems to be the predominant species till mid July when it progressively disappears and *P. fusca* becomes the main species infecting cucurbits till the end of October. Only chlamydothecia of *P. fusca* were collected in October-November. This peculiar behavior suggests a seasonal alternance in the cucurbit powdery mildew species composition that could be explained both by the different climatic requirements of the two species and by their different overwintering strategies. Further investigations are in progress to confirm these results.

MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF *FUSARIUM OXYSPORUM* ISOLATED FROM CHICORY. A. Poli, G. Gilardi, D. Spadaro, M.L. Gullino and A. Garibaldi. AGRONNOVA-Centro di Competenza per l’Innovazione in Campo Agroambientale, Università degli Studi di Torino, Via L. da Vinci 44, Grugliasco, Italy. E-mail: davide.spadaro@unito.it

Chicory (*Cichorium intybus*) is a wild perennial plant belonging to the family Asteraceae, which has been selected, domesticated, and cultivated in Europe. In summer 2009, wilt symptoms, including chlorosis and poor development of the root system were observed on chicory cultivars in northern Italy. The causal agent isolated from symptomatic tissues was identified as *F. oxysporum* on the basis of morphological features and molecular analyses. In this work, we characterized the isolates of *F. oxysporum* from *C. intybus* both with biological and molecular approaches. Pathogenicity trials performed on five species of family Asteraceae with isolates of *F. oxysporum* from chicory were identical to one another. On the basis of these results we propose to designate this organism as *Fusarium oxysporum* f. sp. *cicorii*.

DETECTION OF BOIS NOIR PHYTOPLASMA IN GRAPEVINE ROOTS BY REVERSE TRANSCRIPTION-REAL TIME TAQMAN ASSAYS. R. Polizzotto1, F. De Marco1, S. Palmaro2, S. Santi2 and R. Musetti2. 1Dipartimento di Scienze Agroeur e Ambientali, Università di Udine, Via delle Scienze 208, 33100 Udine, Italy. 2Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: rachele.polizzotto@uniud.it

Bois noir (BN) is a phytoplasma disease of grapevine spread in the Mediterranean basin, causing relevant economic losses. BN phytoplasma diagnosis is currently carried out by detecting the pathogen DNA sequences in the leaf. Even if reliable and usable in routine analyses, molecular diagnosis is nevertheless restricted to the late summer, when BN symptoms become evident. This is because the above methods are not enough sensitive to carry out diagnosis in other periods of the year, when symptoms are not evident, or on other plant organs such as roots. This consideration suggests that more sensitive and focused detection methods for BN would be needed. Because of the lack of diagnostic methods for root tissues, it is not known where the pathogen overwinters inside the host. We aim at expanding diagnosis effectiveness to root tissues in BN-infected grapevine, to better understand phytoplasma/grapevine interactions and to give new insights to BN epidemiology. Different root samples from healthy, BN-infected and recovered grapevines, have been collected and RNA was extracted. As the low concentration of template in the host, we performed a nested Real-Time PCR using a BN specific TaqMan probe, following the method already described by Margaria et al. (Plant Pathol. 58:838-845, 2009). Preliminary analyses showed positive signal in roots of symptomatic and recovered plants, whereas no amplification was observed in healthy samples. This result suggests that BN phytoplasma persists in the root phloem tissues of recovered individuals. The epidemiological significance of this finding will be discussed.

APPLICATION OF NEXT-GENERATION SEQUENCING FOR TRANSCRIPTOMICS STUDIES IN PHYTOPATHOGENIC FUNGI. S. Pollastro, R.M. De Miccoli Angelini, D. Gerin, C. Roto1, M. Masiello, M. Miazzi, A. Santomauro, M. Ferrara, L. Pentimone, F. Fallanaj, S.M. Sanzani, A. Ippolito, F. Nigro and F. Faretra. Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: stefania.pollastro@agr.uniba.it

In the framework of Project No. 14 “Laboratory network for the selection, characterization and conservation of germplasm and for preventing the spread of economically-relevant and quarantine pests (SELGE)”, founded by the Apulia Region, PO FESR 2007-2013, an Illumina HiScan Seq platform was acquired. The next generation sequencing technologies (NGS) can be applied to improve knowledge in several contexts, such as genotyping, whole-genome sequencing, targeted resequencing, gene expression, discovery of transcription factor binding sites, noncoding RNA expression profiling, etc. With particular regard to transcriptomics, the application of NGS, commonly RNA-seq, allows the nearly complete characterization of transcriptomic events occurring in a specific biological system. Quantitative and qualitative differences in gene expression are detected by comparing multiple mRNA populations, and the understanding of fungal plant diseases can be improved. The analysis of gene expression during infection, together with expression profiling in a multitude of different in vivo and in vitro growth conditions can greatly enhance the understanding of fungal pathogenicity. The Illumi-
na NGS was applied to gain insight into a wide range of transcriptional events related to: (i) plant-pathogen interaction (i.e. grapevine-Phomopsis viticola); (ii) morphogenesis and development in Botryotinia fuckeliana, with particular regard to mycelial growth, sclerotia differentiation, occurrence of the sexual process, and ascospore development; (iii) synthesis of ochratoxin A in Aspergillus carbonarius grown under inductive and non-inductive condition; (iv) defoliating and non-defoliating pathotypes of Verticillium dahliae growing in a synthetic simulated xylem medium; (v) induced resistance to postharvest decay in oranges treated with electrolyzed water.

**PRODUCTION OF CERTIFIED POME FRUIT PROPAGATION MATERIAL: NEED FOR SOILS FREE FROM PSEUDOMONAS SYRINGAE pv. SYRINGAE. N. Pucci, A. Gallelli and S. Loreti. CRA, Centro di Ricerca per la Patologia Vegetale, Via C. G. Bertero 22, 00156 Roma, Italy. E-mail: nicoletta.pucci@entecra.it**

The Italian Ministry of Agriculture, based on EU indications to harmonize the marketing of the propagation material of fruit, ornamental and horticultural species, published in 2006 the “Technical guides for the production of certified fruit plant propagation materials”. In this document it is outlined that the production of propagation material of pome fruit tree has to be performed on soils free from several fungal pathogens and from Pseudomonas syringae pv. syringae van Hall (Pss), causal agent of bacterial canker and blast of pome fruit trees, but also a polyphagous pathogen, well adapted to an epiphytic phase. Present, no suitable protocol for the detection of Pss from soil is available, and the molecular detection is complicated by the high genomic variability of Pss. Within the frame of the ARNADIA project, in 2011 we screened for the presence of Pss 8 soil samples collected from areas suited to pome fruit cultivation (Northern Italy, Val d’Isarco, BZ) that were previously interested by severe outbreak of the disease. Subsamples of 20 g were shaken with sterile extraction buffer for 30 min. Isolation performed on KB and DL-lactate media gave fluorescent colonies, which were transferred onto NSA medium to check levan-positive morphology. Two levan-positive isolates were tested for the presence of syrB gene and hypersensitive tobacco response, but the result was negative. These results, together with the lack of evidence in the literature on the recovery of Pss from soil of pome fruit cultivation, raises some doubts about the usefulness of testing the presence of Pss in soil, even for the production of certified propagation material.

**EFFECT OF SILICATES FROM INDUSTRIAL WASTES ON POWDERY MILDEW OF ZUCCHINI. M. Pugliese, M.T. Moreno Alvarez, D. Spadaro, 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, 10095 Grugliasco. E-mail: massimo.pugliese@unito.it**

Silicon is the second most abundant element on the earth’s surface and its use can stimulate natural defense mechanisms in plants. The effect of silicate from industrial wastes against powdery mildew on zucchini (Cucurbita pepo) was evaluated under greenhouse conditions. Potted plants were inoculated with a spore suspension containing $1 \times 10^9$ CFU/ml. The following treatments were carried out, 3 and 10 days before pathogen inoculation. Treatments: (i) control; (ii) Cu-based fungicide (propiconazole, TILT 25 EC, Syngenta); (iii) Bacillus subtilis (250 g/l, Serenade, Intrachem); (iv) 1% and 0.1% sodium silicate (r=1, granular formulation); (v) tap water as control. Disease incidence and severity were assessed 7, 14 and 21 days after pathogen inoculation. The application of 1% and 0.1% sodium silicate significantly reduced the powdery mildew and increased above-ground biomass of plants at a level similar to chemical control. The other treatments, including Bacillus subtilis, reduced disease severity compared to water control, but were less efficient than chemical. The use of silicates from industries is a valid alternative for the control of powdery mildew on zucchini, in particular in organic farming. However, silicates might not be sufficient at higher disease incidence levels, and their use is more suitable within an integrated disease control strategy.

**CONCENTRATED EXTRACTS OF VEGETATION WASTE WATER FROM OLIVE MILLING AGAINST PHYTOPATHOGENIC OOMYCETES AND FUNGI. M. Quaglia1, G. Cappelletti1, S. Urbani2 and A. Taticchi2. 1Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. 2Dipartimento di Scienze Economico-Estimative e degli Alimenti, Università degli Studi, Via San Costanzo, 06121 Perugia, Italy. E-mail: mara.quaglia@unipg.it**

Annually, about 10 million tons of olives, mostly used for oil production, are collected in the Mediterranean basin. An average of 10 million m$^3$ of waste, mainly as vegetation waters, result from the oil extraction processes. The olive mill wastewaters (OMW) have disposal problems due to their high content in organic matter and in phenols. These last compounds also confer antimicrobial activity to OMW and to OMW-derived products, suggesting their possible use in pest management. Here, a phenolic concentrate, obtained from OMW (CEOMW) through a membrane filtration process, was characterized by HPLC-DAD. The effect of CEOMW, used at different doses expressed as mg ml$^{-1}$ of phenols, was tested in vitro against phytopathogenic oomycetes and fungi. Compared to the untreated controls, CEOMW significantly reduced the number of Penicillium expansum colonies at the doses of 0.25 to 4 mg ml$^{-1}$, the mycelial growth of Phytophthora infestans (2 and 4 mg ml$^{-1}$) and of Alternaria sp., Fusarium oxysporum f. sp. lycopersici and Monilia sp. (4 mg ml$^{-1}$). Instead, at the lowest doses (from 0.125 to 2 mg ml$^{-1}$), CEOMW significantly increased the mycelial growth of the last three fungi. The mycelial growth of Botrytis cinerea was not affected by CEOMW at all the tested doses (from 0.125 to 4 mg ml$^{-1}$). On apple fruits, CEOMW (4 mg ml$^{-1}$) was not able to contain $P.$ expansum infection, both when used for its antimicrobial activity and when used as resistance inducer. The inability to induce resistance has been also verified through analysis of PR-1a gene transcripts.

**BLACK ROT OF GRAPEVINE IN ITALY: EPIDEMIOLOGICAL OBSERVATIONS. P. Rinaldi1, M. Skaventzou1, M. Rossi1, C. Comparini1, D. Molitor1 and L. Mognini1. 1Dipartimento di Biotecnologie Agrarie, Sezione Protezione delle Piante, Piazzale delle Cascine 18, 50144 Firenze, Italy. 2Department Environment and Agro-Biotechnologies, Centre de Recherche Public Gabriel Lippmann, 41 Rue du Brill, 4422 Belvaux, Luxembourg. E-mail: pietroantonello.rinaldi@unifi.it**

In the last 5 years several countries in Europe, such as Germany, Hungary, Portugal, experienced a sudden and sometimes strong increase in the incidence and severity of damages on grapevine caused by black rot (Gniigartia bidwillii). This ascomycetous fungus can cause a full drying out of the cluster, so
that berries typically mummify. Heavy damages were recorded in 2010 and even more in 2011, leading up to 100% crop losses, also in Tuscany, a region where the disease had never caused losses before. In 2012, symptom development was then carefully monitored in two vineyard located in the surroundings of Florence, Tuscany, assessing the relationship between infection events and meteorological data, in order to understand the factors affecting infection process in the Mediterranean climate. Field observations showed that primary infections are almost exclusively given by ascospores produced in overwintering perithecia, while conidial infections seem to have a minor role. Infections started even at bud burst, requesting the need of protection with chemical much earlier than it is done for downy mildew. First field surveys and observations suggest that leaf infections could be not related to the disease incidence and to the severity of the infections on the berries. Nevertheless, heavy infections on grape organs other than leaves and berries were recorded in some vineyards before flowering, differently from what reported elsewhere. Cankers were formed on the young shoots, the leaf petiols and the cluster rachis, causing a partial crop loss. The biology and epidemiology of the pathogen in the Mediterranean region - with unusual climatic conditions for this fungus - need to be fully clarified.

LOOKING FOR NEW MOLECULAR AND CELLULAR TARGETS FOR CHV1 MYCOVIRUS INFECTION OF CRYPHONECCTRIA PARASITICA, THE ASCOMYCETOUS FUNGUS CAUSAL AGENT OF CHESTNUT BLIGHT. M. Rossi, S. Abbà, M. Moretti and M. Turina. Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: m.rossi@ivv.cnr.it

Cryphonectria parasitica is the causal agent of chestnut blight, a chestnut tree disease controlled by the widespread presence of mycovirus-containing hypovirulent strains. CHV1 infection was shown to have molecular pleiotropic effects, but a number of questions still remain open, such as the specific nature of the viruses hijacked for its replication and what is the specific fungal molecular target that results in hypovirulence. The biological function(s) of cpkk1, cpkk2 and cpkk3 genes, encoding three mitogen-activated protein kinase kinases (MEKs) of Cryphonectria parasitica were examined through specific knock-out strains. Our analyses confirmed that each MEKs to belong to the proper signalling cascade with typical defects in the null mutants already identified for the homologues of phylogenetically related filamentous fungi. Virulence on chestnut cuttings was only affected in Δcpkk1 and Δcpkk2 mutants. A successful CHV1 infection through natural anastomosis with a virus-donor line was obtained in Δcpkk1 and Δcpkk3 with common symptoms associated to hypovirus infection. Surprisingly, on the contrary, no infection was possible in Δcpkk2 by anastomosis or transformation with an infectious clone of CHV1, suggesting its important role for maintaining a proper cellular environment for virus replication. For this reason a proteomic approach was established to further characterize the specific perturbation present in Δcpkk2. Currently we are evaluating other possible cellular targets of CHV1 infection, such as the components of the autophagy pathway and the role of a putative GipC-like protein in C. parasitica biology, which is specific to the kingdom Mycota and strongly upregulated in Δcpkk2.

INVENTORY OF PLANT ABC TRANSPORTERS POTENTIALLY INVOLVED IN PLANT-MICROBE INTERACTIONS. M. Ruocco1, R. Marotta2, G. Andolfo3, S.L. Woo4, M. Lorito5, F. Scala2 and M.R. Ercolano2. 1Istituto per la Protezione delle Piante del CNR, Via Università 133, 80053 Portici, Italy. 2Dipartimento del Suolo, della Pianta, dell’Ambiente e Scienze delle Produzioni Animali, Università degli Studi di Napoli Federico II, Via Università 100, 80053 Portici (NA), Italy. 3Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università 100, 80053 Portici, Italy. E-mail: ruocco@ipp.cnr.it

The ATP-binding cassette (ABC) transporter superfamily consists of a large group of proteins whose members participate directly in the bidirectional transport of a wide range of molecules across membranes. Plants have a number of ABC proteins, which are associated with a number of processes such as xenobiotic detoxification, disease resistance, transport of phytohormones, primary and secondary products, lipid catabolism, heavy metal tolerance, organoleptic and nutritional qualities of food. In Arabidopsis thaliana 132 ABC proteins have been identified via sequence similarity to known ABC transporters in other organisms. Recently, we cloned four pleiotropic drug resistance (PDR) transporter genes from Solanum tuberosum and studied their function during infection by Phytophthora infestans. The growing availability of sequence data offers the possibility to conduct studies about ABC genes to produce additional information on their genetic relationships, identification of variants and of new functional copies. In the present work, we analyzed and annotated new ABC transporters from four plant species (Solanum esculentum, Solanum tuberosum, Oryza sativa and Vitis vinifera) and produced a detailed inventory. Genomes of the various species differed significantly in the number of ABC proteins, which is especially expanded in S. tuberosum. The obtained data will contribute to a better understanding of the diversity of the plant ABC proteins providing clues to prioritize species-specific proteins for further plant-microbe interaction analysis.

UNUSUAL OCCURRENCE OF SCHIZOPHYLLUM COMMUNE ON YOUNG TOPGRAFTED CITRUS. M. Russo1, G. Liciardello3 and P. Bella2. 1Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Blocco Palma I, Via V. Lancia, 95121 Catania, Italy. 2Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli Studi, Via Santa Sofia 100, 95123 Catania. Italy. E-mail: mrusso@pstsicilia.it

Many fungal organisms colonize dead wood of old or wounded citrus and are morphologically detected by the large sporocarps they produce. Among them are some species of the genera Daldinia, Ganoderma, Polyporus, Stereum, Fomes and Schizophyllum commune as well. Here we report about a severe collapse of living citrus associated to rootstock infection by S. commune. These symptoms were observed in a 15 year-old Fortune Mandarin on Troyer citrange, spaced 3 m by 4 m. Failed topgrafted trees started to show vertical cracking of bark and discoloration of the wood within six months. In cross sections of the trunk the area of affected wood was usually confined to a section. An abundant production of basidiocarps of S. commune were also observed on the rootstock. Direct DNA extraction from lyophilized fruit bodies was carried out. ITSII gene region was amplified using specific Schizophyllum spp. primers pairs Schi2R/ITIS2 revealing the presence of the expected size amplicon. S. commune is a sap-rot basidiomycete and cosmopolitan species, reported to be a pathogen of humans and trees. Usually it adapts a saprobic lifestyle by causing white rot. In specific conditions it behaves as plant pathogen of living trees and many plant species, including fruit and ornamental trees. To our knowledge, this is the first report of S. commune causing disease on living citrus in Italy.
**THE BIOLOGY OF ARTICHOKE ITALIAN LATENT VIRUS IN NICOITANA PLANTS: IS RECOVERY A STATE OF APPARENT QUIET?** E. Santovito1, T. Masca2, and D. Galtelli1. 1Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Via Amendola 163/A, 70126 Bari, Italy. 2Istituto di Vegetologia del CNR, UOS Bari, Via Amendola 163/A, 70126 Bari, Italy. E-mail: elisa.santovito@uniba.it

Artichoke Italian latent virus (AILV) in a nepovirus listed among the ten most detrimental plant viruses for globe artichoke production. Like other nepoviruses, AILV genome consists of two ssRNA molecules encapsidated separately in virus particles and both necessary for infectivity. Starting from the observation that AILV invades shoot apical meristem and is transmitted through seeds we studied the AILV infection pathway in tobacco. Plants recover from viral symptoms by 21 days post-inoculation (dpi) although the virus is still present and infectious in recovered tissues. This suggests that AILV ability to interfere with RNA silencing (RS) is rather weak. The agropatch biosays on plants expressing GFP showed that the virus seems to be able to interfere with cell-to-cell movement of the silencing signal rather than with its long-distance movement. Followings the dynamic of virus accumulation we also estimated that the threshold for triggering plant RS is reached by 14 dpi in the first systemically infected leaf and by 19 dpi in newly emerged leaves. After reaching the threshold, virus accumulation/replication is dramatically reduced but not abolished and new vegetation undergoes recovery. Virus titre never rises again up to 60 dpi. Changes of expression level of plant key genes, involved in RS were also studied. On the whole our results provide circumstantial evidence that AILV does not possess a suppressor of systemic silencing signal but seems able to interfere with its cell-to-cell spread.

**TRICHODERMA GAmti 6085 AS BIOCONTROL AGENT OF FUSARIUM HEAD BLIGHT IN THE FIELD. S. Sarrocco1, L. Moncini2, G. Pachetti2, A. Moretti2, A. Riteni2 and G. Vannacci2. 1Dipartimento di Coltivazione e Difeza delle Specie Legnose G. Saramuzzo, Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 54, 36124 Pisa, Italy. 2Centro Ricerche Strumenti Biotecnici nel Settore Agricolo-forestale, c/o IRIS Leopoldo II di Lorena, Cittadella dello Studente, 38100 Grosseto, Italy. E-mail: g.vannacci@agr.unipi.it

Fusarium head blight (FHB) represents one of the most economically devastating disease of wheat, causing significant reduction of grain yield and quality. Among fungal species associated with FHB, *Fusarium graminearum* and *F. culmorum* are the most prevalent. Along with crop losses, contamination of grain with mycotoxins, particularly trichothecenes, is a very serious consequence of FHB. Different strategies are used to control FHB, including fungicides and resistant cultivars, but none of them are able to reduce the impact of the disease. Since biological control offers an additional strategy, aim of this work was to use the antagonistic strain *Trichoderma gamsii* 6085, alone and in combination with the antagonist *Pythium* sp. SC-1-14a as inoculants of wheat, to test biocontrol efficacy against FHB under field conditions during the 2010-2011 season. Antagonistic strains were inoculated in soil, alone and in combination or, as for *T. gamsii* 6085, on spikes during anthesis. Statistical analysis of Disease Incidence, Disease Severity, FHB Index and productivity indicates that both antagonists reduce FHB and increase the 100 seeds weight. After harvesting, presence of mycotoxins was measured on wheat kernels. The very low amount of trichothecenes in all treatments did not allow us to speculate on a potential ability to reduce mycotoxins contamination by our antagonists. Anyway, the interesting disease control effects of both *Trichoderma* and *Pythium* open the possibility to use our *Trichoderma* strain, also in combination with other organisms, in order to develop a multi-trophic approach in the biocontrol of FHB.

**IDENTIFICATION OF ASPERGILLI ISOLATED FROM DRIED WINE FRUITS FOR THE PRODUCTION OF OTA IN SANTO.** A. Scala1, N. Parisi2, C. Comparini2, C. Fanelli2, M. Reverberi2 and A. Ricelli1. 1Dipartimento di Biotecnologie Agrarie, Università degli Studi, Piazzale delle Cascine 18, 50144 Firenze, Italy. 2Dipartimento di Biologia Vegetale, Università degli Studi La Sapienza, Largo Cristina di Svezia 24, 00163 Roma, Italy. E-mail: annel.lo.scafo@unifi.it

Aspergillus carbonarius, *A. niger* and *A. tubingensis* are known to produce ochratoxin A (OTA), a secondary metabolite with very dangerous effects in animals and in humans. The International Agency for Research on Cancer has classified OTA as a possible carcinogen to humans (group 2B). As a consequence, the European Commission has imposed regulatory limits for the maximum tolerable presence of this toxin in different foodstuffs. After cereals, grape products are accounted as a considerable source of human OTA intake. Based on the fungal requirements of environmental temperature and relative humidity, it is easy to suppose that wines, as the Tuscan “Vin Santo”, obtained from grapes partially dried for several months to concentrate sugar content to at least 30% (w/v), are potentially at risk more than table wines. In the present work, performed in the farm “Azienda Agricola Montepaldi srl” (San Casciano Val di Pesa, Florence), we isolated from grape berries about 6x107 colony-forming units divided into six major fungal morphotypes. Among those were 24 typical colonies of *Aspergillus* spp. These colonies were processed in parallel using: (i) conventional culture methods that allowed their morphological description; (ii) a molecular approach based on the sequencing of the internal transcribed spacer (ITS) region. All the Aspergilli seem to belong to the species *A. tubingensis*. The sequencing of the β-tubulin and calmodulin genes is in progress. We also report the amount of OTA produced *in vitro* by these 24 isolates.

**EFFECTS OF FUNGAL SECONDARY METABOLITES ON IN VITRO GROWTH OF PHYTOPHthora spp.** B. Scana1, B.T. Linaldedda1, A. Franceschini2, A. Evidente2 and L. Maddau1. 1Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. 2Dipartimento di Scienze del Suolo, della Pianta, dell’Ambiente e delle Produzioni Animali, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. E-mail: lmaddau@unisrs.it

The genus *Phytophthora* De Bary includes some of the most important pathogens causing disease to agricultural, forest, ornamental, and natural systems world-wide. Within the disease management strategies, there is a need for new effective compounds owing to the development of fungicide resistance by pathogens and adverse effects on environmental ecosystems. Microbial bioactive secondary metabolites, which have historically been of great importance in medicine and agriculture, could be expected...
to overcome these critical points. In this study, seven bioactive metabolites produced by phytopathogenic fungi and/or biocontrol agents were examined against several *Phytophthora* species isolated from horticultural and forest ecosystems in Sardinia (Italy). The effects of these metabolites were assessed *in vitro* on mycelial growth. The fungicide metalaxyl-M (48% active ingredient) was used as positive control. All metabolites were assayed at increasing concentrations and EC$_{50}$ values were evaluated. Interestingly, mycelial growth inhibition was observed, with sphacelo-sidin A, which showed to be the most active metabolites. The EC$_{50}$ for sphacelo-sidin A was ranging 1-10 µg/ml depending on the *Phytophthora* species, and it was tenfold higher than that of metalaxyl-M in some species. The EC$_{50}$ for the other metabolites tested exceeded 100 µg/ml. Although the chemical control of these pathogens is impractical in natural ecosystems, our results may provide a basis for the development of new compounds that may have useful applications in diseases control caused by *Phytophthora* spp. in agriculture and nurseries.

**FIRST DETECTION OF LEAF AND TWIG BLIGHT OF ILEX AQUIFOLIUM IN ITALY CAUSED BY PHYTOPHTHORA ILICIS.** B. Scano, B.T. Linaldeddu, L. Maddau and A. Franceschini. Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: bcsano@units.it

*Illex aquifolium* is an evergreen tree or shrub native to Europe and western Asia. In Sardinia this species grows exclusively inland between 400 and 1500 m a.s.l., particularly in the Gennargentu area where it forms pure and ancient forests. In 2010, severe defoliation and dieback were observed on *Ilex aquifolium* trees located along a water course. Close inspection revealed the occurrence of leaf black spots and twig blight, especially on the lower parts of the canopy. Large limb and trunk cankers also occurred, often girdling the stem and resulting in death of the terminal portion. Culturing from margins of infected tissues on *Phytophthora* selective medium consistently yielded a *Phytophthora* species. Based on morphology and growth rate at 20°C and 25°C on carrot agar (CA), it was identified as *Phytophthora ilicis*. Identity was confirmed by sequencing of ITS rDNA regions. One of these isolates was pathogenic to *Ilex aquifolium* in the Mediterranean basin.

**PHYTOPHTHORA SPECIES ASSOCIATED WITH CORK OAK DECLINE IN SARDINIA.** S. Seddaiu, B. Scano, B.T. Linaldeddu, C. Sechi and A. Franceschini.* Dipartimento della Ricerca per il Sogno e la Silecultura, Agris Sardegna, Via Limbara 9, 07029 Tempio Pausania, Italy. Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: bcsano@units.it

*Quercus suber* is an evergreen tree native to the Mediterranean basin with a great economic and ecological value. In Italy, 87% of the total cork oak forests is located in Sardinia with nearly 85,000 ha. Since 2011, during a large scale survey on the occurrence of *Phytophthora* species in Sardinian forest ecosystems, three cork oak stands have been investigated. Rhizosphere soil samples were collected from ten declining cork oak trees and bailed using oak leaflets. Necrotic leaves were then planted onto a *Phytophthora* selective medium and pure cultures grown on carrot agar. Based on morphology and growth rate, isolates were identified as *Phytophthora cinnamomoni* and *Phytophthora gonapodyides*. Identity was confirmed by sequencing of ITS rDNA regions. One *Phytophthora* isolate is still under identification since its morphological and molecular properties did not match any formally described species or informally designated taxon. *P. cinnamomoni* was the most frequently isolated species, while *P. gonapodyides* was isolated at one site from one cork oak tree. Koch’s postulates for *P. cinnamomoni* and *P. gonapodyides* were met by wound inoculation of freshly cut logs of *Q. suber* and soil infestation of six-months old *Q. suber* seedlings. Both *Phytophthora* species demonstrated to be pathogenic towards *Q. suber*, with *P. cin- namomoni* being the most aggressive species with both methods used. To our knowledge this is the first report of *P. gonapodyides* on *Q. suber*. This pathogen has been recently associated with the decline of *Quercus ilex* in xeric conditions in Spain.

**A PECTIN METHYL ESTERASE IS A VIRULENCE FACTOR OF FUSARIUM GRAMINEARUM WHEN IT INFECTS COMMON AND DURUM WHEAT.** L. Sella, K. Gazzetti, M. Janni, C. Volpi, W. Schäfer, R. D’Ovidio and F. Favaron. 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. Biocenter Klein Flottbek, Molecular Phytopathology and Genetics, University of Hamburg, Hamburg, Germany. E-mail: francesco.favaron@uni-pd.it

Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most dangerous disease of wheat crops worldwide. Trichothecene toxins are virulence factors during spike infection and degradation of plant cell wall components are visible at early stages of infection. In order to understand the contribution of cell wall degrading enzymes (CWDEs) to virulence, we are following two approaches: the production of deletion mutants of some fungal CWDEs, and the production of transgenic wheat plants expressing inhibitors of CWDEs. By these approaches we obtained some fungal mutants knocked out for pectin methyl esterase (PME) activity and, on the other side, transgenic durum wheat plants expressing a PME inhibitor (PMEI) able to reduce the plant PME activity and to increase the methylesterification degree of cell wall pectins. Infection experiments of common and durum wheat spikes showed that the fungal PME deletion mutants are less virulent than the wild type strain, mostly at early stages of infection. Transgenic durum wheat expressing PMEI showed an increase of resistance when infected with wild type *F. graminearum*, but no further increase of resistance was observed when the transgenic plants were inoculated with the fungal PME deletion mutant. These results clearly indicate a contribution of PME to virulence of *F. graminearum* and that methylesterification of pectin in the wheat cell wall plays a role in contrasting the infection of *F. graminearum*.

**THE DETECTION OF PSEUDOMONAS SYRINGAE pv. PHASEOLICOLA ON FRENCH BEAN SEEDS USING OFFICIAL METHODS.** THE INRAN LABORATORY EXSP-
RIENCE. V. Senape, L. Sigillo, G. Serratore and R. Bravi. Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN), Sezione di Battipaglia, SS 18 - Km 77,700, 84091 Battipaglia (SA), Italy. E-mail: senapeveronica@libero.it

Pseudomonas syringae pv. phaseolicola (Psp), causing the halo blight disease, is a worldwide severe seedborne bacterial pathogen of French bean. In the last years, seed companies used to check the French bean seed lot health to assure the phytosanitary quality of their production. The Laboratory of Phytopathological Analyses of INRAN (Battipaglia Unit) offers to seed companies and growers a phytodiagnostic service on seeds and growing materials. From 2010 to 2012, the laboratory analysed 122 French bean seed samples provided by seed companies for Psp detection. The analyses were carried out using an internal procedure developed according to the validated ISTA protocol n. 7-023. The samples were separated into two 1000 seed sub-samples, soaked overnight in a buffer, and their extracts were plated on semi-selective media (MSP, MT); typical colonies were purified on King B medium. The suspected isolates were tested for tobacco hypersensitivity reaction and the pathogenicity was confirmed with in vivo tests. At this stage, the technician experience is a critical point for result interpretation: the symptoms developed on seedlings were visually examined and compared with those observed on positive and negative controls. Using this procedure a high percentage of infected samples was detected; almost 30% of them resulted strongly infected by Psp. According to Italian and European legislation the phytosanitary certification of vegetable seeds is not provided. Moreover, it should be considered that many dangerous pathogens are transmitted by seeds and that the seed health is an important condition for a high quality production. This survey allowed the seed companies to improve the phytosanitary quality of their seed production. Moreover the results emphasized the importance of the phytosanitary controls regarding the quality of propagating material and the need of a seed health certification program.

A STANDARDIZED METHOD TO EVALUATE THE RESISTANCE TO FUSARIUM OXYSPORUM f. sp. LACTUCAE IN LETTUCE. L. Sigillo, V. Senape, G. Serratore and R. Bravi. Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Sezione di Battipaglia, SS 18 - Km 77,700, 84091 Battipaglia (SA), Italy. E-mail: l.sigillo@ense.it

Fusarium oxysporum f. sp. lactucae (Fol) is the causal agent of fusarium wilt of lettuce. The widespread distribution of the disease is increasing especially in the fourth range productions. Three pathogen races are known but in Italy only race 1 affects cultivations. Traditional control methods are not effective and the use of resistant varieties can reduce yield losses. The characteristic “resistance to Fol” is becoming more and more important in commercial varieties. However its evaluation is not included in the official technical protocol of lettuce varieties for listing in National and European Catalogues and granting of Plant Breeders’ Rights. A method to evaluate lettuce variety resistance to Fol was developed on demand of seed companies. The test is based on the dipping root inoculation method and is performed in climatic room conditions. The observation of symptoms was reported according to a disease scale and the symptoms developing on the samples were compared with those observed in reference varieties. After statistical analyses, results showed that variety behaviour vary from resistant to intermediate resistant to susceptible. Therefore, considering several experimental conditions, a standardized simple and rapid test was established. The inoculation plant stage and the choice of the reference varieties seems to be the critical control points for a reliable evaluation. Recently, the characteristic “resistance to Fol race 1” was proposed for DUS lettuce test for granting of Plant Breeders’ Rights and UPOV (Union for the Protection of new Varieties of Plants) has been evaluating the analytical protocol reported in this work.

CITRUS TRISTEZA VIRUS INFECTION ON PLANTS OF TAROCCHIO O.L. GRAFTED ON SOUR ORANGE: EVALUATION OF THE EFFECT ON SOME PRODUCTIVE AND PHYSIOLOGICAL PARAMETERS, BY SIMPLE VARIANCE AND DISCRIMINANT ANALYSIS. G. Sorrentino1, M. Guardo1, M.P. Russo1, S. Davino2, G. Iacono3, M. Davino3 and A. Caruso1. 1CRA, Centro di Ricerca per l’Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Actreale, Italy. 2Dipartimento DEMETRA, Università degli Studi, Viale delle Scienze, 90124 Palermo, Italy. 3Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli Studi, Via Santa Sofia 100, 95123 Catania. E-mail: guidosorrentino@enteca.it

In this paper the effect of Citrus tristeza virus (CTV) on yield, fruit quality and some plant physiological index are evaluated. The observations were carried out on a plot located in Catania province constituted by 500 plants 30-years-old of Tarocco O.L. grafted on sour orange, combination susceptible to CTV. The citrus grove, upon screening by immunoenzymatic test, was infected by CTV. The virus characterization revealed the presence of CTV-DS2, virulent and easily transmitted by aphid populations. The percentage of infection was 32% and showed different decline levels. The sampling was carried out on four groups of 30 trees each: group 1, CTV infected showing severe decline; group 2, infected showing initial symptoms of decline; group 3, infected with good vegetative growth; group 4 CTV-free. On each plant we determined the mean yield, and analyzed fruit quality (average fruit weight, total soluble solids, percentage, total acidity and ripening ratio, peel, flesh and juice colour). Plant physiological index as chlorophyll contents in leaves were measured by SPAD 502plus Konica Minolta. The obtained data were elaborated through simple variance analysis and discriminant analysis. The results have underlined that all the considered parameters are influenced by the level of infection. Among all the mean yield, the weight of the fruits and the index of green (SPAD) determination showed the most meaningful values to the statistic and discriminating analysis; particularly interesting are the differences of these last values between the healthy plants and the CTV infected plants with good vegetative growth and without symptoms.

QUANTITATIVE REAL-TIME PCR FOR FUSARIUM FUKUIROI AND FUSARIUM PROLIFERATUM ON RICE. D. Spadaro, M.T. Amatulli, 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, Italy. E-mail: davide.spadaro@unito.it

Due to the reduction in pesticide availability, in recent years the incidence of bakanae disease on rice has grown in Italy. Fusarium fujikuroi is the causal agent of bakanae disease and a species in the Gibberella fujikuroi species complex (GFSC). Other Fusarium spp. also occur on rice including two other species of the GFSC, Fusarium verticilloides and Fusarium proliferatum. In particular, F. proliferatum is morphologically indistinguishable from F. fujikuroi and can only be distinguished by making tests of sexual cross-fertility or through DNA sequencing. Multiple alignment of translation elongation factor (TEF) gene sequences of different Fusarium spp.,
showed a deletion of six nucleotides in *F. fujikuroi* sequence and a two nucleotide polymorphism in the same region of *F. proliferatum* sequence. These elements of variability were used to develop a conventional and real-time PCR assay for diagnosis. The species specific primer pairs (FugiEF/TEf1R and Proli1F/TEf1R) gave a product of 179 and 188 bp for *F. fujikuroi* and *F. proliferatum*, respectively. Primer specificity was confirmed by analysing the DNA of the most representative species of the GFSC and 298 strains of *Fusarium* spp. isolated from rice plants and seeds in Italy. The specific primers were also successfully used to detect fungal presence directly from infected rice tissues and seeds, providing a rapid tool for the early detection of pathogen contamination. The early and correct identification of this species is very important for the rice seed industry to set up, when necessary, good control strategies, and to sell disease-free certified seeds.

**PHYTOPLASMAS ASSOCIATED TO SPONTANEOUS AND CULTIVATED PLANTS.** R.E. Spallino1, S. Rizza1, G. Causarano1, C. Oliveri1, C. Marzachi1 and M. Tessitori1, 1Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Sezione Fitopatologia e Genetica Vegetale, Via S. Sofia 100, 95123 Catania, Italy. 2Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10133 Torino, Italy. E-mail: mtessitori@unict.it

Symptoms caused by phytoplasmas occur worldwide in many crops and spontaneous plants. The latter play an important role in these diseases as pathogen reservoirs. A study on symptoms such as fasciations, witches' broom, virenescence and internode shortening and their association with phytoplasma infections has been conducted in Sicily. Symptoms on plants belonging to several species, genera and families (annual or perennial, herbaceous or woody, spontaneous or cultivated) were observed. The detection and molecular characterization of phytoplasmas carried out through analysis of the 16S ribosomal RNA gene amplified by nested-PCR using universal primer pairs (P1/16S-SR followed by R16F2/R2) revealed 16 new sequences on *Brassica oleracea* L. subsp. *botrytis* (L.) Metzg, *Austrocedrus excelsa* (A. Berger) Backeb, "monstruosa", *Opuntia ficus-indica* (L.) Mill., *Opuntia subulata* Engelh. "monstruosa", *Spartium junceum* L., *Ramunculus bucephalophorus* L. subsp. *bucephalophorus*, *Linaria botryoides* Dode., *Fedia corniculata* (L.) Gaertner, *Pennisetum undulatum* L. Isolates were classified, on the basis of virtual RFLP analysis performed by PhyClassifier, in seven different groups and subgroups. The 16S rRNA sequences obtained were deposited in GenBank (JQ181539-JQ181554). The study demonstrates that among the investigated species some, to our knowledge, are new phytoplasma hosts. As expected, phytoplasmas belonging to group I are hosted by different plant species in Sicily. Unexpectedly, during the two year survey of the spartium witches' broom, this disease evolved into an epidemic, probably due to the presence of an efficient vector in the region.

**FUNCTIONAL GENOMICS ANALYSIS OF *FUSARIUM CULMORUM* BY RANDOM INSERTION MUTAGENESIS WITH A MIMPI1/IMPALA DOUBLE-COMPONENT SYSTEM.** F. Spanu1, M. Passualli2, B. Scherm2, V. Balmas1, A. Marcelli1, G. Ortu2, M. Dufresne3, L. Hoffman4, M. J. Dubouzet1 and Q. Miglì1, 1Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. 2Centre de Recherche Public Gabriel Lippmann, 41 Rue du Brill, 44226 Belfaux, Luxembourg. 3Institut de Genétique et Microbiologie, Bâtiment 400, Université Paris Sud 11, 91405 Orsay Cedex, France. 4Institut de Biologie des Plantes, Bâtiment 630, Université Paris Sud 11, 91405 Orsay Cedex, France. E-mail: qmigli@uniss.it

*Fusarium culmorum* is an ubiquitous soil-borne fungus incitant of crown and foot rot and *Fusarium* head blight especially in durum wheat and a type B trichothecene producer. The genome of *F. culmorum* is being sequenced but for many genes the function is yet unknown. Here we report for the first time on the functionality and effectiveness of the double component system mimpi1/impala as high-throughput strategy for gene identification and for functional genomics analysis. This study shows for the first time the use in a filamentous fungus of the splinkerette-PCR approach to clone the flanking sequences of mimpi1. New acquired data show that mimpi1 transposes by a cut-and-paste mechanism into TA dinucleotides, which are duplicated upon insertion, similar to *Fusarium graminearum*. Furthermore, mimpi1 reinserts throughout the entire genome as indicated by the analysis of distribution of the homologous genes in *F. graminearum*. This system allowed also to identify a putative aurofusarin gene and two genes putatively involved in oxidative stress coping capabilities in *F. culmorum* as well as a sequence specific to this fungus. This suggesting that the transposon tagging approach with the mimpi1/impala double component system is an efficient method for randomly marking genes in *F. culmorum* and may represent a powerful mutagenesis tool for the functional analysis of this genome. Further validation of the double component system will be performed once the complete genome sequence shall be available, in order to understand the role and function of tagged genes with respect to the pathogenicity and mycotoxigenic potential of this pathogen.

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**EXPRESSION ANALYSIS AND QUORUM SENSING REGULATION OF GENES INVOLVED IN THE CYCLIC LIPOPEPTIDE CORPEPTIN PRODUCTION IN *PSEUDOMONAS CORRUGATA*.** C.P. Strano1, P. Bella1, A. Fiore2, G. Lisciandrello1, V. Fogliano2, V. Venturi3, A.R. Lo Porto1 and V. Catara1, 1Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli Studi, Via Santa Sofia 100, 95131 Catania, Italy. 2Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli Studi, Via Santa Sofia 100, 95131 Catania, Italy. 3Parco Scientifico e Tecnologico della Sicilia Zona Industriale, Blocco Palma I, Stradale Lancia 57, 95121 Catania, Italy. 4Bacteriology Group, International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano, 34149 Trieste, Italy. E-mail: vcatara@unict.it

An ABC (ATP-binding cassette) transport system consisting of two genes and a truncated ORF encoding part of a putative non ribosomal peptide synthetase (NRPS) were identified in a cosmid insert of a genomic library of *Pseudomonas corrugata*, downstream of the PcoI/PcoR quorum sensing system and the transcriptional regulator rfiA. The genetic organization of this locus shows high similarity with other *Pseudomonas* cyclic lipopeptide (CLP) biosynthetic clusters. MALDI-TOF MS analysis of cultural filtrates showed that the wild type strain produced two kind of CLPs, comycin A and corpeptins, whereas derivative mutants of the ABC transport system and NRPS produced only comycin, thus suggesting that these genes are part of the compeptin biosynthesis cluster. Moreover, RT-PCR with two set of primers overlapping the three genes demonstrated that they work as an operon. Quantitative real-time RT-PCR showed the presence of NRPS gene transcript in the
monitoring health status in forestry nurseries of western Sicily. L. Torta1, G. Conigliaro1, S. Lo Piccolo1, V. Mondello1, A. Sidoti2 and S. Burrano1. 1 Dipartimento DEMETRA, Viale delle Scienze, 90124 Palermo, Italy. 2 Regione Sicilia, Dipartimento Regionale Azienda Foreste Demaniali, Servizio 7°, Via Selafani 34, 95024 Acireale, Italy. E-mail: livio.torta@unipa.it

In autumn 2011 and spring 2012, six forestry nurseries of western Sicily, managed by the Dipartimento Regionale Azienda Foreste Demaniali, producing seedlings used primarily for reforestation of the regional state-owned forests, were monitored in order to evaluate their health status. Particularly, the nurseries were located as follows: two in the province of Agrigento, two in Palermo, one in Trapani and one in Caltanissetta. Many different plant species (cypress, strawberry tree, Aleppo pine, mountain ash, myrtle, holly oak, oleander, mastic, Japanese cheesewood, pine, downy oak, thuja, laurel, cork oak, golden chain, Nebrodi pine, carob, rosemary) showed alterations, such as root rots, foliar spots and wilting, twig drying and cankers, browning of stems and a general decline. Samples of symptomatic seedlings were collected and subjected to isolation techniques. Fungal isolates belonging to genera known as etiological agents of alterations were first separated based on gross colony morphology from other isolated fungi. Morphological and molecular identification assays have been carried out on pure colonies of selected fungi. First results suggest that fungal biodiversity is associated with alterations depending on the considered organ rather than the host species or the localization of nursery. In particular, genera Alternaria and Rhizoctonia were recurrent in foliar symptoms, Fusarium, Cylindrocladium and Rhizoctonia were associated with root rot and Alternaria, Rhizoctonia, Verticillium and Cladosporium were often isolated from drying twig and cankers. Molecular analysis and pathogenicity assays are in progress to elaborate the most effective defence strategies towards these pathogens.

Effects of soil texture and water regime on the inoculum density of Verticillium dahliae microsclerotia. V. Turci2, M. Ferrara1, I. Pentimone1, A.D. Palumbo2, A. Ippolito1 and F. Nigro1. Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Viale Fanini 44, 40127 Bologna, Italy. E-mail: stefano.tonti@unibo.it

The effects of soil texture and water content on the inoculum density (ID) of Verticillium dahliae microsclerotia was tested in a series of 24 lysimeters containing clay (C) or sandy (S) soil artificially infested with the pathogen. In the lysimeters, seedlings of artichoke cv Opal were transplanted and two water regimes (33% and 100% of the evapotranspiration, ET) were compared. The experimental design was a split plot with three replications (soil texture as the main treatment and water regime as the secondary treatment). Lysimeters were periodically sampled over a 3-yr period, and the ID was determined by the plate-dilution method. Regardless of the water regime and soil texture, ID showed a seasonal variation, with significant increase between February and April; conversely, the lowest ID values were found in July and September. At 0-25 cm depth, ID values showed a larger fluctuations in the S than in the C soil for both water regimes tested. Moreover, irrespective of the water regime applied, higher ID of microsclerotia was found in the S soil as compared to the C soil. At 25-50 cm depth, the water regime exerted opposite effects, determining significantly lower and significantly higher ID values in S than in C soil at 33% and 100% ET, respectively. Information obtained in this study may represent a starting point for a better understanding of the factors affecting the population dynamics of V. dahliae microsclerotia in the soil, thus allowing the development of more effective integrated strategies for controlling Verticillium wilt of artichoke.

Characterization of novel microsatellite markers in Fusarium fujikuroi from Italian rice cultivation areas. M.T. Valente, A. Santori, A. Infantino and M. Aragona. CRA, Centro di Ricerca per la Patologia
**Fusarium fujikuroi** Nirenberg, (teleomorph: Gibberella fujikuroi Sawada) is the causal agent of Bakanae disease of rice, causing increasing losses to Italian production in the last years. The aim of this study is to understand the genetic structure of Italian *F. fujikuroi* populations identifying genetic and genotypic diversity by developing microsatellite molecular markers. A collection of about 240 isolates of *F. fujikuroi* was established starting from the infected culms of plants showing the typical Bakanae symptoms, collected during 2011 from cultivated fields in different Italian regions. Morphological and molecular analysis allowed to identify *F. fujikuroi* as the major responsible for the disease. All the available nucleotide sequences of *F. fujikuroi*, published in NCBI database, were used to find microsatellite motifs (SSRs) through a microsatellite finder software (WebSat; http://wsmartins.net/websat/). Perfect mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide motifs with a repeat of ≥6 times were identified. The longest microsatellite repeats were selected and were amplified with primers designed by the primer3 software. Moreover, some of the SSRs used for *Fusarium verticillioides* and available from literature, are going to be tested for their suitability in identifying polymorphisms in *F. fujikuroi*, taking into account that both these species belong to the *Gibberella fujikuroi* complex. The length polymorphisms were detected by Polyacrilamide Gel Electrophoresis (PAGE) and analysed by statistical tools. The genetic variation of pathogen populations opens up new challenges for rice breeders in developing Bakanae-resistant rice varieties. Understanding the genetic structure of *F. fujikuroi* may facilitate to understand its biology and evolution and can be useful to improve the disease management.

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

The hypersensitive response (HR) triggered by an avirulent pathogen in resistant plants is characterized by the simultaneous production of nitric oxide (NO) and reactive oxygen species (ROS), both involved in the onset of cell death. Among other things NO can react with O$_2$ in a diffusion-limited reaction to produce peroxynitrite, the increase of which has been recently demonstrated in Arabidopsis plants challenged with an avirulent pathogen with a timing that correlates with an increase in tyrosine-nitrated proteins. In plants, peroxynitrite is not responsible for NO-mediated cell death as observed in animals and till now its physiological function is poorly understood. However, it is emerging as a potential signaling molecule during the induction of defense responses against pathogens. In order to decipher the role of peroxynitrite during the HR, we attempted to identify specific targets of nitration displaying signaling functions during this process. In particular we focused our interest on MAPK cascades, a complex network of phosphorylation events involved in plant defense responses and known to be regulated by nitration in animals. We identified AtMKK4 as specifically nitrated by peroxynitrite, leading to an inhibition of its activity in vitro as well as in vivo. Interestingly, despite 78% of sequence homology with AtMKK4, AtMKK5 is not nitrated by peroxynitrite and its activity is not modulated by such a treatment. Therefore peroxynitrite produced during the HR could block selectively the activity of AtMKK4, raising the question of AtMKK4 and AtMKK5 function redundancy in mediating defense signals in plants.

**CLASSICAL AND MODERN TECHNIQUES TO ASSESS THE EFFECTS OF CROP ROTATION ON THE SOIL MICROBIAL COMMUNITY.** R. Varlese¹, D.G. Crispo³, M. Ruocco², F. Vinale², S. Lanzuise, M. Nigro¹, A.M.E. Eid¹, A. Pascale¹, G. Manganelli¹, V. Matteoli¹, D. Stellitano¹, R. Marra¹, M. Lorito³ and S.L. Wool³. ¹Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici, Italy. ²Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. E-mail: wool@unina.it

Microbe-plant interactions in the soil have a large impact on agriculture. Beneficial effects include disease control, plant growth promotion, induced resistance, increased nutrient availability. The objectives of this study were to evaluate the highly complex microbial diversity in soils where legume and wheat crop rotations have been applied, in order to understand the biology and the mechanisms involved in this agricultural system. Samples tested included soils from pea-wheat rotation, no crop, legume only, cereal only. Different approaches using classical and modern molecular techniques were compared. Soil was plated to determine the number of colony forming units (CFUs), the abundance and morphology of the “culturable” microorganisms on selective substrates. Total DNA was extracted for use in molecular techniques of PCR-DGGE and metagenomic analysis; two methods increasingly applied in the field of soil microbiology. Preliminary screening of PCR primers was conducted, and those producing satisfactory results were synthesized with a GC-Clamp for DGGE analysis. The microbial DNA complex was sequenced for metagenomic analysis and functional studies including: identification of genes, metabolic pathways, as well as gene annotation and species identification and abundance. Similarities were noted in from the comparison of traditional and modern methods. Both approaches were able to distinguish microbes from the various crop rotation systems, but only the molecular methods provided the possibility to analyze the non-culturable microflora. Soil microbial diversity has important implications for sustainable agricultural production and management.

**CHARACTERIZATION OF A MUTANT KNOCKED OUT FOR A POLYGALACTURONASE GENE IN TRICHODERMA VIRENS.** M. Vergara¹,², S. Sarrocco¹, S. Brizolara¹, B. Seibold³, V. Seidl-Seiboth³ and G. Vannacci³. ¹Dipartimento di Arboricoltura, Entomologia e Patologia Vegetale Giovanni Sarracino, Sezione di Patologia Vegetale, Via del Borghetto 80, 56124 Pisa, Italy. ²Scuola Normale Superiore, Piazza dei Cavalieri 7, 56126 Pisa, Italy. ³Molecular Biotechnology, Institute of Chemical Engineering TU, Getreidemarkt 9, 1060 Vienna, Austria. E-mail: rvergara@age.unipi.it

The ability of *Trichoderma* spp. to antagonize plant pathogens, to induce plant resistance and to promote growth in plants has been widely described. Endo-PG produced by *Trichoderma* spp. can assist root penetration and play a pre-eliciting role in systemic resistance (SIR), a beneficial effect detected in plants colonised by *Trichoderma*. Two endopolygalacturonase (endo-PG) genes, *Tvpg1*
and Teppg2, have been identified and partially characterised in a T. vires isolate previously investigated for its antagonistic ability in several biological systems. An expression analysis pointed out a different regulation of those genes: Teppg1 gene proved to be induced in response to pectin or plant cell walls (in vitro) and to tomato roots (in vivo), while Teppg2 was constitutively expressed. The molecular cross-talking between tomato roots and T. vires was investigated by checking the expression of a tomato PGIP (Leppg1) in colonised roots. The Leppg1 transcript was induced at times coincident with Teppg1 expression suggesting a functional correlation between the two gene products. Gene targeting by homologous recombination has been applied to the T. vires constitutive gene by exploiting an available Dbea70 transformed isolate in order to generate a double mutant Dbea70DTcppg2 with the relative gene displaced. Some transformants are currently being checked for basic physiological properties. Then, the selected knock-out strain will be used to study the involvement of endoPG genes in the interaction with plants.

**ASSESSMENT OF GENETIC VARIATIONS OF DIVERGENT CITRUS TRISTEA VIRUS RNA POPULATIONS IN ITALY UPON VECTOR TRANSMISSION PROCESS.** D. Yahiaoui1,2, K. Djelouah1, A.M. D’Onghia1 and A. Catara1, 1Istituto Agromonomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano, Italy. 2Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 3Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Blocco Palma I, Stadale Lancia 57, 95121 Catania, Italy. E-mail: yahiaouidor@yahoo.fr

Citrus tristeza virus (CTV) causes one of the most disastrous citrus diseases worldwide. In Italy, tristeza outbreaks were reported from Sicily (Catania and Siracusa provinces) and Apulia (Taranto province), where two divergent RNA genome variants (seedling yellows and mild isolates), have been found. However, information on the mechanisms involved in the rapid spread of CTV populations and on their genomic changes following vector transmission is still limited. In the present work, representative CTV haplotypes were successfully transmitted by local aphid biotypes. Afterwards, the response of aphid-derived CTV variants to MCA13 strain-specific antibody has been studied and gene mutations have also been assessed by single strand conformation polymorphism (SSCP) of viral p18 and p25 and sequence analysis of the coat protein. Results revealed a high spread potential of CTV variants using *Aphis gossypii* (about 50%), while transmission efficiency by *A. pisum* (< 3%) and *Toxoptera auranti* (< 2%) was lower. The Apulian isolate reacted negatively to MCA13 even after transmission, while the Sicilian isolate and its sub-isolates cross-reacted with MCA13. The molecular cross-talking between tomato roots and *A. gossypii* was investigated by checking the expression of a tomato PGIP (*Leppg1*) in colonised roots. The *Leppg1* transcript was induced at times coincident with *Teppg1* expression suggesting a functional correlation between the two gene products. Gene targeting by homologous recombination has been applied to the *T. vires* constitutive gene by exploiting an available Dbea70 transformed isolate in order to generate a double mutant Dbea70DTcppg2 with the relative gene displaced. Some transformants are currently being checked for basic physiological properties. Then, the selected knock-out strain will be used to study the involvement of endoPG genes in the interaction with plants.

**EVALUATION OF ANTIFungal ACTIVITY OF “KARMA”, A FORMULATE BASED ON POTASSIUM BICARBONATE, AGAINST POSTHARVEST DECAY OF FRESH FRUIT AND VEGETABLES.** K. Youssel3, S.M. Sangani2, F. Fallanaj1, A. Ligorio2, F. Nigro2, A. Ippolito2 and A. Myra1, 1Agricultural Research Center, Plant Pathology Research Institute, 9 Gamna St., 12619 Giza, Egypt. 2Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. 3Certi Europe, Boulevard de la Woluace 60, 1200 Brussels, Belgium. 4Certi Italy, via Josemaría Escrivá de Balaguer 6, 21047 Saronno, Italy. E-mail: youseffela@uol.com; antonio.ippolito@uniba.it

Safe alternative treatments have become an essential requirement for the management of postharvest pathogens due to concerns regarding the negative impact on human and environmental health and development of fungicide resistant strains. The efficacy of a novel formulation of 85% potassium bicarbonate (Karma, Certi Europe B.V.) as a possible alternative to synthetic fungicides for controlling postharvest pathogens was evaluated. Karma at increasing concentrations (0, 0.1, 0.2, 0.3, 0.4, and 0.5%, w/v) mixed with Potato Dextrose Agar (PDA) medium was tested; the concentration causing 50% growth reduction (ED<sub>50</sub>, SAS probit analysis) and the minimum inhibitory concentration (MIC) values were recorded. The ED<sub>50</sub> was 0.12, 0.12, 0.075, 0.12, 0.08, 0.05, and 0.11 % (w/v) for *Penicillium digitatum*, *P. italicum*, *P. expansum*, *Phytophthora nicotianae*, *Monilia laxa*, and *Botrytis cinerea*, respectively; a complete growth inhibition for the same pathogens was achieved at 0.3, 0.2, 0.3, 0.2, 0.2, and 0.3% (w/v), respectively. The effect of Karma was primarily fungistatic since fungal discs, re-seeded onto the fresh PDA medium, revived their growth. In *in vitro* experiments, carried out on ‘Tarocco’ and ‘Valencia late’ oranges, Karma confirmed its activity against the pathogens, significantly reducing the incidence of *Penicillium rot* as compared to water-treated fruits. The incidence of *Penicillium decay* in fruit dipped in 3% salt solution was reduced by 38 and 100% in ‘Tarocco’ and ‘Valencia late’, respectively. The results show potential benefits of Karma as an alternative to fungicides for controlling postharvest pathogens attacking fruit and vegetables.

**IRIS YELLOW SPOT VIRUS: AN INCREASING PROBLEM FOR ITALIAN ONION CROPS.** S. Zicca1, M. Giau2, A. Mangli1,2, M. Turina2 and L. Tomassoli1, 1CRA, Centro per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. 2Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. 3Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89122 Reggio Calabria, Italy. E-mail: laura.tomassoli@centra-it

Iris yellow spot virus (IYSV, family Bunyaviridae, genus *Tospovirus*), is a *Thrips tabaci*-transmitted, devastating pathogen for several *Allium* spp. worldwide. In the last four years, surveys of onion (*Allium cepa*) crops showed the presence of this disease also in Italy. In fact, characteristic symptoms of IYSV infection including diamond-shaped lesions, as well as circular to irregular, chlorotic or necrotic spots were reported first in Emilia Romagna (seed crops) followed by Piedmont, Veneto and Marche (bulb crops). In 2012, new surveys confirmed the presence of the virus in Piedmont and Veneto and its introduction in Calabria where infected plants (cv Cipolla Rossa di Tropea) were found both in bulb and seed crops. During these past years, IYSV has been detected in every onion production areas we surveyed. Furthermore, a recent Real time RT-PCR assay was shown to detected virus in some of the samples that tested negative using traditional diagnostic methods. Therefore, the actual IYSV incidence in Italy is probably underestimated and much effort is required to know the current phytosanitary status of onion crops in every Italian regions. IYSV is not reported to be seed–borne and seed transmitted while bulb infection and its role as overwintering material and for long distance spread is still debated. Proper cultural and thrips management, clear and rapid diagnosis and specific epidemiological studies are necessary to provide good surveillance of the disease in Italy and to support phytosanitary control of imported planting materials, and to improve genetic breeding or screening of local varieties for virus resistance.