IDENTIFICATION OF RESISTANCE TRAITS TO FUSARIUM AND VERTICILLIUM WILT IN ITALIAN TOMATO LANDRACES. **N. Acciarri[1], G.L. Rotino[2], E. Sabatini[2], S. Voltattorni[1], D. Valentino[3] and G. Tamietti[3].** *[1]CRA, Istituto Sperimentale per l'Orticoltura, Via Salaria 1, 63030 Monsampolo del Tronto (AP), Italy. [2]CRA, Istituto Sperimentale per l'Orticoltura, Via Paullese 28, 26836 Montanaso Lombardo, (LO), Italy. [3]Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Patologia Vegetale, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: giacomo.tamietti@unito.it*

A representative number of tomato landraces belonging to different typologies, collected in the main Italian tomato-growing areas were evaluated for resistance to *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae* by root dip inoculation method. None of the screened landraces was completely resistant to Fusarium wilt. Nevertheless high levels of tolerance were found in 'Ovalino', 'Locale Valle d'Agri', 'Patataro pop. 29' (a typical accession from Campania), 'Sorrentino' (a pink tomato selected in the Sorrento area) and 'Mezzo lungo', and intermediate tolerance in additional 11 landraces. Similarly, most of the genotypes were highly susceptible to *V. dahliae*, but 'Eraldo 4', belonging to Cuore di Bue di Albenga type, 'Rosa di Rotonda' and 'Locale Valle d'Agri' (an accessions with pink fruit from Basilicata) and the breeding line 'L 16-94', appeared to be tolerant, showing disease incidence ranging from 72.2 to 100% and disease severity from 26.6 to 30%, whereas 'Sorrentino' was as resistant as 'Florida'. Two new molecular markers linked to Verticillium resistance genes were developed on the sequence information in GenBank for Ve genes and validated on several resistant varieties. Genetic analysis carried out using these markers demonstrated that the same resistant alleles are present both in 'Sorrentino' and 'Eraldo 4'. Two years of individual selection and self-fertilization yielded breeding lines of 'Sorrentino' resistant to *V. dahliae* and highly tolerant to *F. oxysporum*. 'Eraldo 4' is currently used as parent for the production of resistant and typical HF1 hybrids of the 'Cuor di Bue di Albenga' which is one of the Italian most popular typology for fresh consumption.

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IDENTIFICATION AND CHARACTERIZATION OF *APPLE DIMPLE FRUIT VIROID* IN LEBANON. **M. Afechtal[1], E. Choueiri[2], S. El Zammar[2], F. Jreijiri[2], C. Hobeika [2], A. Myrta[1]* and F. Di Serio[3].** *[1]Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy. [2]Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, P.O.Box 287, Zahlé, Lebanon. [3]Istituto di Virologia Vegetale del CNR, Sezione di Bari and Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: f.diserio@ba.ivv.cnr.it*

Apple trees of cv. Starking Delicious producing fruits with depressed green spots 3-4 mm in diameter, scattered on the whole fruit surface and merging around the calyx, were observed in the Bekaa valley (Lebanon). These symptoms resembled those of apple dimple fruit and dapple apple diseases induced by *Apple dimple fruit viroid* (ADFVd) and *Apple scar skin viroid* (ASSVd), respectively. Dot-blot hybridization using digoxigenin-labelled riboprobes, multiplex RT-PCR amplification assay specific for detecting these viroids in both single and mixed infections and sequencing of the generated amplicons showed that the symptoms were associated exclusively with the presence of ADFVd, whereas ASSVd was never detected. Full length ADFVd cDNAs from one symptomatic apple tree were also amplified and cloned. Sequencing of four independent clones identified ADFVd sequence variants composed by 307 nt with limited variability. Multiple sequence alignment of these variants with those reported previously in databank allowed the identification of one additional polymorphic position in the viroid genome. To our knowledge, this is the first report of ADFVd in Lebanon and the first in a country other than Italy. Tissue-printing hybridization was successfully used for a large-scale survey for the presence of ADFVd and ASSVd in Lebanon, the results of which are presented and discussed.

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IDENTIFICATION AND CHARACTERIZATION OF *PEAR BLISTER CANKER VIROID* IN MALTA AND IN BOSNIA AND HERZEGOVINA. **M. Afechtal[1], B. Lolic[1], S. Matic[2], D. Attard[3], A. Myrta[1]* and F. Di Serio[2].** *[1]Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy. [2]Istituto di Virologia Vegetale del CNR, Sezione di Bari and Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. [3]Plant Biotechnology Center, Ministry for Rural Affairs and the Environment, Lija, Malta. E-mail: f.diserio@ba.ivv.cnr.it*

Preliminary surveys to investigate the presence and spread of pome fruit viroids in Bosnia and Herzegovina and in Malta were carried out by a tissue printing hybridization (TPH) method. More than 300 samples of apple and pear trees from Bosnia and Herzegovina were tested for the presence of *Apple scar skin viroid* (ASSVd), *Apple dimple fruit viroid* (ADFVd) and *Pear blister canker viroid* (PBCVd). Whereas ASSVd and ADFVd were not detected, almost 17% of assayed pear samples, belonging to 13 different cultivars, gave positive TPH signals when hybridized with a PBCVd-specific cRNA probe. In parallel experiments, a total of 113 pear samples (mainly cv Bambinella) from Malta were tested for PBCVd showing an infection rate of 12%. These results were largely confirmed by Northern-blot hybridization assays and by RT-PCR followed by cloning and sequencing of the amplified cDNAs. No symptoms were observed in infected field-grown trees. Seedlings of the pear indicator LA62 were graft-inoculated with bark tissues from several new PBCVd isolates from both countries. Six month post-inoculation, molecular hybridization assays and RT-PCR followed by direct sequencing of the amplicon detected the viroid in the inoculated plants which remained symptomless. Further molecular characterization of several of the new PBCVd isolates allowed the identification of previously unreported polymorphic positions in the viroid genome. Altogether, these data show for the first time that PBCVd infects pear trees in Bosnia and Herzegovina and in Malta, and validate the TPH method for large scale surveys of pome fruit viroid infections.

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***PHYTOPHTHORA CINNAMOMI* ON *QUERCUS ILEX* IN CENTRAL ITALY.** **T. Annesi, L. D'Amico, G. Mazza and E. Motta.** *CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: t.annesi@ispave.it*

A decline was observed in a *Quercus ilex* nursery in central Italy where, three years ago, about 200 five-year-old trees had been planted to produce trees for urban streets and gardens. During winter 2006-2007 several trees showed necrosis of the

collar and roots and wilting, while scattered trees had already died in previous years. To detect the causal agent, isolations were made from altered tissues of symptomatic plants on PDA and on a PARPNH selective medium. Further isolations were attempted from soil samples collected near diseased and symptomless plants. The fungal isolates obtained were investigated for both morphological characters and for the sequence of the ITS region of rDNA and were identified as *Phytophthora cinnamomi* Rands. Pathogenicity was confirmed by successful inoculation on three-year-old holm oaks. *P. cinnamomi* is an aggressive and polyphagous pathogen, associated with severe decline of *Q. ilex* in Spain and Portugal. It was found also in Italy in a holm oak forest but in soil samples only. To our knowledge, this is the first report of *P. cinnamomi* on *Q. ilex* in Italy.

CORRELATION BETWEEN THE OCCURRENCE OF PATHOGENIC FUNGAL ENDOPHYTES IN HEALTHY OAK TREES AND OAK DECLINE. N. Anselmi, M. Nasini, A. Mazzaglia, A. Librandi, E. Rocco and F. Ravaioli. *Dipartimento Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: anselmi@unitus.it*

This research is part of studies on fungal endophytes involved in oak decline. We analyzed the endophytic microflora in healthy trees of 11 oak stands of central Italy characterized by different levels of decline. Four healthy plants were selected per site, and 100 samples of one- and three-year-old branches were collected from each plant. After surface sterilisation, fragments of these samples were transferred to an appropriate substrate. The fungal isolates were subcultured and identified on morphological and molecular bases. Most of the isolated species in the different sites were the same, including pathogens, i.e. *Discula quercina*, *Biscognauxia mediterranea*, *Coryneum quercinum*, *Cytospora* sp., *Phomopsis* spp., *Diplodia corticola*, etc. and non-pathogens, i.e. *Acremonium* sp., *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Epicoccum* sp., *Gliocladium roseum, Thrichoderma* spp., *Verticillium lecanii*, etc. The incidence of endophytic pathogens, agents of bark necrosis, particularly *B. mediterranea* and *P. quercina*, was higher in declining oak stands, whereas non-pathogenic endophytes (e.g. *A. pullulans* and *G. roseum*) were less represented in the same conditions. The increase of inoculum pressure due to the dissemination of propagules of bark necrosis agents in declining oak trees, most likely leads to an increase of new infections to healthy plants. The possible use of these results to define a "forest decline risk index" based on the abundance of the endophytic bark pathogens in healthy plants is discussed.

MICROBIAL RESPONSE TO ORGANIC MATTER AMENDMENTS MODULATES PHYTOTOXICITY AND BIOLOGICAL CONTROL EFFECTIVENESS. V. Antignani, G. Puopolo, G. Bonanomi, C. Pane, A. Zoina and F. Scala. *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: antignani_vincenzo@libero.it*

Recent studies demonstrate that the use of organic amendments for the control of plant diseases can produce suppressive or conducive effects. Disease enhancement has often been associated to the release of phytotoxic compounds from decaying organic matter in the presence of microorganisms. In this work we evaluated the phytotoxicity of *Medicago sativa* residues (MSR) using the *Lepidium sativum* test. Seeds of this species were sawn in pots filled with quartz sand amended with MSR at four concentrations (0%, 0.1%, 0.3%, 1% w/w). Successively, pots were inoculated with three different concentrations of microorganism suspensions. Ten fungi, ten bacteria and three formulations of a commercial microbial consortium were individually used for these trials. At the same time, the antagonistic activity of the ten bacteria toward *Rhizoctonia solani* was evaluated in a dual culture method, on a growth medium amended with MSR at 0.1% and 0.3% final concentration. The analysis of the data obtained in the first group of tests showed that phytotoxicity on *Lepidium sativum* increased with the amount of incorporated MSR and with the density of the microbial inoculum. *Arthrobacter* sp., *Enterobacter aerogenes* and *Trichoderma harzianum* produced the highest phytotoxicity effects, while the microbial consortia were the least phytotoxic. The antagonistic activity of the bacteria against *Rhizoctonia solani* was significantly influenced, either positively or negatively, by MSR content in the growth medium. These preliminary results show that the phytotoxicity of MSR depends both on the microbial species and on its density.

EVALUATION OF THE ANTAGONISTIC ACTIVITY OF SOME BACTERIA AGAINST MELON SOIL-BORNE PATHOGENS. M. Antonelli, M.P. Aleandri, N. Vinci, G. Chilosi and L. Varvaro *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: antonell@unitus.it*

Repeated melon cropping and different soil sterilisation treatments cause a biological impoverishment of soil fertility and an increase of diseases caused by soil-borne pathogens. To develop effective and sustainable strategies for soil-borne pathogen management, we have sampled soils from plots given over to melon in the province of Viterbo and have isolated and selected fluorescent and spore-forming bacteria. These isolates, were characterized biochemically and tested for their *in vitro* activity against *Monosporascus cannonballus*, *Fusarium oxysporum* f. sp. *melonis*, *Rhizoctonia solani*, *Verticillium dahliae* and *Erwinia carotovora* subsp. *Carotovora,* and for their ability to produce toxic volatile compounds (*i.e.* HCN). Bacterial isolates of *Pseudomonas putida*, *P. fluorescens* and *Bacillus* spp. that gave positive responses were used for *in vivo* tests to verify their role as plant growth promoters or as deleterious rhizobacteria with plant growth inhibitory effects through bacterization of melon, lettuce and tomato seed. The bacterial isolates analysed during this study could be a potential tool for biological or integrated pest management programs for farmers in the area of Viterbo.

NEEDS FOR PHYTOBACTERIOLOGICAL ANALYSES TO SUPPORT THE REGIONAL PHYTOSANITARY SERVICES IN ITALY. M. Antonelli, G. Balestra, C. Bazzi, P. Bella, R. Benedetti, E. Biondi, R. Buonaurio, M. Calvi, A. Calzolari, V. Catara, G. Cirvilleri, D. D'Ascenzo, A. Fabi, M. Fiori, G. Gianetti, M. Guelfi, N.S. Iacobellis, P. Lancioni, G. Licciardello, L. Lindner, P. Lo Cantore, S. Loreti, C. Lucchese, C. Marcone, L. Marinoni, E. Mariotti, P. Martini, U. Mazzucchi, P. Minardi, C. Moretti, C. Morone, D. Pasqua di Bisceglie, G. Petris, A. Quattrucci, A. Rossetti, A. Saccardi, M. Scortichini, F. Sesto, A. Sisto, E. Stefani, G. Surico, S. Tegli, F.G. Troiano, L. Varvaro, A. Vincenzi, M. Zaccardelli, V. Zagari, R. Zasso and A. Zoina. *Gruppo di Lavoro di Batteriologia della Società Italiana di Patologia Vegetale. E-mail: umberto.mazzucchi@unibo.it*

The Legislative Decree no. 214 dated 19-9-2005 introduced in

Italy the Council Directive 2000/29/CE, as amended by the Council Directive 2002/89/CE. The aforesaid Decree enforced protective measures against the introduction and spread into the Community of organisms harmful to plants/plants products. In the annexes of the Decree, there are 20 phytopathogenic bacteria. The key measure foreseen in the Decree is the strengthening of plant health checks, carried out by the Regional Phytosanitary Services (Servizi Fitosanitari Regionali, SFR) at the place of production and on imported/exported plants/plant products. In this respect, laboratory analyses are essential for the detection of latent infections. Analyses are currently carried out in laboratories belonging to SFR or, under the supervision of each SFR, in accredited private or public laboratories. The Decree 214/2005 lays down a national laboratory network (art.53) to support SFR in performing the analyses. This network operates under the supervision of a central Phytosanitary Committee (art.52). In 2006, a specific enquiry showed that SFR are characterised by different needs as they carry out different bacteriological analyses for the crops grown in their areas and for controls of materials imported through the relevant points of entry. However, all SFR had as a common task the detection of three quarantine bacteria, *Ralstonia solanacearum, Clavibacter michiganensis* and *Erwinia amylovora.* The national laboratory network, envisaged by Decree 214/2005, should take into account the demand for analyses expressed by the SFR, that have already provided useful data regarding their bacteriological needs. This national laboratory network could provide the SFR with an effective support also in the control of regulated non quarantine pests.

EFFECT OF PHYSICAL AND CHEMICAL TREATMENTS ON BACTERIAL POPULATIONS PRESENT IN MELON-CULTIVATED SOILS. M. Antonelli, R. Reda, G. Chilosi and L. Varvaro *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: antonell@unitus.it*

In recent years melon crops in the north of Latium were dramatically affected by several soil-borne pathogens, especially fungi. As part of a monitoring disease project, we started a microbiological study to: (i) isolate from the soil culturable bacteria with antagonistic activity and, (ii) secure information on the qualitative and quantitative composition of bacterial populations in soils treated with steam or solarisation (physical treatments) or metham sodium (chemical treatment). We focused our attention on two bacterial groups with a well known antagonistic activity: fluorescent and spore-forming bacteria. Soil was sampled before treatments and after some months, inside and ouside different plastic tunnels. The data obtained showed that in steam treated or solarisated soils, fluorescent bacterial populations were not isolated from inside the tunnel, whereas no significant variations were observed in the chemically treated soil. In solarisated soil an increase of spore-forming bacterial populations was found. In other tunnels no significant variations were observed. More biological complexity and variability was found in soils collected outside tunnels, where weed roots grew in the apparent absence of the effect of treatments.

TRANSPOSON TAGGING IN *FUSARIUM CULMORUM*. V. Balmas[1], M. Dufresne[2], G. Ortu[1,2], M.-J. Daboussi[2] and Q. Migheli[1]. *[1]Dipartimento di Protezione delle Piante, Centro interdisciplinare di eccellenza per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. [2]Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France. E-mail: qmigheli@uniss.it*

Crown and foot rot of wheat is an important soil-borne disease caused by several species of filamentous fungi. *Fusarium culmorum* (W.G. Smith) Sacc. is one of the most common incitants of this disease worldwide and is able to produce type B trichothecenes. Aiming at deciphering mechanisms involved in pathogenicity and mycotoxin production of *F. culmorum*, we have recently started a transposon-based mutagenesis approach to identify genes governing these characters without an *a priori* knowledge on their function. We first used the *impala* element, which was already shown to transpose in a wide range of Ascomycete species. A collection of 300 revertant strains was generated and tested for pathogenicity on wheat under glasshouse condition. Following two rounds of wheat assays, 7 mutants, more or less altered in pathogenicity, were identified among 175 strains with a reinsertion event. To date, one strain has been characterised in detail. Loss of pathogenicity results from the insertion of *impala* in a region close to the 5' end of a gene encoding a putative HMG Co-A reductase, a function already described as involved in *F. graminearum* pathogenicity (Seong *et al. Fungal Genet. Biol.* **43**:34-41, 2006). In parallel, a systematic recovery of *impala* insertion sites was initiated in order to determine the insertional preference of this transposable element. More recently, we introduced the double component *mimp/impala* to compare the tagging efficiency of both systems.

Work funded by the Ministry of University and Research (PRIN 2005: Fusarium crown and foot rot of wheat: effect of plant defense mechanisms on pathogenicity and on mycotoxin production).

SOILS OF A MEDITERRANEAN HOTSPOT OF BIODIVERSITY AND ENDEMISM (SARDINIA) ARE INHABITED BY PAN-EUROPEAN AND LIKELY INVASIVE SPECIES OF *HYPOCREA/TRICHODERMA*. V. Balmas[1], B. Scherm[1], R. Caria[1], S. Fiori[1], A. Marcello[1], M. Komoń-Zelazowska[2], A.G. Kopchinskiy[2], I. Druzhinina[2], C.P. Kubicek[2] and Q. Migheli[1]. *[1]Dipartimento di Protezione delle Piante, Centro interdisciplinare di eccellenza per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. [2]Institute of Chemical Engineering, Division of Applied Biochemistry and Gene Technology, Vienna University of Technology, Getreidemarkt 9/E1665, 1060 Vienna, Austria. E-mail: balmas@uniss.it*

To investigate the occurrence and biodiversity of *Trichoderma* spp. in Sardinia (Italy), we isolated 483 strains of *Hypocrea/Trichoderma* from 15 non-cultivated soils including forest, shrublands, undisturbed and extensively grazed grass steppes. Isolates were pre-screened by RAPD-PCR, and identified at the species level by sequence analysis of internal transcribed spacer regions 1 and 2 (ITS1 and 2) of the rRNA gene cluster and the long intron of translation elongation factor 1-alpha, using oligonucleotide BarCode for *Hypocrea/Trichoderma* (*Trich*OKEY), sequence similarity analysis (*Trich*oBLAST) and phylogenetic inferences. The majority of the isolates were positively identified as pan-European and/or pan-global *Hypocrea/Trichoderma* species from sections *Trichoderma* and *Pachybasium*. Recovered species comprised: *H. lixii/T. harzianum*, *T. gamsii*, *T. spirale*, *T. velutinum*, *T. hamatum*, *H. koningii/T. koningii*, *H. virens/T. virens*, *T. tomentosum*, *H. semiorbis*, *H. viridescens/T. viridescens*, *H. atroviridis/T. atroviride*, *T. asperellum*, *H. koningiopsis/T. koningiopsis*, and *Trichoderma* sp. Vd2 *sensu* Jaklitsch *et al.* (*Stud. Mycol.* 55: 135-177, 2006). Only one isolate comprised a new, undescribed species belonging to the

Harzianum-Catoptron Clade. Analysis of ITS1 and 2 and *tef1* sequences, revealed one potentially endemic ITS1 allele of *T. hamatum*, although this species is known from several locations in Europe, Africa and North America. All other species exhibited genotypes which were already found in Eurasia or other continents. Our data show either absence or suppression of native *Hypocrea/Trichoderma* diversity due to extensive colonisation of Sardinia by highly adaptive ecological opportunistic species from Eurasian, African and Pacific Basin. This evidence documents Sardinian soil biodiversity as vastly disturbed and therefore unshielded against further environmental and anthropogenic pressure.

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IDENTIFICATION AND CHARACTERIZATION OF *BACILLUS AMYLOLYQUEFACIENS* STRAIN M123 AS A POTENTIAL ANTAGONIST OF PHYTOPATHOGENIC FUNGI. V. Battaglia[1], G. Puopolo[1], V. Cappio[1], A Raio[2] and A. Zoina[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università, 100, 80055 Portici, Italy. [2]Istituto per la Protezione delle Piante del CNR, Via Università, 133, 80055 Portici, Italy. E-mail: puopolo@unina.it*

Bacillus spp. are used for preventing fungal plant diseases in organic agriculture although concern exists on the use of some species, such as *B. cereus*, since they may be associated with human diseases. The spore-forming Gram-positive isolate M123, recovered from wheat straw, was selected for its high antibiotic activity observed in the isolation plates. The identification of this isolate was done through the analysis of its 16S rDNA sequence. The isolate belongs to the species *B. amyloliquefaciens* an harmless bacterial species included in *B. subtilis* group. Strain M123 showed strong antagonistic activity *in vitro* against *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum.* f. sp. *radicis lycopersici.* It produced proteases and siderophores while no chitinolytic activity was detected *in vitro*. *B. amyloliquefaciens* strain M123 was evaluated for the protection of tomato plantlets from *R. solani*, *F. oxysporum.* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis lycopersici*, using an *in vivo* assay. The severity of disease and the percentage of dead plantlets was reduced in all cases by the presence of M123. At the same time, M123 was able to reduce the gravity of *R. solani* attacks to greenhouse-grown tomato plants. These preliminary results assess the potential of strain M123 in the biological control of fungal diseases of tomato plants and represent a first step in its possible exploitation in organic agriculture.

PHYTOPATHOLOGICAL PROBLEMS OF BIOMASS POPLAR AND MISCANTHUS. G. Beccari and L. Covarelli. *Dipartimento di Scienze Agrarie e Ambientali, Sezione di Arboricoltura e Protezione delle Piante, Area Patologia Vegetale, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: lorenzo.covarelli@unipg.it*

The phytosanitary status was monitored of poplar (*Populus* spp.) and miscanthus (*Miscanthus x giganteus* Greef *et* Deuter) grown as energy crops in an experimental field in Umbria (central Italy). In 2006, natural late summer rust infections were observed on four different poplar clones which proved susceptible to the disease, but with significant differences in disease incidence and severity. Poplar leaves showed the typical rusty coloration due to the presence of many hypophyllous, less frequently epiphyllous, uredinia with urediniospores. As the season progressed, a high number of telia with teliospores appeared on both leaf surfaces. Microscopic observations of the morphological characteristics of urediniospores and teliospores and of infected leaf sections and a statistical cluster analysis of spore size revealed that the attacks were caused by two rust species, *Melampsora larici-populina* and *M. allii-populina*. In May 2007, miscanthus rhizomes coming from experimental field plots showing a very low emergence and a soft and dry rot, with brownish and necrotic tissues, were examined. Isolation on PDA and microscopic observations showed that *Fusarium* spp. and *Rhizopus* spp. were consistently associated with diseased material with an incidence of 70% and 55%, respectively. Pathogenicity tests are in progress.

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***PLEOSPORA ALLII* AGENT OF WILTING AND ROTTING ON RADISH PLANTLETS IN ITALY. A. Belisario[1], S. Talevi[2], S. Vitale[1], L. Luongo[1], S. Nardi[2] and F. Corvi[2].** *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]Agenzia Servizi Settore Agroalimentare Marche (ASSAM), Via Alpi 21, 60131 Ancona, Italy. E-mail: a.belisario@ispave.it*

Pleospora allii (anamorph *Stemphylium vesicarium*) is a polyphagous fungal pathogen particularly harmful to succulent organs. In Italy, it is common on *Allium* spp., but it has also reported on tomato and pear. On European pear this fungus is the agent of brown spot, a destructive disease in the Mediterranean area. A consistent contamination of *Stemphylium* sp. was detected on radish seeds by seed blotter. Seeds were to be exported for sprout production to Asian markets where they are appreciated for human consumption. No chemical dressing was allowed against seed-borne pathogens or contaminants. *Stemphylium vesicarium* was identified on the basis of morphological characters of conidia and conidiophores. Species identification was also supported by ITS (accession Nos. AM 746020 to AM746023) and *gpd* sequence comparison. Pathogenicity tests were carried out on sprouts grown in infested soil, and on plantlets at the 1st-2nd true leaf stage by spraying a conidial suspension. Epicotyl darkening and rotting and root rotting developed on sprouts, while wilting and death occurred on infected young plants. The pathogen reisolated from the inoculated plants was morphologically identical to the original isolates, which confirmed *S. vesicarium* as the causal agent. To our knowledge this is the first report of *S. vesicarium* as agent of wilting and rotting of radish plantlets in Italy.

DETECTION OF ATYPICAL STRAINS OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* IN GREENHOUSE TOMATOES IN SICILY. P. Bella[1,2], G. Ialacci[1], G. Licciardello[1,2], and V. Catara[1] *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Stradale G. Agnelli angolo V. Lancia, 95030 Catania, Italy. E-mail: patrizia.bella@unict.it*

In the last two years, tomato samples from plastichouses of Ra-

gusa (Sicily's most important tomato growing area) have been analysed in our laboratories. Symptoms typical of bacterial canker were found: wilting of the upper leaves, yellow to brown discoloration of vascular tissues, cankers on stem and cavities in the pith. Thus, the samples were subjected to the analyses described in the EPPO diagnostic protocol for *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). Gram positive colonies morphologically similar to *Cmm* were isolated from discoloured vascular tissue. Preliminary identification of isolates from two farms by PCR with CMM5/CMM6 specific primers failed, as did direct PCR analyses on infected tissues. Nevertheless the PCR-negative strains were identified as *C. michiganensis* subsp. *michiganensis* by partial 16S rRNA sequence analysis, Microlog database and agglutination with specific antiserum (Kit Express, Adgen). We tested strain pathogenicity to tomato plants (cv. Roventa), since *Cmm* avirulent strains lacking the *pat*-1 gene on which the PCR assay is targeted have been reported. All strains were virulent when inoculated to tomato plants and within 10-15 days induced unilateral leaf wilting just above the inoculation point, stem cankers and brown discoloration of vascular tissues. The lack of a PCR product with P1-rep/P3-rep primers to amplify the repetitive *pat-1-rep* motif located downstream of *pat-1* further supports the absence of this gene.

GLUCOSE, FRUCTOSE AND SACCHAROSE METABOLISM IN *DIPSACUS SILVESTRIS* INFECTED BY *CUCUMBER MOSAIC VIRUS*. **M.G. Bellardi[1], A. Benni[1], S. Davino[2], S. Grandi[3], M. Davino[2] and R. Piccaglia[3].** *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Alma Mater Studiorum, Università degli Studi, Via G. Fanin 42, 40127 Bologna, Italy. [2]Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via S. Sofia, 100, 95123 Catania, Italy. [3]Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Agronomia, Alma Mater Studiorum, Università degli Studi, Via G. Fanin 44, 40127 Bologna, Italy. E-mail: mariagrazia.bellardi@unibo.it*

Dipsacus silvestris Miller (or Fuller's Teasel) (*Dipsacaceae*) showing stunting, chlorotic leaf mosaic and narrowing of the leaf blades, was found naturally infected by *Cucumber mosaic virus* (CMV) for the first time in Italy. This virus was detected by PAS-ELISA and molecularly characterized by RT-PCR using specific primers for the movement protein gene of RNA-3 (*forward* 5' CTAGGCTTTC-CAAGCTACAG 3'; *reverse* 5' CTAAAGACCGTT AACCACCT-GC 3'). The content of glucose, fructose and saccharose, determined by HPLC analysis, was calculated in both healthy (h) and CMV-infected (i) *D. silvestris* plants during the chronic infection period. An higher content of these carbohydrates was observed in healthy leaves and roots: 5.57% (h) and 1.98% (i) in the leaves; 12.47% (h) and 6.49% (i) in the roots. In particular, roots of CMV-infected plants, had a significantly lower glucose level (5%) than roots of healthy plants (8,73%), A similar behaviour was shown by fructose content (1.5% in infected roots and 3.35% in the healthy ones). These results tally with previous reports indicating that, in virus-infected plants, decreased phytosyntetic activity coupled with increased respiratory rate, produce lower carbohydrate concentration. Considering that some of the most important components of *D. sylvestris*, a medicinal plant, are glucosides (i.e., scobioside), determination of sugar content, in particular glucose level, can be useful for the market value of the drug.

SURVEY OF VIRUS DISEASES OF *POLYGALA MYRTIFOLIA* IN LIGURIA. **M.G. Bellardi[1], A. Benni[1], S. Davino[2], M. Davino[2] and G. Bozzano[3].** *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Alma Mater Studiorum, Università degli Studi, Via G. Fanin 42, 40127 Bologna, Italy. [2]Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di di Patologia Vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [3]Società Cooperativa L'Ortofrutticola, Via Dalmazia 169, 17031 Albenga (SV), Italy. E-mail: mariagrazia.bellardi@unibo.it*

Polygala myrtifolia L. (myrtle-leaf milkwort; *Polygalaceae*) is an attractive evergreen shrub, originating from South Africa, recently introduced in Liguria (northern Italy) where it is propagated by cuttings. In 2006, severe virus-like symptoms consisting of leaf mosaic, narrowing of the leaf blades, yellow spots, vein yellowing, white necrotic spots, rings and line-patterns on oldest leaves and variegation on younger leaves, were observed on plants growing in different nurseries in the Imperia province. No flower symptoms were observed. Since in 2002 *Polygala* was reported in Italy as a new natural host of *Cucumber mosaic virus* (CMV), preliminary PAS-ELISA tests were carried out to check for the presence of this virus. CMV alone was detected in association with chlorotic mosaic symptoms. In plants showing yellow veins and spots, potyvirus-like filamentous particles were observed under the electron microscope. In IEM ("decoration") and PAS-ELISA tests, this virus proved to serologically related distantly to *Soybean mosaic virus* and strongly to *Bean yellow mosaic virus*. *P. myrtifolia* showing rings and line-patterns was positive (PAS-ELISA) to the Batavian lettuce strain of *Tomato spotted wilt virus* (TSWV: PVAS-450; American Type Culture Collection, Manassas, VA, USA). RT-PCR was done using specific primers for the CP gene of TSWV (forward 5'-TTA ACT TAC AGC TGC TTT-3'; reverse 5'-CAA AGC ATA TAA GAA CTT-3'). All samples yielded DNA fragments of the expected size (823 bp). Considering that in 1940 wild *Polygala* in South Africa was reported as a probable host of tospoviruses (unconfirmed report), our study establishes *P. myrtifolia* as a new natural host for TSWV.

SURVEY OF VIRUS DISEASES OF RANUNCULUS HYBRIDS IN LIGURIA. **M.G. Bellardi[1], P. Restuccia[2], M. Zanini[1] and V. Vicchi[3].** *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Alma Mater Studiorum, Università degli Studi, Via G. Fanin 42, 40127 Bologna, Italy. [2]Cooperativa Riviera dei Fiori, Regione Prati e Piscine, 18011 Arma di Taggia (IM), Italy. [3]Servizio Fitosanitario Regione Emilia-Romagna, Via di Corticella 133, 40129 Bologna, Italy. E-mail: mariagrazia.bellardi@unibo.it*

In 2005-2006, two hundred samples of Ranunculus hybrids collected in the Sanremo area (Liguria, northern Italy) were examined for the presence of viruses associated with severe symptoms on the leaves (chlorosis, mosaic, "parsely-like" appearance, vein-yellowing, necrotic spots and rings), flowers (colour breaking, malformations) and/or with premature death of the plants. Through mechanical inoculations to herbaceous plants, electron microscopy ("leaf-dip"), DAS and PAS-ELISA, IEM ("decoration"), and RT-PCR, virus particles of different length (from 700 to 800 nm) and diameter (from 30 to 90-100 nm) were detected singly or in mixed infections. *Cucumber mosaic virus* (CMV), alone in a few cases, more frequently in mixture with *Tomato spotted wilt virus* (TSWV) occurred in plants showing leaf mosaic, necrosis, stunting and severe symptoms on the flowers. Both TSWV and *Impatiens necrotic spot virus* (INSV), in some cases in assoaciation with the Ranunculus strain of *Potato virus Y* (PVY-R), were detected in plants characterized by mosaic, necrosis and "parsely-like" apperance of the leaves. *Turnip mosaic virus* (TuMV) and other unidentified potyvirus-like viruses were also encountered. CMV and all potyviruses prevailed in Autumn 2006 possibly as a consequence of the heavy aphid infestations. Our

resaults clearly show not only that different virus species are widely present in Ranunculus crops in the Sanremo area, but also that a correlation between symptom expression and infecting virus is very difficult to establish. Considering the increased economic damages to cut flower production recently observed, control measures to prevent virus spreading are required.

TOSPOVIRUSES INFECTING *RUSCUS RACEMOSUS* (*DANAE RACEMOSA*) IN LIGURIA. M.G. Bellardi[1], V. Vicchi[2], A. Benni[1] and P. Restuccia[3]. *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Alma Mater Studiorum, Università degli Studi, Via G. Fanin 42, 40127 Bologna, Italy. [2]Servizio Fitosanitario Regione Emilia Romagna, Via di Corticella 133, 40129 Bologna, Italy. [3]Cooperativa Riviera dei Fiori, Regione Prati e Piscine, 18011 Arma di Taggia (IM), Italy. E-mail: mariagrazia.bellardi@unibo.it*

Ruscus racemosus (*Danae racemosa*) is an ornamental species with decorative foliage whose branches are used in flower arrangements. In the Ligurian Riviera (northern Italy) this shrub is cultivated on a surface of 330 ha and the cut foliage produced is almost all exported to Europe and the United States. In 2005-06, surveys of *R. racemosus* crops in the Sanremo area, revealed different types of leaf symptoms consisting of concentric yellow and necrotic rings, necrotic spots (from a few mm to *ca* 1 cm in size), necrosis and bronzing of the leaf lamina. In 2005, the percentage of diseased plants showing necrotic rings in the open field was about 60-70%; similar percentages of symptomatic plants were also observed in 2006. Preliminary electron microscope observations ("leaf dip") did not show the presence of filamentous virus particles. Considering that ten years ago *Impatiens necrotic spot virus* (INSV) was found infecting *D. racemosa* in Portugal (Louro D., *Acta Hortic.* **431**: 99-105, 1996), testing by DAS and PAS-ELISA and RT-PCR was done to check the presence of tospoviruses. Single infections by *Tomato spotted wilt virus* (TSWV) and INSV were detected in plants showing necrosis and spots on the leaves. However, when in mixed infection, these viruses were associated with the other types of leaf symptoms described, and to premature death of the plants. This survey confirms once again the great variability of symptoms induced by tospoviruses in ornamental species and that their spreading in Liguria is increasing.

CONTROL OF QUARANTINE DISEASES IN EMILIA ROMAGNA: THE CASE OF *PEACH MOSAIC VIRUS*. L. Bianchi[1], A. R. Babini[2], C. Ratti[1], A. Pisi[1], G. Filippini[1], V. Vicchi[2], L. Giunchedi[1] and C. Rubies Autonell[1]. *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Alma Mater Studiorum, Università degli Studi, Viale Fanin 40, 40127 Bologna, Italy. [2]Servizio Fitosanitario Regione Emilia Romagna, Via di Corticella 133, 40129 Bologna, Italy. E-mail: concepcion.rubies@unibo.it*

In Italy, quarantine pest controls on plant material introduced from non UE countries are in charge of the Regional Plant Protection Service (D.L.214/2005) which collaborates with accredited laboratories whenever the need arises. In 2006, three peach plants, obtained from budsticks of accession 32-3 imported in Emilia-Romagna from the United States, and maintained in a quarantine screenhouse, showed in spring a chlorotic yellow mottling of the leaves which did not match symptoms induced by viruses known in Europe. Following graft inoculation, the indicator GF 305 showed leaf symptoms similar to those described above. Leaf dip observations by electron microscope of samples collected from infected GF 305, showed the presence of filamentous virus particles morphologically similar to those of the trichoviruses *Peach mosaic virus* (PcMV), a quarantine pest reported in EPPO list (Annex 1) and in D.L. 214/2005 (Annex 1, A, 1) and *Cherry mottle leaf virus* (CMLV). RT-PCR assays using PcMV specific primers yielded amplicons of the expected size. A sequence of 2.3 kbp, corresponding to the 3' region of the PcMV and CMLV genome was subsequently obtained from the PCR-amplified products which shared 87% and 72% identity with published sequence of PcMV and CLMV, respectively, confirming the presence of PcMV in imported budsticks. The case of PcMV confirms the efficient interaction of the Regional Plant Protection Service and scientific laboratories capable of handling advanced diagnostic tools, in preventing the introduction and dissemination of quarantine pests.

BIOLOGICAL CONTROL OF CITRUS GREEN MOULD WITH *PSEUDOMONAS SYRINGAE*. A. Bonaccorsi and G. Cirvilleri, *Dipartimento di Scienze e Tecnologie Fitsanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: abonacco@unict.it*

Pseudomonas syringae strains were tested for their antagonistic properties to control the post-harvest pathogen *Penicillium digitatum.* Several strains and their culture filtrates reduced the growth of the pathogen in *in vitro* assays. The incidence and severity of green mould on lemons (*Citrus lemon* Burm cv. Femminello) and oranges (*Citrus sinensis* Osbeck cv. Tarocco) were consistently reduced when antagonists were co-inoculated in citrus wounds with the pathogen. One of the most active strain, *P. syringae* 48SR2, was genetically tagged with the promoterless *lux* operon *Tn4431* to monitor population dynamics *in vivo.* Four hundred thirteen mutants were obtained and diverse bioluminescent activities were observed according to the insertion of *Tn4431* into a wide variety of regions of the chromosome. A selected strongly bioluminescent mutant (*lux* 176) and the wild-type strain equally reduced the growth of *P. digitatum in vitro* and the severity and incidence of citrus decay *in vivo*. Population size of both wild-type and *lux*-mutant strains, followed over time with dilution plating techniques and with bioluminescence detection systems, remained similar over 1 week in citrus wounds. These results indicate that *P. syringae* strain 48SR2 could be considered a biological control agent for citrus green mould and that bioluminescence can be a sensitive detection method to study population dynamics and antagonistic behaviour during fruit storage.

SOIL AMENDMENT WITH CROP RESIDUES, ORGANIC WASTE, COMPOST AND PEAT FOR THE CONTROL OF SOIL-BORNE FUNGAL PATHOGENS. G. Bonanomi, V. Antignani, C. Pane and F. Scala. *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: giulianobonanomi@hotmail.com*

The utilization of organic matter (OM) has been proposed, both for conventional and biological agricultural systems, to decrease the incidence of plant diseases caused by soil-borne pathogens. In this work we reviewed the literature on the suppressive capacity of different OM materials and the response of different soil-borne pathogens to OM amendments. A total of 252 articles were included in the analysis, with 1964 experimental study cases. The effect of OM amendments was suppressive in

45% and non-significant in 35% of the cases, respectively. In 20% of the cases, a significant increase of disease incidence was observed. Compost was the most suppressive material, with more than 50% of the cases with effective disease control. Activity of crop residues was more variable. Disease incidence was suppressed in 45% but enhanced in 28% of the cases. Finally, a significant disease suppression with peat was recorded only in 4% of the experiments. OM suppressivity largely varied with different pathogens: it was observed in more than 50% of the cases for *Verticillium*, *Thielaviopsis*, *Fusarium*, *Phytophthora* and *Pythium*. In contrast, an effective control of *Rhizoctonia solani* was achieved only in 26% of the cases. From this review it emerges that application of OM amendments has a great potential but, at the same time, it presents some inconsistencies. Further investigations on the mechanisms by which OM acts on disease suppression are needed to make the use of these materials predictable.

EFFECTIVENESS OF SOIL SOLARIZATION WITH BIODEGRADABLE MATERIALS AND IMPACT ON THE SOIL MICROBIAL COMMUNITY. G. Bonanomi[1], M. Chiurazzi[2], G. Del Sorbo[1], G. Moschetti[3] and F. Scala[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055, Portici (NA), Italy. [2]Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli, Via Università 100, 8005 Portici (NA), Italy. [3]Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. E-mail: giulianobonanomi@hotmail.com*

The increasing concern about the impact of mineral fertilizers, fungicides and herbicides on the environment and human health requires the development of alternative agronomic techniques that may reduce the use of these products. Soil solarization, based on the use of plastic sheets to increase soil temperature, seems one of the most promising techniques for the control of soil-borne pathogens. However, an important limitation to the diffusion of this technique comes from the drawbacks regarding the disposal of the used plastic materials. A possible solution to this problem may be found in the use of biodegradable plastics. These materials degrade gradually when plowed-down in the soil because of microorganism activity. The aim of this study was to compare the impact of soil solarization with plastic films and biodegradable materials on crop productivity, soil-borne disease incidence, weed suppression, and soil chemical and microbial parameters. We carried out field experiments on two types of soil with different textures (clay and sandy) located in southern Italy, which were artificially inoculated with *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotinia minor*. The results showed the potential of the use of the biodegradable solarizing materials in place of plastic films, but indicate the need to improve their properties to afford performances comparable to those of other pest management techniques.

FUNGAL ENDOPHYTIC COMMUNITIES DETECTED IN DECIDUOUS OAK TREES FROM SOUTHERN ITALY. E. Boncaldo, G. Sicoli, F. Mannerucci and N. Luisi. *Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: luisin@agr.uniba.it*

In four oak woods located in different regions of southern Italy, a study was carried out focusing on the composition and seasonal variation of fungal endophytic communities inhabiting epigeous tissues of healthy and declining trees of *Quercus pubescens* Willd. and *Q. cerris* L. In total, 17 fungal taxa were detected including potentially pathogenic organisms, many of which were agents of bark necrosis, as well as indifferent or probable antagonists. The most common pathogen was *Discula quercina* (West) von Arx, that has an isolation frequency (IF) of 10.7% and occured in both oak species, more specifically in buds, followed by *Biscogniauxia mediterranea* (De Not.) O. Kuntze (IF = 5.4%), which was prevalent in leaves and bark, and *Diplodia corticola* A.J.L. Phillips, Alves et Luque (IF = 4.3%). Lower values of IF were shown by *Phomopsis quercina* (Sacc.) Hohn and species in the genera *Cytospora* and *Phoma. Aureobasidium pullulans* (de Bary) G. Arnaud (IF = 25%) was the most common nonpathogenic fungus, isolated primarily from the leaves. By comparing IF values from spring to autumn, most fungal species seemed to prefer autumn as the most suitable season for host tissue colonization.

DEVELOPMENT OF PCR PRIMERS FOR THE IDENTIFICATION OF *CYLINDROCLADIUM PAUCIRAMOSUM*. I. Camele and L. Altieri. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: camele@unibas.it*

Cylindrocladium spp. are reported worldwide as the incitants of root and crown rot, stem canker, leaf spot and seedling and shoot blight of wild and cultivated plants. *Cy. pauciramosum* C.L. Schoch et Crous (teleomorph *Calonectria pauciramosa*) has a wide host range and attacks many ornamental plants. In southern Italy, the fungus has caused extensive losses requiring chemical control measures. It was recently reported in Italy on *Polygala myrtifolia* and identified on the basis of morphological characters. However, these diagnostic procedures are expensive and time consuming. For improving identification and detection of *Cy. pauciramosum* PCR primers CPAUCIR (GCTTTCTGGCAGACCATTTC) and CPAUCIF (TGTCAGTTGGCTTGTGCTTC) were designed on the sequence of the β-tubulin gene. These primers amplified a 161pb fragment from all *Cy. pauciramosum* pure cultures tested but failed to amplify DNA from isolates of many other fungi or stramenopiles used as control. Specificity of the primer pair was verified by PCR assay using the annealing temperature of 55°C. Further studies will be conducted to verify the possibility to distinguish *Cy. pauciramosum* from closely related species (*Cy. scoparium* and *Cy. candelabrum*) by molecular methods.

STUDIES ON POST-HARVEST ORANGE ROT CONTROL WITH ESSENTIAL OILS. I. Camele[1], V. De Feo[2], L. Altieri[1], E. Mancini[2] and G.L. Rana[1]. *[1]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. [2]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Mellillo, 84084 Fisciano (SA), Italy. E-mail: camele@unibas.it*

Twelve essential oils, extracted by hydrodistillation, were tested at different concentrations against four fungi known to cause post-harvest rot of orange fruit, i.e. *Botrytis cinerea, Penicillium expansum*, *Phytophthtora citrophthora* and *Mucor* spp. Plant species used as a source of these volatile fractions were *Carum carvi*, *Foeniculum vulgare, Pimpinella anisum* (*Apiaceae*), *Hyssopus officinalis*, *Lavandula angustifolia*, *Majorana hortensis*, *Melissa officinalis, Ocimum basilicum*, *Origanum vulgare*, *Salvia officinalis*, *Thymus vulgaris* (*Lamiaceae*), and *Verbena officinalis* (*Verbenaceae*). Preliminary experiments carried out *in vitro* showed that only essential oils from *V. officinalis*, *T. vulgaris* and

O. vulgare showed some fungistatic action against the above fungi. Thus, only these three essential oils were used in successive *in vivo* tests to protect healthy orange fruits of cv. Washington from artificial infection by the same micromycetes. Essential oil from *T. vulgare* at 2000 ppm controlled fruit rot by *B. cinerea*, *P. citrophthora* and *Mucor* spp. but was ineffective against *P. expansum*. Essential oils from *V. officinalis* and *O. vulgare* inhibited infection by the former couple of fungi and by *P. citrophthora* only, respectively.

MYCOFLORA ASSOCIATED WITH DURUM WHEAT KERNELS PRODUCED IN SICILY. V. Campanella and C. Miceli. *Ente Nazionale delle Sementi Elette, Viale Regione Siciliana S-E 8669, 90121 Palermo, Italy. E-mail: ense-palermo@ense.it*

The results of investigations on the mycoflora associated with durum wheat seeds, are reported. Analyses were done on 443 durum wheat samples, collected in 2003-05, from conventional and organic crops, in the main cereal growing areas of Sicily. Fungi were isolated plating surface disinfested seeds on a semi-selective media and identified on the basis of colony morfology and fruiting structures produced on the seeds. No substantial differences were observed on seed-borne fungi contamination between conventional and organic samples. Ubiquitary, saprophytic or weakly pathogenic species, among which *Alternaria* spp., *Cladosporium* spp., *Gonatobotrys* spp., *Nigrospora* spp., *Penicillium* spp., *Rhizopus* spp. and *Stemphylium* spp., were found in all samples from both cropping systems. *Alternaria* spp., was the most frequently isolated microorganism ranging from 45.1% to 90.6% in conventional samples and from 36.7% to 82.0% in organic samples. A progressive yearly increase of pathogenic *Fusarium* spp. was observed. In particular, these pathogen occurred on 18.2%, 52.2% and 60.6% in 2003, 2004 and 2005, respectively. *F. poae* was the predominant species found in samples from both cropping systems. Seed germination was not significantly affected by the presence of fungal contaminants, being in average 91.2%, 90.9% and 91.9% in 2003, 2004 and 2005, respectively. Our data show a satisfactory sanitary condition of durum wheat kernels, characterized by the low presence of the most aggressive species of *Fusarium*, associated with head blight, foot rot, and seedling blight.

EFFECTS OF GRAIN LEGUMINOUS CROPS ON POPULATION AND GENETIC VARIABILITY OF SOIL SPORE-FORMING BACTERIA WITH ANTIBIOSIS ACTIVITY. F. Campanile, D. Villecco, A. Del Galdo and M. Zaccardelli. *CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy. E-mail: massimo.zaccardelli@entecra.it*

Spore-forming bacteria, one of the most important group of microorganisms with high antibiosis activity, are interesting for biological control. In this work, in a soil of the Sele Valley (southern Italy), spore-forming bacterial populations were evaluated in May and July 2006 during cultivation of wheat and three leguminous crops (pea, faba bean and chickpea) and in November, during cultivation of fennel that followed the previous crops. Samples (three for each crop and time of collection) were collected from the first 20 cm of soil in proximity of the roots, sieved through 2 mm screens and, washed for 1 h in physiological solution. Ten-fold dilutions were made an aliquot of which was plated and incubated after treatment at 90°C for 10 min. The colonies counted were purified and tested for antibiosis activity against *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phoma* sp. and *Ascochyta* spp. The bacterial isolates that showed antibiosis activity were then characterized for DNA polymorphism by M13-PCR. No difference in the number of soil spore-forming bacteria was observed among the four crops in May, whereas their number was higher (1.8×10^6 g^{-1} of fresh soil) from soil samples cultivated with chickpea collected in July. A high number of spore-forming bacteria collected during cultivation of wheat, leguminous crops and fennel, showed antibiosis activity against the tested fungi. Genetic variability of these potentially antagonistic bacteria was very high, in fact, 28 haplotypes were obtained on the whole.

ROOT ROT DISEASE BY *HETEROBASIDION ANNOSUM* ON *FAGUS SILVATICA* STANDS IN SOUTHERN ITALY. P. Capretti, N. Luchi and G. Mazza. *Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: paolo.capretti@unifi.it*

Occurrence and persistence of the root rot pathogen *Heterobasidion annosum* (Fr.) Bref. in infested areas and its transfer to different host species was studied in different forest sites in Calabria (southern Italy). This region is characterized by annual rainfalls ranging from 1500 to 1900 mm, mostly concentrated during the dormant season, the mean annual temperature is comprised between 10,6 and 11,5°C and soils are mostly sandy. The sites consisted of adult (*ca* 50-60 year-old) mixed stands established with *Acer platanoides, Fagus sylvatica*, and *Pinus laricio* planted on former pasture or agricultural land. The investigation showed that *H. annosum,* which was present in contiguous *Pinus laricio* stands, was able to persist in the root systems of pines for long time and was able to infect nearby beech trees via root contacts and to colonize beech stumps after occasional felling. Damage mainly consisted of uprooting of large beech trees which is unusual for this species in Italy. Isolations from trees and stumps of different sites showed high frequencies of the fungus on declining trees which produced large carpophores on the roots and on the stumps. Infected trees often exhibited top die-back connected with the occurrence of *Biscogniauxia nummularia* ascomata on dead branches.

***RHIZOCTONIA SOLANI* ANASTOMOSIS GROUPS PATHOGENIC TO WHITE LUPIN (*LUPINUS ALBUS*) IN CAMPANIA. A. Carella, E. Cozzolino and R. Nicoletti.** *CRA, Istituto Sperimentale per il Tabacco, Via Vitiello 108, 84018 Scafati (SA), Italy. E-mail: rosario.nicoletti@entecra.it*

The polyphagous pathogen *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris*) is a collective species including at least 13 anastomosis groups (AGs). AGs 1 through 5, AG-8, AG-10 and AG-11 have been reported from *Lupinus* spp., but so far no definite relationship between AG-belonging and virulence of the isolates has been established. *R. solani* and other soil-borne fungi causing crown and root rot represent the major phytopathological problem of white lupin in the Caserta area (souhern Italy) where this crop species has been proposed as an alternative to tobacco. Isolates recovered from diseased plants proved to belong to AG-2-1 and AG-4, with the latter resulting more virulent in greenhouse tests. Both AGs had been previously recovered from tobacco in the same area, but AG-2-1 isolates were ascribed to a new infraspecific group (AG-2-1-Nt) on the account of a peculiar zymogram and low anastomosis frequency with standard AG-2-1 tester isolates. Unlike AG-4 isolates, for which a substantial similarity in fusion frequency and pectic zymograms was observed with isolates from tobacco and wormweed collected in the same area, no correspondence with AG-2-1-Nt was found for AG-2-1

isolates from lupin. In fact, an equivalence of the latter to the standard AG-2-1 tester isolates resulted in both anastomosis frequency and zymography. These results are at least in part indicative of the absence of an epidemiological role of tobacco precession, and confirm Nt-isolates as a distinct and phylogenetically diverging grouping within AG-2-1.

EFFECTS OF PHYSICAL, BIOLOGICAL, AND INTEGRATED CONTROL MEASURES ON THE REDUCTION OF *VERTICILLIUM DAHLIAE* SURVIVAL AND OF *PYRENOCHAETA LYCOPERSICI* INFECTIONS. I. Castello, A. Vitale and G. Polizzi. *Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail:gpolizzi@unict.it*

During 2006-2007, two experiments were carried out in tomato plastic-houses in south-eastern Sicily. Trials allowed to study the effectiveness of soil solarization treatments using innovative plastic mulching films alone or in combination with biological antagonists (*Trichoderma harzianum* T22*, T. harzianum* ICC 012, and *T. viride* ICC 080) and amendments (*Brassica juncea* and Biofence). Solarization of the soil of plastic-houses with no film covering lasted 2 months. The survival of microsclerotia of *Verticillium dahliae* was reduced in all solarized or amended plots after 22 days and 43 days treatment, respectively. The highest reduction was obtained in soil plots solarized with the film "Fumè" and in those covered with the film "Green coextruded" in combination with *Brassica juncea*. Solarization alone and in combination with biological antagonists and amendments resulted in the effective control of infections by *Pyrenochaeta lycopersici.* Solarization integrated with *T. harzianum* T22 was significantly more effective than the corresponding solar treatment alone. Treatments with biological antagonists or with amendments alone did not reduce the severity of tomato corky rot in comparison to control plots. Our results confirmed the efficacy of soil solarization in open plastic-houses with innovative plastic films for the control of corky rot infections and for reducing the survival of microsclerotia of *V. dahliae.*

***IN VIVO* ANTIBIOGRAM TO TEST THE ACTION OF SYNTHETIC AND NATURAL COMPOUNDS ON PHYTOPLASMAS. S. Chiesa, S. Prati, G. Assante, D. Maffi and P.A. Bianco.** *Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: serena.chiesa@unimi.it*

With the aim of finding new strategies to control phytoplasmal diseases, a series of natural and synthetic compounds were tested to study their activity in *Catharanthus roseus* L. healthy and graft-infected with two phytoplasmas (*Candidatus* Phytoplasma asteris and *Candidatus* Phytoplasma ulmi). A preliminary investigation was carried out by testing the responses of plants to treatment with tetracycline. The compound was applied in several ways (spraying, root absorption and scion dipping), and at different concentrations and number and timing of treatments. The results obtained showed that scion dipping was the most suitable method in our experimental conditions, as it needed the lowest amount of compound and permitted the quantification of the compound absorbed. Thus, this procedure was adopted to test some natural substances never assayed before in this host-pathogen system: (i) Cercosporin, Spirolaxin and Cladosporol, organic compounds of fungal origin with alleged antibiotic activity against phytopathogenic bacteria; (ii) Carvone and Pulegone, two active terpenoids of plant origin; (iii) the mineral salt Potassium Alum; (iv) the synthetic basic Dienes stain and (v) a Methionin-Riboflavin mixture. The activity of these compounds was evaluated on the basis of phytotoxicity, symptom evolution and microscope observations. This testing method is likely to provide a sustainable and rational procedure for investigating the triangle host-phytoplasma-compound. It will be adopted in further research to estimate the anti-phytoplasmal activity of other known and novel chemicals, both synthetic and natural.

INTEGRATED MANAGEMENT OF MELON COLLAPSE CAUSED BY *MONOSPORASCUS CANNONBALLUS*. G. Chilosi, R. Reda, M.P. Aleandri, M. Antonelli, L. Varvaro and P. Magro. *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: chilosi@unitus.it*

In recent years melon crops in the province of Viterbo (central Italy) were subjected to significant losses in yield and quality due to infections by the soil-borne fungal pathogen *Monosporascus cannonballus*. The objective of this 3-year field study was to evaluate different management strategies for controlling this disease. Among sanitation methods, the destruction of crop residues including roots effectively reduced disease incidence. Soil treatments with steam contributed to disease control in the short-term, whereas, the use of soil fumigation and solarisation did not provide satisfactory results. Melon cultivars differed in terms of tolerance. Grafting proved to be an effective measure only when more tolerant varieties were grafted on squash, whereas grafting on melon rootstocks was consistently ineffective. In recent years, chemical resistance inducers became an attractive alternative for controlling soil borne pathogens. Among a set of inducers tested, methyl jasmonate decreased disease incidence. Mycorrhizal fungi and antagonistic fungi and bacteria contribute to soil fertility and crop yield by limiting the development of pathogens and inducing resistance in plants. We have isolated from non-cultivated soils of the area, a group of beneficial bacteria able to inhibit growth of *M. cannonballus*. The treatment of composts with local biocontrol agents may provide an additional tool for controlling the pathogen.

REVERSE-TRANSCRIPTION REAL-TIME PCR ASSAY TO DETECT VIABLE PROPAGULES OF *PHYTOPHTHORA RAMORUM* IN INFECTED PLANT TISSUES. A. Chimento[1,2], S.O. Cacciola[2] and M. Garbelotto[1]. *[1]Department of Environmental Science, Policy and Management, University of California, 137 Mulford Hall, Berkeley, CA 94720-3114, USA. [2]Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. E-mail: antchimento@yahoo.it*

A new reverse-transcription real-time PCR (RT-PCR) assay was developded, based on the use of mRNA as a marker to detect viable propagules of *Phytophthora ramorum* Werres, De Cock & Man in't Veld. This method is based on the rapid degradation of mRNA compared to DNA. New primers specific for *P. ramorum* were designed in the cytochrome oxidase gene encoding subunits I (COXI). To evaluate the specificity of the method, four isolates of *P. ramorum* and reference isolates of eleven different *Phytophthora* species were tested. Sixty leaves of Californian bay laurel [*Umbellularia californica* (Hook. & Arn.) Nutt.] with symptoms of sudden oak death disease from three different sites in California (USA) and sixty symptomatic leaves of Californian bay laurel collected in three different seasons of the year from China Camp State Park (CA) were plated on PARP selective medium and test-

ed with the RT-PCR assay in comparison with a TaqMan and a SybrGreen Real-Time PCR assay, after a classical DNA extraction. Results showed that after nine days RNA of *P. ramorum* killed by freeze-drying was undetectable while DNA gave a positive signal. Furthermore, differently from method based on DNA analysis, the results of the RT-PCR assay were correlated with positive isolations on selective media, thus indicating that the risk of PCR false positives can be circumvented by using this RT-PCR method.

GENE EXPRESSION PROFILING IN TOMATO PLANTS INFECTED BY FOUR COMBINATIONS OF *CUCUMBER MOSAIC VIRUS* AND ITS SATELLITE RNAs: A MICROARRAY ANALYSIS. F. Cillo[1], A. Polverari[2], M. Pasciuto[1], D. Glissant[3], M. Delledonne[3] and D. Gallitelli[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. [2]Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino, Villa Ottolini-Lebrecht, 37029 San Floriano di Valpolicella (VR), Italy. [3]Dipartimento Scientifico e Tecnologico, Università degli Studi, Strada Le Grazie 15, 37134 Verona, Italy. E-mail: f.cillo@ba.ivv.cnr.it*

An analysis of transcriptional changes in tomato plants, induced by the infection of *Cucumber mosaic virus*, alone or in combination with satellite RNA (satRNA) variants, has been made by microarray analysis. The analysis was performed on the newly developed CombiMatrix platform at the University of Verona, on a tomato chip carrying 20200 specific probes in quadruplicates from assembly of Tentative Consensus of the last Tomato Gene Index (LeGI), release 11.0 (June 21, 2006). The CombiMatrix CustomArray ^{TM} technology is characterized by an exclusive *in situ* oligo (up to 40 mers) synthesis driven by electrochemistry and by the reusability of the same microarray chip, all factors that confer high flexibility to the system and reduce remarkably the costs of microarray analysis. Tomato plants cv. UC82 were infected with the aggressive strain CMV-Fny and three more strains, in which CMV-Fny was associated with three different satRNAs (benign variant: CMV-Fny/Tfn-satRNA; stunting variant: CMV-Fny/TTS-satRNA; necrogenic variant: CMV-Fny/77-satRNA). Mock-inoculated plants were used as negative controls. Gene expression was examined at 2 and 9 days post inoculation, to analyse transcriptional changes associated with the different disease phenotypes in both locally and systemically infected leaf tissues. Hybridisations were carried out with samples deriving from three independent biological replicates. Differentially expressed genes were selected and analysed using the multi experiment Significance Analysis of Microarray test, and gene clustering was performed using Genesis software. This is the first time that the CombiMatrix platform for microarray analysis has been applied to the study of tomato-virus interactions.

***POTATO VIRUS Y*-LF, A PUTATIVE NEW RECOMBINANT PVY ISOLATE. S. Comes, P. Tarantino, R. Pacella, A. Fanigliulo and A. Crescenzi.** *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: aniello.crescenzi@unibas.it*

Serious symptoms of leaf necrosis, necrotic mottling along leaflet veins, rachis distortion and irregular fruit ripening were observed in tomato idroponics in Calabria (southern Italy) in the last five years. Preliminary serological tests associated these symptoms with infection by *Potato virus Y* (PVY). The virus was isolated on *Nicotiana tabacum* "Xanthi" and characterised biologically by mechanical inoculation to several herbaceous hosts. Molecular characterisation was performed by the amplification, cloning and sequencing of two genomic regions: P1 and coat protein (CP). The identity of the virus was ascertained with monoclonal antibodies (Mab) specific for PVY^N, PVY^O and PVY^C: the isolate reacted only with the PVY^C -specific Mab. Immuno capture reverse transcription polymerase chain reaction (IC-RT-PCR) was carried out on TNA extracted from PVY-LF infected plants, using two couples of primers specifically designed on the P1 and CP of other fully sequenced PVY isolates. PCR products of the expected size were directly cloned into a vector and sequenced. Analysis of P1 and CP coding regions suggests that PVY-LF originated from a putative recombination event between a virus of the PVY^O type and another parental virus, maybe a PVY^{NP} isolate, because of the high similarity shared by PVY-LF with "non potato" PVY isolates in the CP coding region and PVY^O isolates in the P1 coding region.

USE OF ANTAGONISTIC MICROORGANISMS FOR THE CONTROL OF FUSARIUM SEEDLING AND HEAD BLIGHT OF BREAD WHEAT. L. Covarelli, M. Quaglia, E. Pannacci, G. Beccari and C. Cappelli. *Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno, 74, 06121 Perugia, Italy. E-mail: lorenzo.covarelli@unipg.it*

In 2006 and 2007 the effect of several antagonists in laboratory and field experiments against Fusarium seedling blight and Fusarium head blight were carried out in the province of Perugia (Umbria, central Italy). After soil inoculation with mycelium of *Fusarium graminearum* and *F. culmorum* grown on PDA, seed dressing treatments with *Pseudomonas chlororaphis*, *Trichoderma viride*, *Bacillus subtilis*, a commercial hypovirulent strain of *F. oxysporum* and a commercial mixture of bacteria, mycorrhizae and *Trichoderma* spp., were compared in the laboratory with chemical seed dressing treatments with carboxin+maneb and triticonazole+guazatine. One day before head inoculation with a *F. culmorum* spore suspension at mid anthesis, *Trichoderma viride*, *Bacillus subtilis*, *F. oxysporum*, *Ampelomyces quisqualis* and *T. harzianum*, were sprayed in the field, on wheat plots and compared with chemical treatments with prochloraz+propiconazole and azoxystrobin. Results showed that all the utilised antagonists did not give a satisfactory control of Fusarium seedling and head blight in comparison with chemical treatments. However, the pedo-climatic characteristics of the experimental areas were not favourable to natural infections, as shown in other host-pathogen combinations (i.e., barley-*Ustilago nuda*, linseed-*Alternaria linicola*, etc.). Therefore, in some areas of Umbria, a satisfactory prevention of the diseases in question for production of healthy wheat seed in organic farming, could be afforded by sowing pathogen-free seed and by decreasing fungal inoculum with agronomic practices, such as crop rotation with non-host species and soil tillage.

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HORIZONTAL GENE TRANSFER FROM GENETICALLY MODIFIED TOMATO PLANTS TO *PSEUDOMONAS SYRINGAE* pv. *TOMATO* AND *RALSTONIA SOLANACEARUM*. L. Cozzolino[1], A. Zoina[1] and. A. Raio[2]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Istituto per la Protezione delle*

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Since antibiotic resistance markers are most frequently used for plant transformation, there is considerable concern about the possibility that these genes are transferred from transgenic plants to bacteria. In fact, bacteria that have developed a state of competence, may integrate foreign DNA into their genome by natural transformation and then express the newly acquired genes. To appraise the possibility that recombinant DNA will be transferred from genetically modified (GM) tomato plants to *Pseudomonas syringae* pv. *tomato* and *Ralstonia solanacearum*, both bacteria were first made competent to recombination, then transformed by inserting two different broad host range plasmids containing a deleted *nptII* genes into the cells. For these experiments transgenic tomato plants (cv. BetterBoy) transformed with prosystemin c-DNA and with *nptII* as gene marker were used. Restoration of 317 bp deleted *nptII* gene in *Pseudomonas syringae* pv. *tomato* (pBBRiTp Δ*npt*II) was detected, with extremely low frequency, after experiments of filter transformation with DNA from GM tomato plants conferring Kanamycin resistance. No kanamycin-resistant transformants were detected for *Ralstonia solanacearum* (pLaf Δ*npt*II). Probably this species did not develop DNA uptake competence under our experimental conditions. These preliminary results suggest that horizontal gene transfer may occur if homologous sequences are present both in the plant genome and in the phytopathogenic bacterium DNA.

HETEROBASIDION ANNOSUM IN THE NATIONAL PARK OF CIRCEO. L. D'Amico, M. Scirè, T. Annesi and E. Motta. *CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: e.motta@ispave.it*

During a survey for *Heterobasidion annosum* in central Italy, ten infection centres were investigated in *Pinus pinea* stands of the Circeo national park. Basidiocarps or mycelia were collected and airborne spores were trapped. The identification of the fungal isolates (about 200) was based on the taxon-specific size of the introns in the ML5-ML6 DNA region of the mitochondrial large ribosomal RNA (mtLrRNA). Sequence analyses for the nuclear elongation factor 1-α were also made in a sub-sample of homokaryotic isolates. Isolates identified in six infection centres were assigned to the Eur-P group, and to the NAm-P group in four centres. Mitochondrial introns of two size are known (1.5 kb and 1.8 kb) in isolates collected in North American forests, regardless of the geographical origin. By contrast, until now, all the NAm-P isolates collected in central Italy and studied by us showed the presence of the 1.5 kb intron only. It was therefore supposed that the Italian NAm-P population originated by a single introduction from North America. However, a group of windborne spores collected in the Circeo national park showed a 1.8 kb mitochondrial intron, whose sequence is not homologous with that of the 1.5 kb intron. Since mitochondrial DNA have an uniparental origin, this new record was taken as evidence that at least two separate introductions have taken place. NAm-P *H. annosum* is causing heavy damage in the park, especially in dry areas, such as the coastal dunes with *Juniperus* spp. and dunes with *Pinus pinea* and/or *Pinus pinaster*.

A NEW METHOD FOR DETECTING VIRUSES THAT CAUSE TOMATO YELLOW LEAF CURL DISEASE IN THE MEDITERRANEAN REGION AND THEIR RECOMBINANTS. S. Davino[1], M. Davino[1] and G.P. Accotto[2]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via S. Sofia, 100, 95123 Catania, Italy. [2]Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: wdavinonict.it*

In the Mediterranean basin two viruses have been associated with epidemics of tomato yellow leaf curl disease (TYLCD), i.e. *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) in the last two decades. Both viruses belong to the genus *Begomovirus*, family *Geminiviridae*. In the last five years, interspecific hybrids between TYLCV and TYLCSV, also known as recombinants, have been described in Spain, where the two species have coexisted for several years. Both species are currently present in tomato fields also in other Mediterranean countries, including Italy. This creates ideal conditions for recombinants to appear and spread, so new methods for detecting all viruses present in the region are necessary. We have developed a multiplex PCR/RFLP protocol that amplifies the intergenic region of the genome of all TYLCSV and TYLCV isolates known, including interspecific hybrids. After PCR, the sample is digested with *Psp*1406I restriction enzyme, which yields DNA fragments of specific size (800 to 410 bp) for each virus species and each recombinant. This new method gives, with a single reaction, an overview of the species present in the sample and will be useful for screening the causal agents of TYLCD, as well as in breeding programs for resistance.

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YELLOWING VIRAL DISORDER IN TOMATO: OCCURRENCE OF _TOMATO INFECTIOUS CHLOROSIS VIRUS_ IN SICILY. S. Davino[1], M. Meneghini[2], C. Boccongeli[3], G. Di Modica[3] and L. Tomassoli[2]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [2]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [3]Seminis Vegetable Seeds Italia. E-mail: wdavino@unict.it*

The yellowing viral disorder of tomato, consisting of interveinal leaf mottling and yellowing, is associated with two whitefly-transmitted viruses belonging to the genus *Crinivirus*, family *Closteroviridae*. *Tomato infectious chlorosis virus* (TICV) was the first causal agent of this disease to be characterized, followed by *Tomato chlorosis virus* (ToCV) identified as a distinct species. Whereas TICV is transmitted by *Trialeurodes vaporariorum*, three species, *Bemisia tabaci*, *T. abutilonea* and *T. vaporariorum,* are vectors of ToCV. ToCV has been reported in Sicily since 2001 but, even though *T. vaporariorum* is widespread in Sicily, TICV had not been detected so far. In spring 2007 different tomato samples, showing interveinal yellowing of older leaves, combined with thickening and brittleness, were collected in several greenhouses in the province of Ragusa and analyzed for virus identification. Total RNA was extracted from leaf tissue and screened by RT-PCR for the presence of both ToCV and TICV using primer sets specific for the HSP70 gene. A fragment of the expected size of *ca* 500 bp was obtained with TICV primers from samples from different greenhouses. Virus identification was also confirmed by nucleotide sequence and analysis using the BLAST programme. The virus was found either in single infection or together with ToCV. The presence of TICV aggravates the phytosanitary status of greenhouse tomato production in Sicily which is already affected by other economically important disease.

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OBSERVATIONS ON THE RAPID SPREAD OF SEVERE ISOLATES OF *CITRUS TRISTEZA VIRUS* BY *APHIS GOSSYPII*.
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The discovery of a large focus of *Citrus tristeza virus* (CTV) in Sicily has allowed to process a prediction model of CTV spreading at Baé in the province of Catania. Investigations were carried out in three different groves of sweet orange cv. Tarocco O.L. grafted on sour orange. All trees in each grove were sampled yearly in May and September from 2002 to 2007. Four young apical shoots were collected from each tree and analyzed by DAS-ELISA and DTBIA. Molecular tests, SSCP and sequencing of P20 and P23 genes, showed that the viral isolates present in these groves belonng to the seedling yellow strain (CTV-DS2). Monitoring of aphid population in all plots confirmed the results reported in preceding works, indicating that *Aphis gossypii* Glover represented 85-90% of the aphid population. Our results about the percentage infected trees are very similar to nonlinear predictive model estimation of CTV spread, reported from other citrus areas where the vector is *A. gossypii* until the 7^{th}-8^{th} year from the beginning of infection. However, after eleven years the percentage of infected trees in all plots in our nonlinear prediction model was *ca* 98%, a figure which, elsewhere, is reached in the 14^{th} -15^{th} year. This more rapid spreading of CTV in Sicily, just after the 8^{th} year of infection, may depend on the severe CTV isolate present (seedling yellow) combined with the high susceptibility of Tarocco O.L. grafted on sour orange.

ANTIFUNGAL ACTIVITY OF LEAF EXTRACTS OF LAUREL, SWEET ORANGE, AND OLIVE, OBTAINED USING THE SUPERCRITICAL CARBON DIOXIDE TECHNIQUE.
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Several plants synthesize substances (phenols, sesquiterpenes and glucosides) which possess antifungal properties and may be of interest for use in crop protection. The purpose of this work was to obtain leaf extracts with antifungal properties by an innovative process that utilise a supercritical CO_2 technique. A pilot plant at ENEA was utilized for antifungal substance extraction from dried leaves of three Mediterranean plant species: olive (*Olea europaea* L.), sweet orange (*Citrus sinensis* Osbeck) and laurel (*Laurus nobilis* L.) at conditions of 110 bar and 40°C. Antifungal activity tests were conducted *in vitro* on seven species of plant pathogenic fungi: *Alternaria alternata* (Fr.) Keissler, *Botryosphaeria dothidea* (Moug. *ex* Fr.) Ces *et* De Not., *Botrytis cinerea* Pers., *Fusarium culmorum* (W.G. Sm.) Sacc., *Penicillium digitatum* (Pers.) Sacc., *Rhizoctonia solani* J. G. Kühn and *Sclerotinia sclerotiorum* (Lib.) de Bary grown in Petri plates on potato dextrose agar (PDA). Each extract was tested at three different concentrations: 50, 125 and 250 μg ml^{-1} of PDA. The percentage of mycelial growth inhibition (MGI) was calculated as follows: %MGI = [(DMA – DE) / DMA] × 100; where DE = mycelial growth diameter in treatment sets, DMA = in control sets. The index values were statistically analysed using Duncan's test (Probability = 0,01). The highest antifungal activity was shown by *L. nobilis* extracs against *B. cinerea*. In particular, MGI was 85% when the laurel extract was given to *B. cinerea* cultures at a concentration of 250 μg ml^{-1}.

BIOLOGICAL AND INTEGRATED CONTROL OF WHEAT POWDERY MILDEW.
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Powdery mildew of wheat, caused by *Blumeria* (= *Erysiphe*) *graminis* f. sp. *tritici* (anamorph: *Oidium monilioides*), is recognized as one of the main wheat diseases. Present control measures against this pathogen are based on the use of fungicides at the pre-flowering stage. In order to find new ecocompatible control methods against wheat powdery mildew, the yeasts *Rhodotorula glutinis* (isolate LS11) and *Cryptococcus laurentii* (isolate LS28) and the yeast-like fungus *Aureobasidium pullulans* (isolate LS30), previously selected and tested for their wide range of activity against different fungal pathogens, were evaluated. In a two-year experiment carried out in wheat fields of the Molise region, the biocontrol agents (BCAs) were applied alone or in combination with a low dosage of common fungicides or with natural adjuvants. Treatments were applied by spraying them twice at (i) flag leaf stage and (ii) pre-flowering stage. Percentage of infected leaves, disease severity and the population level of BCAs on the leaves were periodically assessed and the data were subjected to variance analysis. Results showed that the highest protection from powdery mildew was given by BCAs applied in combination with some adjuvants (i.e. calcium citrate, calcium chloride, calcium propionate, soybean oil and humic acid) as well as with a low dosage of fungicides. Moreover, highest levels of the antagonist population were found on leaves treated with BCAs plus mineral salts.

MOLECULAR CHARACTERIZATION OF *BOTRYOTINIA FUCKELIANA* MUTANTS RESISTANT TO DICARBOXIMIDES AND TO THE NEW CARBOXAMIDE BOSCALID.
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The molecular bases of resistance to dicarboximide fungicides and to the new chemical boscalid were investigated in *Botryotinia fuckeliana* (de Bary) Whetz. (*Botrytis cinerea* Pers.), the agent of grey mould on numerous crops. PCR-amplified fragments of the genes encoding the target proteins, i.e. a two-component histidine kinase and succinate dehydrogenase, respectively, were sequenced and compared in sensitive and resistant strains. Different point mutations were found in the histidine kinase gene from representative field and laboratory mutants with different level of resistance to dicarboximides. In particular: (i) the substitutions at codon 365 of isoleucine with serine (I365S) or asparagine (I365B) were detected in low-resistant mutants; (ii) the two substitutions of glutamine with proline at position 369 (Z369P) and asparagine with serine at position 373 (B373S) were associated with moderate resistance; (iii) the substitutions of glycine with asparagine at position 357 (G357B) or glycine with serine at position 446 (G446S) were present in highly resistant mutants displaying different sensitivity to high osmotic pressure (a pleiotropic effect). Laboratory mutants resistant to boscalid showed single amino-acid substitutions in highly conserved cysteine-rich clusters of the iron-sulfur protein of the succinate dehydrogenase, whereas the sequences of the flavoprotein and the two transmembrane sub-

units of the enzyme did not differ in parental sensitive strains and derived mutants. The mutation from histidine to tyrosine at codon 272 (H272Y), already known for inducing resistance to carboxin in other fungi, was detected in low-resistant mutants. Proline to leucine or to phenylalanine replacements at position 225 (P225L or P225F) were found in high-resistant mutants.

A RHABDOVIRUS ASSOCIATED WITH HIBISCUS VEIN CLEARING DISEASE IN SOUTHERN ITALY. A. De Stradis[1], G. Parrella[2], C. Vovlas[3], A. Ragozzino[4]. *[1]Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. [2]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. [3]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Sudi, Via Amendola 165/A, 70126 Bari, Italy. [4]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: parrella@ipp.cnr.it*

Several commercial *Hibiscus rosa-sinensis* cultivars showing vein clearing symptoms were observed since 2005 in Campania and Calabria (Southern Italy) from which a virus was mechanically transmitted to herbaceous hosts. *Nicotiana occidentalis* and *N. clevelandii* reacted with yellow local lesions followed by systemic vein clearing, mottling, mosaic and stunting, whereas *N. benthamiana* and *N. glutinosa* developed only systemic symptoms and *Gomphrena globosa* reacted locally with reddish local lesions. No symptoms were observed in *N. rustica*, *Chenopodium quinoa*, *C. amaranticolor* and *Citrullus vulgaris*. Systemic symptoms in *Nicotiana* spp. appeared often about one month after inoculation. Electron microscope observation of leaf dips and ultrathin sections of chlorotic perninerval leaf areas revealed the consistent presence of rhabdovirus-like particles. Virions measured 230x70 nm and in thin sectioned cells were localized between the inner and outer membrane of the nuclear envelope. A similar disease of *H. rosa-sinensis* has been previously reported from Morocco, Tenerife and Rhodes. The associated virus was identified as an isolate of *Eggplant mottled dwarf virus*. This appears to be the first record of the natural occurrence of a nucleorhabdovirus in Italy in the same host. Since *Hibiscus* spp. are vegetatively propagated, the introduction of infected materials is strongly suspected as the cause of the widespread presence of *Hibiscus* vein clearing in southern Italy.

EARLY DETECTION OF AFLATOXIGENIC FUNGI ON MAIZE SEEDS. A. Del Fiore[1], S. Serranti[4], A. Ricelli[3], M. Reverberi[2], A.A. Fabbri[2], A. Bonifazi[4] and C. Fanelli[2]. *[1]Dipartimento Biotecnologie, Agroindustria e Protezione della Salute, ENEA, Centro di Ricerche della Casaccia, Via Anguillarese 301, 00123 Santa Maria di Galeria (Roma), Italy. [2]Dipartimento di Biologia Vegetale, Università "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. [3]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. [4]Dipartimento di Ingegneria Chimica dei Materiali, delle Materie Prime e Metallurgia, Università "La Sapienza", Via Eudossiana 18, 00184 Roma, Italy. E-mail: antonella.delfiore@casaccia.enea.it*

Fungi can grow on many food commodities. Some fungal species that belong to the genus *Aspergillus* are able to synthesise and release in the environment carcinogenic toxins, such as aflatoxins. *A. flavus* and *A. parasiticus,* produce, under suitable conditions, aflatoxins, secondary metabolites which are toxic for human and animals. Aflatoxigenic fungi are a real issue, especially for the cereal industry. Several studies have tried non-destructive, spectral methods to detect fungal contamination and toxins on cereals. Many methods have been utilized to measure fungal contamination and the presence of these toxins. The aim of this work is the early detection of aflatoxigenic fungi in cereals, such maize, the discrimination between healthy and diseased cereals and the determination of the extent of damage caused by the use of hyperspectral imaging and DNA-based methods. A hyperspectral imaging system, ImSpectorTM, V10 and two aflatoxigenic species, *A. parasiticus*, and *A. flavus* were used in this work. Both fungi were inoculated on maize and were imaged from 24 h to 7 days of growth. Changes in reflectance of maize were observed during fungal growth. This approach could be a rapid method for detecting the aflatoxigenic fungi on cereals. New methods developed in recent years include DNA-based analytical methods (PCR) for detection of fungi. We present a molecular method, based on PCR amplification of the *omt* gene, which encodes the enzyme necessary for the last step of aflatoxin biosynthesis. Specificity was assayed with DNA extracted from 7 different fungal species. Amplification was obtained only with DNA from aflatoxigenic A. *flavus* and A. *parasiticus*.

EPIDEMIOLOGICAL ASPECTS OF WOOD DECAY OF KIWIFRUIT (*ACTINIDIA DELICIOSA*). S. Di Marco and F. Osti. *Istituto di Biometeorologia del CNR, Via P. Gobetti 101, 40129 Bologna, Italy. E-mail: s.dimarco@ibimet.cnr.it*

A new form of wood decay caused by several fungi has recently been observed on kiwifruit (*Actinidia deliciosa* var. *deliciosa*). Trunk and shoots of diseased vines show decayed areas surrounded or preceded by wood discoloration. Symptoms appear on the foliage of the current season as chlorotic areas that soon become necrotic; leaves tend to curl, wilt and drop prematurely. In some cases, fruits do not reach full maturity. Repeated surveys were conducted in a typical Emilia-Romagna (northen Italy) kiwifruit-growing area in order to investigate the incidence of the disease and the relation between foliar symptom expression and environmental factors. The disease proved to be widespread throughout the surveyed area. Foliar symptoms did not appear regularly every season, as they failed to appear in a certain years or for some years in succession, even though vines were still infected. The number of infected vines increased since the first year of the survey. The time of appearance and the development of the disease during the growing season correlated with plant physiology and climatic factors. In particular, the annual incidence of the disease was affected by the June-August temperatures in terms of symptomatic shoots and vines. A similarity between esca of grapevine and wood decay of kiwifruit was hypothesized.

INFLUENCE OF POLYAMINE TREATMENTS ON *CUCUMBER MOSAIC VIRUS* INFECTION IN TOMATO. E. Di Nicola-Negri[1], P. Tavladoraki[2], L. Salandri[1] and V. Ilardi[1]. *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Rome, Italy. [2]Dipartimento di Biologia, Università deli Studi "Roma Tre", Viale G. Marconi 446, 00146 Rome, Italy. E-mail: v.ilardi@ispave.it*

Cucumber mosaic virus (CMV) is one of the most detrimental pathogens of tomato causing important yield losses worldwide. In the last twenty years different approaches, besides the use of transgenic plants, have been explored to control CMV infection but none of them proved to succeed in controlling this viral pathogen. Polyamines are low-molecular weight organic compounds involved in plant defence responses elicited by biotic and

abiotic stresses. Moreover, H_2O_2 production through polyamine oxidation is correlated with oxidative burst, cell death, as well as lignification and suberization processes occurring during defence responses. In order to analyse the influence of polyamines on CMV infection four tomato cultivars (Corbarino, Galatino, Vesuviano and UC82B) and one hybrid (Tomito) were sprayed with putrescine and spermidine and with the polyamine analogue guazatine and viral infection was monitored by visual inspection of CMV-inoculated plants and ELISA. Results of the impact of polyamines treatments on CMV infection are reported and discussed.

SUSCEPTIBILITY OF DIFFERENT FABABEAN GENOTYPES TO *UROMYCES FABAE* IN THE OPEN FIELD. B. D'Onofrio and M. Zaccardelli. *CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy. E-mail: massimo.zaccardelli@entecra.it*

Uromyces fabae (Pers.) de Bary is the causal agent of blight of fababean and other wild and cultivated leguminous plants. Typical symptoms consist of pustules on the leaves surrounded by a chlorotic halo. The use of tolerant varieties is the best strategy to control the pathogen. In an experimental field located in the Sele Valley in Southern Italy, heavy attacks of *U. fabae* developed at the end of winter 2007. Using a severity index from one to five (1 = no symptoms; 2 = slight symptoms on the leaves; 3 = heavy symptoms on the leaves; 4 = symptoms on the leaves and partially on the stem; 5 = heavy symptoms on the leaves and stem), disease severity was assessed in April. Among the twelve different genotypes of fababean under trial, the strongest disease symptoms were observed on "Betty" (mean index 3.6), "Espresso" (mean index 3.45), "Lady" (mean index 3.0) and "Vitabon" (mean index 3.0). The lowest disease severity was observed on "Prothabon 101" (mean index 1.1), "Prothabat 69" (mean index 1.6), "Scuro di Torre Lama" (mean index 1.6) and "Merkur (mean index 1.9). The genotypes "Chiaro di Torre Lama", "Sicania", "Irena" and "Castel", showed a mean severity index of 2.5, 2.75, 2.75 and 2.9, respectively.

DEVELOPMENT AND EVALUATION OF A CULTURE-INDEPENDENT SEMI-NESTED PCR ASSAY FOR DETECTION OF *BOTRYOSPHAERIA* spp. DNA IN GRAPEVINE TISSUES. S. Essakhi, G. Marchi, L. Mugnai, F. Peduto, A. Spagnolo and G. Surico. *Dipartimento di Biotecnologie Agrarie, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: guido.marchi@unifi.it*

In recent years fungi from the genus *Botryosphaeria* Ces. & De Not. have become increasingly important worldwide among the fungi that cause decline and other diseases in grapevine trunks. Diagnosis of the diseases caused by these fungi is complicated because most of the symptoms (cankers, dieback, decline, wood necrosis, graft-union failure, fruit rot, ect.) induced by one or another of the 11 *Botryosphaeria* species so far reported as potentially phytopathogenic to grapevine, are aspecific, and because the identification to the species level is difficult, especially when it relies only on the morphology of the isolates. Currently, there have been only two reports in Italy of *Botryosphaeria* species attacking grapevine, both attributed to *B. obtusa*, a ubiquitous species with a quite variable virulence. In order to better understand the presence and spread of the various species of *Botryosphaeria* in vine propagation material and in Italian vineyards, a culture-independent semi-nested PCR assay was developed that targeted the internal transcribed spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA of *Botryosphaeria*. The specific identity of the amplified DNA was determined by sequencing both strands and by pairwise comparisons with the homologous sequences available in GenBank database. Results showed that the most common *Botryosphaeria* species were by far *B. dothidea* and *B. obtusa* both of which are not considered as the most virulent of the *Botryosphaeria* species that attack grapevine.

CONTROL OF OCHRATOXIGENIC *ASPERGILLI* AND OCHRATOXIN A BIOSYNTHESIS IN GRAPES BY ANTAGONISTIC YEASTS AND AN ANTIFUNGAL NATURAL COMPOUND. M. Favilla[1], M. Pascale[1], A. Ricelli[1], A. Evidente[2], C. Amalfitano[2] and C. Altomare[1]. *[1]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. [2]Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: claudio.altomare@ispa.cnr.it*

Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin. Lately, there has been a growing interest in the occurrence of OTA in grapes and derivatives, including wines, mainly caused by fungi of the *Aspergillus* section *Nigri*. Management of the sanitary state of grapes is a critical point in a strategy aimed at preventing OTA occurrence in wine. We examined the ability of fusapyrone (FP), an antifungal metabolite of *Fusarium semitectum*, and of antagonistic yeasts to inhibit ochratoxigenic *Aspergillus* species and prevent OTA production. Artificially wounded grapes (cv. Negroamaro) were inoculated with *A. carbonarius* and treated with: (i) a solution of FP at 100 mg/l, (ii) biocontrol yeasts, (iii) a combination of FP at 50 mg/l and yeasts. Grapes were incubated in moist chambers at 25°C in the dark for 10 days. Significant inhibition of fungal growth was observed in the 100 mg/l FP-treated grapes. Ergosterol content was 72% less than control and conidiation was almost completely inhibited. The OTA content was reduced by 99%. At 50 mg/l, FP reduced both the viable mould count (by 99%) and OTA production (by 79%). The efficacy of antagonistic yeasts was dose-dependent and the best performance was observed at the highest cell concentration tested (10^8 cells/ml). Neither additive nor synergistic effect was observed with biocontrol yeasts plus 50 mg/l of FP. Results show that both FP and yeasts were able to control *A. carbonarius* and OTA contamination and suggest that the use of FP in rotation with antagonistic yeasts is a feasible strategy for management of OTA occurrence in grapes.

PHYLOGENETIC CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* pv. *TABACI* AND THE ROLE PLAYED BY THE EFFECTOR PROTEIN HOPQ1-1 IN DETERMINING ITS VIRULENCE AND HOST SPECIFICITY. P. Ferrante[1], R. Buonaurio[1] and B.A. Vinatzer[2]. *[1]Dipartimento di Scienze Agrarie e Ambientali, Sezione di Arboricoltura e Protezione delle Piante, Università degli Studi, Via Borgo XX Giugno 74, 06121 Perugia, Italy. [2]Department of Plant Pathology, Physiology and Weed Science, 551 Latham Hall (0390), Virginia Tech, Blacksburg, VA 24061, USA. E-mail: patferrante@tiscali.it*

Pseudomonas syringae pv. *tabaci* strains, responsible for wildfire and blackfire diseases of tobacco, were phylogenetically characterized via multilocus sequence typing (MLST). MLST analysis based on the sequence of four gene fragments of eight *P. syringae* pv. *tabaci* strains, *P. savastanoi* pv. *phaseolicola* 1448A and eleven *P. syringae* strains already characterized via MLST by Hwang *et*

al. (*Appl. Environ. Microbiol.* **71***:* 5182-5191, 2005) revealed that our *P. syringae* pv. *tabaci* strains are all closely related to the completely sequenced *P. savastanoi* pv. *phaseolicola* 1448A strain. Moreover, we confirmed our previous rep-PCR results that all analyzed *Pseudomonas syringae* pv. *tabaci* strains are representatives of the same clonal group. The presence of 21 *P. savastanoi* pv. *phaseolicola* 1448A effector genes was determined in the *Pseudomonas syringae* pv. *tabaci* strains by PCR. The effector genes *hopR1*, *hopAE1*, *hopAH2*, *hopAN1*, *hopAS1*, *hopAJ2*, *hopD1*, *hopI1*, *hopX1* and *hrpK1* were found. *P. syringae* pv. *tabaci* transformants were obtained introducing individually six effector genes of *P. savastanoi* pv. *phaseolicola* 1448A which are absent in the *P. syringae* pv. *tabaci* genome. Virulence was tested in bean and *Nicotiana benthamiana* plants. We observed that the growth *in planta* of the transformant bearing the *hopQ1-1* gene was significantly increased in bean and reduced in *N. benthamiana*. Virulence of the other five transformants was not changed. From our results, we can deduce that hopQ1-1 is an important virulence determinant of *P. savastanoi* pv. *phaseolicola* and that it triggers a defence response in *N. benthamiana*.

SAFFRON (*CROCUS SATIVUS* L.) DISEASES IN ITALY. M. Fiori[1], G. Falchi[1], M. Quaglia[2] and C. Cappelli[2]. *[1]Dipartimento di Protezione delle Piante, Università degli Studi Via E. De Nicola 9, 07100 Sassari, Italy. [2]Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: fiorim@uniss.it*

Surveys carried out in different Italian saffron-growing areas showed the presence of some fungal and bacterial diseases. *Fusarium oxysporum* f. sp. *gladioli,* a fungus transmitted through infected corms, was the major responsible of severe yield losses, especially in central Italy and, recently, also in Sardinia (Medio Campidano). Primary symptoms occurred during flowering. They included basal stem rot, yellowing and wilting of the shoots and corm rot. The rapid spread of the disease is caused by movement of contaminated and/or infected corms. The Italian fungus isolates from saffron did not differ by those obtained from gladiolus or saffron grown in Spain as shown by VCs groups studies. *Macrophomina phaseolina* was found in Sardinia. The fungus was transmitted by infected corms and caused wilting of plants and charcoal rot of corms. *Penicillium* spp. and *Stromatinia gladioli* were also found in/on the corms at the time of transplanting. Recent investigations carried out in Sardinia showed that *Burkolderia* sp. and *Pseudomonas* spp. are the causal agents of a disease characterized by rotting of the leaves, flowers and corms. Smooth and winkled colonies were isolated from plants and corms. Bacterial isolates were characterized by pathogenicity, phenotypic and PCR analysis. Most of the isolates were identified as *Burkholderia gladioli* and some as fluorescent pseudomonads. FLP analysis are under way to characterize the pathovar of the *B. gladioli* isolates. Other fungi that were recorded many years ago, such as *Rhizoctonia* spp. and *Phoma crocophila,* were not found.

BIOFUMIGATION: INTERACTION BETWEEN *TRICHODERMA* AND THE GLUCOSINOLATE-MYROSINASE SYSTEM. S. Galletti, E. Sala, P.L. Burzi, O. Leoni and C. Cerato. *CRA, Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, 40129 Bologna, Italy. E-mail: s.galletti@isci.it*

Biofumigation is a biocontrol method based on the exploitation of the glucosinolate-myrosinase system contained in all *Brassicaceae* tissues. Green manuring represents the technique utilised to activate the system but also seed meal incorporation into soil is an attractive alternative. In the presence of water, the enzyme myrosinase degrades glucosinolates into toxic compounds, mainly isothiocyanates, lowering soil-borne pathogen inoculum. The effects of the biocidal compounds on the beneficial soil microflora need to be carefully evaluated. Thus a screening was carried out *in vitro* among 40 *Trichoderma* isolates for tolerance to allyl-isothiocyanate (AITC) released by myrosinase degradation of sinigrin from moistened seed meal of *Brassica carinata*. Most isolates suffered a fungistatic effect, while a few were killed, like two pathogenic isolates of *Pythium ultimum* and *Rhizoctonia solani*. In addition, one *Trichoderma* isolate tolerated very well AITC, showing a short lag phase, due to its ability to reduce AITC concentration in the Petri dish atmosphere, as determined by GC analyses. Further investigation showed that this isolate was also able to grow in liquid and solid culture additioned with pure sinigrin. The presence of AITC in the atmosphere of the cultures, revealed by GC analyses, and the progressive decrease in sinigrin content in the liquid substrate, determined by HPLC analyses, suggest that a fungal myrosinase is involved. These findings account for a general good tolerance of the beneficial fungus *Trichoderma* to biofumigation carried out with sinigrin-containing matrix. However, the existence in the soil of strains able to interact with toxic compounds derived from sinigrin could affect biofumigation effectiveness.

CAPS ANALYSIS: A POSSIBLE EPIDEMIOLOGICAL APPROACH FOR THE STUDY OF *ARMILLARIA GALLICA* ATTACKS TO DECLINING OAK TREES. A. Gatto, G. Sicoli, N. Luisi. *Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: luisin@agr.uniba.it*

Oak decline is the result of one of the most severe forest pathogen outbreaks on a regional scale in Europe over the last decades. Among other fungal attacks observed in southern Italy, root infections have frequently been ascribed to populations of locally widespread isolates of *A. gallica*, a weakly pathogenic species of the genus *Armillaria*, which is responsible for well-known and worldwide occurring root rot and wood decay. Biomolecular techniques have been increasingly acknowledged as powerful tools in the epidemiological approach toward diseases in forest ecosystems. For this purpose, a study was carried out on the intraspecific variability of a population of 96 *A. gallica* isolates sampled from the "Difesa Grande" oak stand located in Gravina di Puglia (southern Italy), based on restriction fragment length polymorphism analysis of the intergenic spacer 1 region of rDNA, namely CAPS (cleaved amplified polymorphic sequence) analysis. Three different banding patterns were yielded by the isolates under study, one of which is commonly reported for this species in the literature. The other two patterns were previously detected only once within a broad-scale Italian population that we had collected before. Therefore, it can be argued that, if the DNA region analysed is more variable than what has been reported till now for *A. gallica*, then the biodiversity within this species is more pronounced than previously thought. Thus, new perspectives seem to emerge for a better understanding of landscape pathology dynamics concerning oak decline.

FIRST REPORT OF *ASCOCHYTA* sp. ON GRASS PEA (*LATHYRUS SATIVUS* L.) IN ITALY. A. Infantino[1], N. Pucci[1], G. Di Giambattista[1], S. Vona[1] and M. Zaccardelli[2]. *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via. C.G. Bertero 22, 00156 Rome, Italy. [2]CRA, Istituto Sperimentale per le Colture Industriali,*

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Yellowish necrotic lesions containing black pycnidia were observed on stems and pods of several plants of grass pea (*Lathyrus sativus* L.) grown in 2006 and 2007 in experimental fields in Battipaglia (SA), and Corleto Perticara (PZ) (southern Italy). Isolations were attempted in the laboratory by placing symptomatic tissues on potato dextrose agar amended with streptomycin sulphate and ampicillin (100 ppm each). Fast growing colonies with abundant sporulating pycnidia producing 1-2 septate conidia (15.5 x 5.7µm) were obtained. Pathogenicity tests were done by spraying a conidial suspension of the isolate ISPaVe ER-1413 (3×10^5 spores ml^{-1}) on 2-week-old seedlings of the following species: grass pea, lentil, fababean, pea, chickpea, alfalfa, lupin, French bean, and vetch. Symptoms similar to those observed in the field appered after 15 days only on grass pea seedlings. *Ascochyta* sp. was reisolated from all infected plants. DNA of two isolates (ISPaVe ER 1413 and ISPaVe ER 1415) was extracted and amplified with ITS specific primers ITS5 and ITS 4. The obtained fragment *ca* 600 bp in size was directly sequenced on both strands. Homology search with BLAST in GenBank sequences revealed 99% identity with a comparable sequence of *Ascochyta lentis* (accession no. AY131201). Nevertheless, the lack of disease symptoms on any leguminous species tested other than *L. sativus* casts doubts on the identification at the species level. Ascochyta blight caused by *Mycosphaerella pinodes* is reported to cause damage to *L. sativus* worldwide. To our knowledge, this is the first report of *Ascochyta* sp. on *L. sativus*.

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SERIOUS OUTBREAKS OF ROOT AND CROWN NECROSIS OF STRAWBERRY IN SOUTHERN ITALY. E. Lahoz[1], R. Caiazzo[1], A. Carella[1], A. Fanigliulo[2,3], S. Comes[2] and A. Crescenzi[2]. *[1]CRA, Istituto Sperimentale per il Tabacco, Via Vitiello 108, 84018 Scafati (SA), Italy. [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. [3]Bioagritest Srl, zona PIP lotto E2, 85010 Pignola (PZ), Italy. E-mail: ernesto.lahoz@entecra.it*

During autumn-winter 2006/2007 a serious disease was observed in young strawberry plants cv. Ventana grown under plastic tunnels in Basilicata and Campania (southern Italy). Symptoms, present on about 50-60% of the plants, consisted in poor development. Roots were necrotic and diffusely corky. Necrosis and brown areas occurred at the base of the crown, which showed a red tinge when sectioned. Isolation on several growth media yielded colonies mainly of *Fusarium sp.* and *Phoma sp.* Based on morphological and molecular characterization the two fungi were identified as *Fusarium tricinctum* (Corda) Saccardo and *Phoma glomerata* (Corda) Wollenweber et Hochapfel. The role of the two agents in the genesis of the disease is discussed. In preliminary pathogenicity tests, the two fungi, when inoculated contemporarily, gave symptoms similar to those observed in the field.

***FOMITIPORIA MEDITERRANEA* ASSOCIATED WITH WOOD ROT OF CITRUS IN SICILY. A. Ippolito[1], F. Nigro[1], L. Schena[2], S.O. Cacciola[3], F. Raudino[2] and G. Magnano di San Lio[2].** *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. [2]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Loalità Feo di Vito, 89060 Reggio Calabria, Italy. [3]Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. E-mail: gmagnano@unirc.it*

Heartwood rot is a chronic disease occurring endemically in most citrus growing areas of the world. It results in decay and death of scaffold limbs and branches and affects old trees where environmental conditions, such as close tree spacing or frost, are conducive to infection. Although this disease is not a limiting factor to the citrus industry it can contribute to the deterioration of the groves. As infection extends downward in the trunk, topworking of the trees may become problematic. Several basidiomycetes have been reported to be associated with wood decay of citrus trees. Early studies attributed heartwood rot of citrus trees to *Fomes applanatus*. However, the fungus described under this name was probably *Fomitiporia mediterranea*, a recently described species which is associated with wood decay of grapevines affected by esca. Recently, *F. mediterranea* was found to be associated with wood decay of citrus trees in Greece. It had previously been reported as *F. punctata* from Apulia (southern Italy). In a survey of citrus groves in Sicily, *F. mediterranea* was found to be associated with heartwood rot in many sites. The fungus was isolated from infected wood and basidiocarps. It was identified by PCR-amplification and sequencing of the ITS regions of rDNA. In old orchards typical resupinate basidiocarps of *F. mediterranea* were found on branches of a high proportion of trees. Interestingly, the incidence of wood rot caused by *F. mediterranea* was high in 20 to 30-year-old orange orchards which had been replanted in sites with a previuous disease history.

OPTIMIZATION OF AN INDUSTRIAL FERMENTATION PROCESS FOR THE PRODUCTION OF NOVEL *TRICHODERMA*-BASED FORMULATIONS. S. Lanzuise[1], M. Ruocco[2], S. Woo[1], V. Aloj[1], D. Turrà[1], F. Vinale[1], R. Marra[1] and M. Lorito[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA) Italy. [2]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. E-mail: lorito@.unina.it*

Several members of the genus *Trichoderma* are being proposed commercially as alternatives to chemical fungicides. However, full-scale application of *Trichoderma* for the biological control of plant pathogens has not been widely implemented also because the technology for mass production of *Trichoderma* spp. spores, enzymes (cellulose, α-amylase, xylanase, chitinase, β-glucanase) and extra cellular metabolites via submerged fermentation still requires improvement for application at an industrial scale. The aim of present study is to optimize culture conditions that influence both enzyme and spore production by *T. harzianum* in large submerged cultures, thus obtaining a new formulation that contains enough of highly active propagules and enzymes. Maximum chitinase, glucanase and xylanase production was obtained when fermentation was carried out at 25°C for 72 h using 72 h old mycelium and lyophilised fruiting body of *Agaricus bisporus* plus cereal fibre as the sole carbon source. Very few spores were observed in cultures grown at 100 rpm even after 120 h of fermentation (HF). In cultures grown at 200 rpm, extensive sporulation and mycelium fragmentation was observed after 72 HF; after 96 HF, also fragmented mycelium began sporulation and at 120 HF a high number of spores were produced. The new

formulation obtained (spore plus enzymes) showed a high biocontrol activity against many foliar and soil pathogens, and also a good biofertilizer effect.

EVALUATION OF COMPOSTS AND AMENDMENTS FOR SUPPRESSIVE ACTIVITY AGAINST *VERTICILLIUM DAHLIAE* ON NURSERY-GROWN OLIVE PLANTS. G. Lima[1], F. De Curtis[1], D. Piedimonte[1], D. Vitullo[1], I. Pentimone[2] and F. Nigro[2]. *[1]Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Sezione di Patologia Vegetale, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso, Italy. [2]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: lima@unimol.it*

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is one of the most severe diseases of olive and other important crops worldwide. In the last decades the disease has occurred with increasing frequency and severity in most olive-growing areas. Its control has relied on chemical biocides but more eco-compatible and safer control mesures are necessary. Preventive methods (i.e. pathogen-free soil and planting material, suppressive substrates and biocontrol agents) have been implemented in the nurseries since they are considered key factors for an efficient disease control. Recently, alternative substrates for plant growth containing natural amendments and plant composted biomasses are being studied not only for agronomic properties, but also for their suppressiveness against soil-borne pathogens. To prevent *V. dahliae* infections our investigations were aimed at evaluating the suppressive activity of different composts and amendments, alone or enriched with biocontrol agents, against microsclerotia (MS) of the pathogen. The experimental products at 15% concentration (v/v) were mixed with a standard substrate and the mixtures were artificially contaminated with 50 MS/g. Trials were conducted under growth chamber and nursery conditions. In blind trial experiments, the pathogen was periodically monitored in the soil by either semi-selective medium and real-time nested Scorpion PCR. Results showed that some composts and amendments reduced significantly the density of *V. dahliae* MS in the rizosphere of young olive plants. Further studies are in progress to optimize the suppressive activity of these alternative substrates in order to protect olive plants from *V. dahliae* infection in the nursery.

ACTIVITY AGAINST *FUSARIUM OXYSPORUM* OF ANTAGONIST BACTERIA ISOLATED FROM SUPPRESSIVE SOILS OR COMPOSTS. [1]G. Lima, [1]D. Vitullo, [1]F. De Curtis, [1]D. Piedimonte, [2]L. Maiuro and [1]V. De Cicco. *[1]Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Sezione di Patologia Vegetale, Università degli Studi del Molise, Via De Sanctis, 86100, Campobasso, Italy. [2]Dipartimento di Scienze e Tecnologie Agro-Alimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso, Italy. E-mail: lima@unimol.it*

The most common approach to biological control involves selection of antagonist microorganisms, elucidation of their modes of action, optimization of the antagonistic activity and development of biocontrol products. The rate of suppressiveness of composts and natural amendments against soil-borne pathogens (*Fusarium oxysporum, Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani*, *Sclerotinia* spp., *Sclerotium rolfsii*, *Verticillium dahliae*, and others) depends on several characteristics of these substrates such as chemical components, composting conditions and microorganic components. Among microorganisms populating different composts and amendments, over 130 bacterial strains were isolated and assayed *in vitro* for their antagonist activity against *F. oxysporum* f.sp. *lycopersici*. Some of the most effective antagonists were studied for their *in vitro* and *in vivo* interaction with both pathogen and roots of tomato plants. Results of these investigations showed that 26 bacterial strains inhibited the radial growth of *F. oxysporum* on agarized medium; moreover, on liquid medium, bacterial culture filtrates reduced both germination and germ tube-elongation of fungal spores. Scanning electron microscopy observations of two antagonist bacteria showed that bacterial cells adhere to *F. oxysporum* hyphae causing collapse of the fungal cell wall. This research gives useful information to optimize the activity of biocontrol bacteria in order to develop new and high effective bioformulates for use in eco-compatible agriculture.

EVALUATION OF NEW BIOCONTROL AGENTS FOR SUPPRESSION OF ARTICHOKE CROWN ROT CAUSED BY *SCLEROTIUM ROLFSII*. B.T. Linaldeddu, L. Maddau, A. Franceschini and F. Marras. *Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: ben@uniss.it*

Sclerotium rolfsii Sacc. is one of the most important soil-borne plant pathogens widespread in the tropic, subtropic and temperate regions on a wide range of economically important crops. In Sardinia (Italy), it causes serious losses on monocultures of artichoke. In recent years, several antagonistic micro-organisms have been used as an alternative to chemical products for controlling *S. rolfsii* attacks under field conditions, often with unsatisfactory results. In previous *in vitro* tests a strong antagonistic activity of two oak fungal endophytes, *Bionectria solani* (Reinke *et* Berthold) Schroers and *Trichoderma* sp. 1, and two soil-borne fungi, *Trichoderma viride* Pers. and *Trichoderma* sp. 2, was observed against *S. rolfsii.* In the present work the capability of these fungi to prevent artichoke crown rot by *S. rolfsii* in greenhouse experiments was tested. Furthermore, the production of secondary metabolites potentially involved in biocontrol mechanisms was evaluated. Results indicate that all the antagonistic fungi significantly reduce disease incidence; in particular, *B. solani* and *Trichoderma* sp. 1 reduced plant mortality by up to 89%. The four fungi tested produced *in vitro* secondary metabolites, the chemical and biological characterization of which is in progress.

PRELIMINARY RESULTS ON THE RESPONSE OF THE TRADITIONAL FRENCH BEAN CULTIVARS "FAGIOLI DI SARCONI" TO THE VARIETIES *FUSCANS* AND NON *FUSCANS* OF *XANTHOMONAS AXANOPODIS* pv. *PHASEOLI*. P. Lo Cantore, G. Figliuolo and N.S. Iacobellis. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: lp460agr@unibas.it*

The cultivation of "Fagioli di Sarconi", a pool of high value traditional French bean varieties grown in Basilicata (southern Italy) for the production of dry seeds, is limited by common blight, caused by the variety *fuscans* of *Xanthomonas axonopodis.* pv. *phaseoli.* Five of the above traditional varieties were artificially inoculated with two selected virulent strains of the varieties *fuscans* and non *fuscans* of *X. a.* pv. *phaseoli* for evaluating their susceptibility-resistance. Bacterial suspensions containing 10^8 u.f.c./ml were injected into the first trifoliate leaf (4 inoculations per leaflet) of bean plants grown in pots in greenhouse. Fourteen, 21 and 28 days post inoculation the diameter of the lesions, which increased in size during the assays, was recorded and the

data obtained were analysed statistically. Bean cultivars were susceptible to both bacterial varieties although a different response was observed. In particular, cvs Tondino bianco, Verdolino and Cannellino were more susceptible that Tabacchino and Ciuoto when inoculated with the strain of variety *fuscans,* while cvs Tondino bianco, Tabacchino and Verdolino were more susceptible than Ciuoto and Cannellino when the strain of the bacterial variety non *fuscans* was used. The different response of bean cultivars to artificial inoculation suggests that several plant factors are involved in the *X. a.* pv. *phaseoli*/French bean interaction, and that these are apparently different among the traditional cultivars of the "Fagioli di Sarconi" pool.

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CHARACTERIZATION OF STRAINS OF THE VARIETIES *FUSCANS* AND NON *FUSCANS* OF *XANTHOMONAS AXONOPODIS* pv. *PHASEOLI*. P. Lo Cantore and N.S. Iacobellis. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: lp460agr@unibas.it*

Sixty strains of the varieties *fuscans* and non *fuscans* of *Xanthomonas axonopodis* pv. *phaseoli*, obtained form typical lesions and seed washings in different geographic areas including Italy, were characterized for different phenotypical features. All strains produced a yellow pigment on GYCA whereas only strains of *X. a.* pv. *phaseoli* variety *fuscans* produced a brown pigment when grown on KB and MT. All strains hydrolysed starch, esculin and casein, grew at 35°C and on minimal medium containing glucose and mannose but not in the presence of arabinose and, in addition, induced hypersensitivity reactions and disease symptoms when injected with a syringe into pepper (cv. Early Calwonder) and bean (cv. Gipsy) leaflets. Virulence, evaluated as size of the lesions, varied among strains of each bacterial variety and, contrary to previous observation, also between strains of the two varieties. The nutritional profile of the strains in question, obtained with the Biolog® Identification System (MicroLog™ System Release 4.2, Biolog Inc., Hayward, CA, USA), showed that the two bacterial varieties utilise in a different way some of the 95 different sources of carbon. A different behaviour between the two varieties was also observed when fatty acid and total protein profiles of strains of both groups were analysed. These features confirm and strengthen recent data on the possible different taxonomic position of the varieties *fuscans* and non *fuscans* of *X. a.* pv. *phaseoli*.

Research partially supported by the Ministry of Agricultural, Alimentary and Forest Politics with funds released by C.I.P.E. (Resolution 17/2003).

PRELIMINARY RESULTS ON THE ANTAGONISTIC ACTIVITY OF FLUORESCENT PSEUDOMONAS spp. TOWARD SOME PATHOGENIC FUNGI OF FORESTRY PLANTS. P. Lo Cantore, A. Viggiano and N.S. Iacobellis. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: lp460agr@unibas.it*

Strains of fluorescent *Pseudomonas* spp., pathogens of the cultivated mushrooms and isolated from the rhizosfere of forestry plants, inhibited *in vitro* the growth of *Phytophthora* spp., *Heterobasidion annosum, Armillaria mellea* and *Cryphonectria parasitica* in antagonistic assays. The antagonistic activity is apparently due to the production of antimicrobial metabolites and to hydrolytic enzymes (i.e. proteases, glucanases, cellulases and chitinases) produced *in vitro* by the majority of the antagonistic bacteria. Chestnut seeds, inoculated with some selected strains of *Pseudomonas tolaasii*, *P. reactans*, *P. fluorescens* biotype C and *Pseudomonas* spp., which were highly active in inhibiting the growth of phytopatogenic fungi in plate antagonism assays, were protected from the pathogenic activity of a strain of *P. cambivora*. In fact, chestnut seeds bacterized with the above bacteria, then inoculated with a virulent strain of *P. cambivora,* germinated and showed a growth similar to the control chestnut seeds untreated or bacterized only with the same antagonistic bacteria. By contrast, chestnut seeds inoculated only with *P. cambivora* did not germinate, did rot and were heavy colonised by the pathogen. These preliminary results suggest that these antagonistic bacteria may have a potential use for seed bacterization practices.

STANDARDIZATION OF A COMMON PROTOCOL FOR OLIVE VIRUS DETECTION. G. Loconsole[1], M. Saponari[1,2] F. Faggioli[3], G. Albanese[4], H. Bouyahia[5], T. Elbeaino[6], M. Nuzzaci[7], V. Prota[8], G. Romanazzi[9] and V. Savino[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola, 165/A, 70126 Bari, Italy. [2]Centro di Ricerca e Sperimentazione in Agricoltura "Basile Caramia", Via Cisternino 281, 70010 Locorotondo (BA), Italy. [3]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [4]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. [5]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. [6]Istituto Agronomico Mediterraneo, Via Ceglie, 9, 70010 Valenzano (BA), Italy. [7]Dipartimento di Biologia, Difesa e Biotecnologie Agroforestali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. [8]Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via De Nicola 9, 07100 Sassari, Italy. [9]Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Via Brecce Bianche 1, 60131 Ancona, Italy. E-mail: giuliana.loconsole@agr.uniba.it*

Sanitary selection and certification of olive require sensitive diagnostic methods and effective sanitation protocols. Although much attention has been paid in the last few years to the development of diagnostic tools for reliable virus identification, the need to define common and consentaneous diagnostic protocols suggested to carry out a ring test with the participation of nine Italian laboratories. Different protocols (one-step RT-PCR, dot-blot hybridization) and reagents (primers, riboprobes), used for the detection of the most common olive viruses, were tested comparatively in each laboratory, using the same batch of positive samples and healthy controls. One-step RT-PCR proved much more sensitive than dot-blot hybridization for all viruses, using as template total nucleic acid extracted with a commercial kit (Qiagen, Valencia, CA, USA). A primer set for olive leaf yellowing-associated virus (OLYaV) was designed for broad-spectrum detection of different viral isolates. Furthermore, a one-step RT-PCR protocol was successfully used for the first time for *Tobacco necrosis virus* (TNV) detection in olive tissues. Results showed that all investigated viruses were not uniformly distributed in the canopy and that at least two sub-samples must to be collected from each

plant. The standardized protocol agreed upon will be used to produce "primary sources" (nuclear stocks) of 70 different Italian olive varieties, in the framework of the national project OLVIVA, which involves 25 national research institutes.

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A HIGH COMPUTING BIOINFORMATIC APPROACH BASED ON GRID FOR DETECTING RECOMBINATION IN WHOLE *CITRUS TRISTEZA VIRUS* GENOME. S. Lombardo[1,2], S. Davino[2], M. Iacono Manno[3] and A. Muoio[3]. *[1]Consorzio Cometa, Via S. Sofia 64, 95123 Catania, Italy. [2]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [3]Istituto Nazionale di Fisica Nucleare, Sezione di Catania, Via S. Sofia 64, 95123 Catania, Italy. E-mail: alelomba@unict.it*

The high computing performance of distributed workstation cluster, like GRID, is useful in bioinformatics because the number of available sequences in GenBank continues to grow at an exponential rate, doubling every 10 months. Furthermore, bootstrapping, the way of testing the reliability of the dataset by resampling requires very long processing times on conventional systems. In our approach we analised *Citrus tristeza virus* (CTV), the causal agent of a major virus disease of citrus. CTV has a single-stranded, positive sense genomic RNA of nearly 20 Kb. Recombination in CTV can occur through either reassortment or "copy-choice" replication. In copy-choice replication, the viral RNA dependent RNA polymerase switches from one RNA molecule to another during replication, generating mosaic genomes. Conventional phylogenetic tree estimation methods assume that all sites have the same evolutionary history. This assumption drops if recombination has occurred among any sequences. Recombination produces mosaic sequences that may cause errors in phylogenetic tree estimation. We have integrated the most innovative computing applications in virology research and bioinformatics on a GRID infrastructure. In particular, we have implemented in a single workflow, three tools for pairwise or multiple sequences alignment and phylogeny tree construction (ClustaW-MPI 1.83), phylogenetic networks generation (SplitsTree 4.6) and recombination detection by phylogenetic methods (TOPALi). The data obtained with this analysis fit with previous works, based on nucleotide identity analysis, with considerable time saving.

INFLUENCE OF NUTRIENTS ON *IN VITRO* GROWTH OF *ARMILLARIA MELLEA*. C.M. Longa and I. Pertot. *Centro SafeCrop, Via Mach 1, 38010 San Michele all'Adige, (TN), Italy. E-mail: claudia.longa@iasma.it*

Armillaria mellea is a slow-growing fungus causing root rot in forest and cultivated plants. Its slow *in vitro* growth results in long lasting and time consuming experiments. The aim of this work was to evaluate the effect of different nutrient sources on *A. mellea* growth in order to speed it up. The effect on *A. mellea* growth was assessed by adding different nutrients to malt extract agar (MEA) in 90 mm Petri dishes. Nutrient sources were added to MEA at a rate of 2 g/l (nitrogen sources) and 5 g/l (carbon sources). Carbon sources tested were glucose, sucrose, mannose, threalose, galactose and corn strep liquor (CSL). Nitrogen sources were peptone and yeast nutrient base (YNB). The pH of growth media and water activity were 5.1±0.1 and 0.998 ±0.001 respectively. The untreated control was MEA without additional nutrient. Four replicates (Petri dishes/nutrient) were inoculated and incubated at 22°C. Effect of nutrients sources was evaluated on rhizomorphs production after seven days, and on colony area and mycelial dry weight after 15 days of incubation. Addition of CSL and sucrose produced a significant higher mycelial dry weight and colony area (Fisher's test, $P \leq 0.05$) if compared with the untreated control. Initial rhizomorphs production was stimulated by the addition of CSL, galactose and peptone. Morphological characteristics and pigment production were not affected by the nutrient sources. CSL, a by-product of the corn wet-milling industry, resulted in the best nutrient source to improve the *in vitro* growth of *A. mellea.*

SOIL MICROCOSM APPROACH TO EVALUATE ANTAGONISTIC EFFECT OF POTENTIAL BIOCONTROL AGENTS AGAINST *ARMILLARIA MELLEA*. C.M. Longa and I. Pertot. *Centro SafeCrop, Via Mach 1, 38010 San Michele all'Adige (TN), Italy. E. mail: claudia.longa@iasma.it*

Armillaria mellea root rots are impossible to control with currently available methods. Biological control, either alone or integrated with other approaches (fumigation, agronomical practices, etc.), may have better perspectives. Results in biocontrol efficacy obtained with *in vitro* dual culture are often not consistent with what found in nature; this is because the soil can influence survival and activity of the antagonist. We present a new, relatively fast and cheap approach to evaluate the antagonistic effect of microorganisms against *A. mellea* using soil microcosms. Soil microcosms (three replicates) were prepared in polypropylene bottles as follows: five plugs of *A. mellea* mycelium and rhizomorphs, protected by sterile lens tissue, were placed between two layers of 100 g of sieved sterile soil inoculated with 10^6 conidia of the testing microorganisms (*Trichoderma atroviride, T. virens* or *T. longibrachiatum)*. Microcosms were maintained for seven days at room temperature. Pathogen plugs were collected, surface sterilised by dipping in NaOCl (0.4%) for 10 min and rinsed in sterile distilled water for 30 min. Pathogen samples were transferred to Petri dishes containing malt extract agar (MEA) and incubated for 15 days at 22°C. Antagonistic effect was evaluated as percentage of pathogen growth failure on MEA (survival reduction). The three *Trichoderma* species significantly reduced pathogen survival (Tukey's test, $P \leq 0.05$) compared to the control. *T. atroviride* and *T. longibrachiatum* grew in the pathogen plug after surface sterilization confirming their mycoparasitic activity.

PRELIMINARY SURVEY OF *RALSTONIA SOLANACEARUM* STRAINS ISOLATED FROM TOMATO IN ITALY. S. Loreti[1], M. Fiori[2], D. De Simone[1], G. Falchi[2], A. Gallelli[1] and A. Schiaffino[2]. *[1] CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2] Dipartimento di Protezione delle Piante, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: s.loreti@ispave.it*

Tomato wilting caused by *Ralstonia solanacearum* (*RS*) to tomato cultivars Arawak, Cuore di Bue and Ikram growing in greenhouses in Southern Sardinia was observed in February and April 2007. Tomato plants were grown in the soil and in soil-less culture in five greenhouses. The percentage of affected plants was up to 70%. Isolations from symptomatic plants and from water used for irrigation onto SMSA and TZCA media gave fluid white colonies with a red centre. Pathogenicity tests were done by inoculating six isolates each on 9 plants of tomato, eggplant and tobacco. Only tomato and eggplant plants wilted within two weeks and died; no symptoms were observed in tobacco and in control

plants. Isolates obtained originally and from artificially inoculated plants were characterized by biochemical and physiological tests, BIOLOG™ system and IFAS. FAME analysis, performed on two isolates, confirmed the similarity with *RS*. PCR with primers OLY-1 and Y-2, specific for *RS*, gave the expected band of 288 bp. Digestion of PCR products with *Ava*II restriction endonuclease gave the same pattern as the reference strain. Rep-PCR and SDS-PAGE of whole-cell proteins confirmed the similarity of our isolates with *RS* reference strain. Furthermore, partial 16S rDNA gene sequences of several isolates were compared with known sequences of *RS* and their similarity was 100%. Sequences were deposited in GenBank (Accession numbers from AM690474 to AM690480). In conclusion, it was ascertained that the bacteria isolated from tomato in Sardinia have the same characteristics of race 3 of *R. solanacearum.*

PATHOGENESIS OF *BRENNERIA NIGRIFLUENS* TO WALNUT. S. Loreti[1], S. Vitale[1], A. Gallelli[1], P. Piccirillo[2] and A. Belisario[1]. *[1]Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero, 22, 00156 Roma, Italy. [2]Istituto Sperimentale per la Frutticoltura, Via Torrino 3, 81100 Caserta, Italy.*

Bark canker of English walnut caused by *Brenneria nigrifluens*, was reported for the first time in USA in 1957. Symptoms consisted in shallow, irregular brown necrotic areas in the bark of the trunk and scaffold branches. Initially, small circular spots are visible in the cortical tissue, and by the enlargement and coalescence of several spots extensive cankers are formed. Watery exudates ooze through cracks on the bark. In Italy, occurrence of the disease has been reported in 1998 in the north of Italy, and in a time period of four years it was observed also in southern-central Italy. Several varieties are known to be susceptible to the disease. A survey in a tree collection of *J. regia* varieties (C.R.A.-ISF of Caserta), in which the disease was widespread, showed that the cvs. Sorrento, Bleggiana, Tehama, Corne, Gustine, Xerta 122, Amigo were susceptible. To assess the disease development on susceptible and resistant *Juglans* species, a pathogenicity test was performed on 4-year-potted *J. regia* seedlings and 11-year-old *J. regia*, *J. hindsii*, *J. sieboldiana* trees. Necrosis progressed equally upward and downward the inoculation point. On *J. sieboldiana* no symptoms were induced on inoculated branches, confirming its resistance. All controls were symptomless. On young plants, inoculations developed sunken brownish areas. Lesions were more extended in width on young plants than on mature trees. Bleeding was never produced. Re-isolations of *B. nigrifluens* were positive at the lesion margins for *J. hindsii* and *J. regia* and at the inoculation point for *J. sieboldiana*.

GENETIC VARIABILITY OF *HETEROBASIDION ABIETINUM* ISOLATES COLLECTED FROM *ABIES ALBA* AND *A. PINSAPO* IN THE MEDITERRANEAN REGION. N. Luchi[1], M.E. Sánchez[2] and P. Capretti[1]. *[1]Dipartimento Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università degli Studi. Piazzale delle Cascine 28, 50144 Firenze, Italy. [2]Departamento de Agronomìa. ETSIAM, Universidad de Cordoba, Apdo 3048, 14080 Cordoba, Spain. E-mail: nluchi@unifi.it*

Heterobasidion abietinum is a fungal pathogen that causes root rot on *Abies* spp. in central and southern Europe. Generally the highest incidence of the disease occurs in areas not optimal for *Abies* and it may vary according to elevation, rainfall pattern, mean temperature and type of soil. Disease is severe along the southern Italian Apennines (Puddu *et al.*, 2003. *For. Ecol. Manag.* **180**: 37-44) and in Spain in some localities of Andalucia, where root damage can be extensive so as to lead to death of the trees of *Abies pinsapo* in Sierra de las Nieves Natural Park (Sánchez, *Forest Pathol.*, in press). The aim of the work was to investigate the genetic variation of *H. abietinum* isolates collected from different abies specie (*A. alba* and *A. pinsapo*) in three different localities of the Mediterranean region: Apennines, Pyrenees and Andalucia, in the far south of the Spanish peninsula. Samples of *H. abietinum* were analyzed on the basis of profiles obtained with direct amplification of minisatellite M13 used as a primer. In a N-J dendrogram, *H. abietinum* isolates separated significantly into clusters, according to their geographical origin.

MOLECULAR CHARACTERIZATION OF *FUSARIUM OXYSPORUM* f.sp. *MELONIS* L. Luongo[1], M. Maccaroni[1], A. Ferrarini[2], S. Vitale[1], A. Polverari[3], M. Delledonne[2] and A. Belisario[1]. *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero, 22, 00156 Roma, Italy. [2]Dipartimento Scientifico e Tecnologico, Strada Le Grazie 15, 37134 Verona, Italy. [3]Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino, Villa Ottolini-Lebrecht, 37029 San Floriano di Valpolicella (VR), Italy. E-mail: a.belisario@ispave.it*

Fusarium oxysporum f. sp. *melonis* is the causal agent of a destructive vascular wilt disease of muskmelon (*Cucumis melo*) worldwide. The fungus penetrates directly the roots and colonizes the vascular system. Symptoms of the disease and the extent of colonization vary according to the host plant, fungal race (pathotype), and infection conditions. A collection of 39 isolates of *Fusarium oxysporum* f. sp. *melonis* representative of all four races (0, 1, 2, and 1,2) was analyzed by several molecular techniques in the attempt to characterize the *formae specialis* and the races. The principal target was to discriminate race 1,2, the most virulent and widespread race in Europe and Italy. Microsatellites (SSR and ISSR), and calmodulin partial sequence analysis were unable to discriminate. In turn, minisatellites (M13 and T3B), translation elongation factor partial sequence analysis, and RAPDs allowed to clearly differentiate race 2 isolates from all other races, confirming its polyphyletic origin. With few exceptions, race 1,2 isolates clustered together in RAPD analysis using 23 primers out of 89 tested. A unique band was identified only in race 1,2 isolates. The corresponding sequence will be used for specific primer design.

EFFECT OF EXOTHERMIC REACTIONS IN STEAMING TREATMENTS: TRIALS ON SCLEROTIA SURVIVAL USING AN *AD HOC* CONSTRUCTED APPARATUS. A. Luvisi and E. Triolo. *Dipartimento Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: etriolo@agr.unipi.it*

Control of soil-borne pathogens using steam combined with exothermic reaction chemicals (Bioflash system) has been widely investigated since 1999, obtaining interesting results in terms of disease reduction in open field condition against *Sclerotinia minor* and *Sclerotinia sclerotiorum*. For securing information on the mechanisms of action of the treatment a specially constructed apparatus was built for laboratory analysis on sclerotia. The central body of the apparatus contains a box for accomodating samples and four pipes for dispensing air-steam mixture, and is filled with soil during testing. The box, containing three sample holders in which sclerotia are mixed with soil, is equipped with sensors for

temperature monitoring. Pipes are connected with an external tube which is in turn connected with a steam generator (0-60 g min^{-1}), while an additional pipe allows air to be pumped into the system. The apparatus was devised for simulating the thermal effects of a mobile steam generator, at mild, medium or high conditions, with Tmax of 60, 70 and 100°C, respectively. These temperatures were maintained for 5 min, then followed by a progressive reduction. Treatments were repeated adding calcium oxide (CaO) for evaluating exothermic reaction effects on the germination of *S. minor* and *S. sclerotiorum* sclerotia. Results confirmed the positive effects of exothermic reactions on sclerotia survival. Steam treatments were strongly effective on *S. sclerotiorum* only at the highest temperature conditions. However, adding CaO, survival was reduced drastically even with medium treatment (germination about 11%). *S. minor* was completely controlled with medium treatment using CaO, while mild temperature conditions lowered germination to about 20%.

CHEMICAL RESISTANCE INDUCERS USED IN GLOBE ARTICHOKE TO CONTROL *SCLEROTINIA SCLEROTIORUM*. **P. Magro and E. Marcucci.** *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: magro@unitus.it*

The aim of this study was to analyze the role of disease resistance inducers in globe artichoke to suggest the alternative use of new chemical compounds with a lower environmental impact. The most common lytic disease of globe artichoke is caused by *Sclerotinia sclerotiorum*, against which three inducers were used, i.e. BTH (acibenzolar-S-methyl), BABA (β-aminobutyric acid) and KH_2PO_4 (potassium phosphate monobasic). Resistance inducers were administered to globe artichoke cvs C3 and Exploter. The effect in resistance activation was determined by the assay of pathogenesis related (PR) proteins with reference to the activity of chitinase, peroxidase and β-1,3-glucanase. Disease severity in treated plants was lower than in the controls artificially inoculated with *S. sclerotiorum*. An increase of peroxidase activity was observed in C3 plants inoculated and treated with KH_2PO_4 and in inoculated Exploter plants treated with KH_2PO_4 and BABA. BABA treatment increased chitinase and glucanase in C3, whereas in Exploter only glucanase was stimulated by KH_2PO_4. The most abundant isoenzymatic pattern of glucanase was observed in inoculated Explorer plants treated with BABA. BABA and KH_2PO_4 induced a new isoform of peroxidase in Exploter, whereas in C3 this isoform was always observed. The isoenzymatic pattern of chitinase did not change among treated and inoculated plants. The experiments have shown that resistance inducers may have good prospects in the sustainable cultivation of globe artichoke.

MOLECULAR DETECTION OF ENDOPHYTIC FUNGI IN GRAPEVINE. M. Martini, R. Musetti, S. Grisan, R. Polizzotto, S. Borselli and R. Osler. *Dipartimento di Biologia Applicata alla Difesa delle Piante, Università di Udine, Via delle Scienze 208, 33100 Udine, Italy. E-mail: Rita.Musetti@uniud.it*

Endophytes are microorganisms (fungi, bacteria, actinomycetes) that live inside host plants without causing disease symptoms. Therefore, they are difficult to detect but identification by molecular means is becoming common. *Epicoccum nigrum* Link and *Aureobasidium pullulans* (de Bary) Arnaud are two ubiquitous microfungi, frequently reported as endophytes of different crops, including grapevine. Fungal endophytes have been isolated in PDA from surface sterilized leaf and shoot fragments of 15 vines. High molecular weight DNA was extracted from fresh mycelia following a modified procedure from Lecellier and Silar (*Current Genetics* 25: 122-123, 1994). ITS1, 5.8S rDNA and ITS2 were amplified by PCR using ITS1/ITS4 primers and sequenced. Two different *E. nigrum* strains and one *A. pullulans* strain were isolated from grapevine tissues. Species-specific primers for *E. nigrum* and *A. pullulans* were designed in variable regions of ITS1 and ITS2. Primers specificity was assessed by PCR using purified DNA from the more representative grapevine fungal endophytes. In order to know the frequency of the two endophytes in grapevine tissues, PCR with species-specific primers was done on DNA extracted from leaves and shoots of 41 grapevines collected in different localities of north-east Italy. All tested plants were positive for both endophytes and the organism identity was further confirmed by RFLP analyses of PCR products. The RFLP patterns obtained from grapevine samples were identical to those of *E. nigrum* and *A. pullulans* reference strains. Thus, the molecular method described allowed a sensitive, specific and reliable identification of the two endophytes in grapevine.

NEW REPORTS OF *PHYTOPHTHORA HEDRAIANDRA*, *P. NIEDERHAUSERII* AND *P. TENTACULATA* IN ITALY. P. Martini[1], S. Scibetta[2], C. Allatta[3], A. Pane[3] and S.O. Cacciola[4]. *[1]Istituto Regionale di Floricoltura, Via Carducci 12, 18038 Sanremo (IM), Italy. [2]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. [3]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 101, 95123 Catania, Italy. [4]Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. E-mail: cacciola@unipa.it*

During a survey of ornamental plant nurseries in Sicily (southern Italy) and Liguria (northern Italy) three uncommon *Phytophthora* species, *P. hedraiandra* de Cock & Man in't Veld (ITS-clade 1), *P. sp. "niederhauserii"* (ITS-clade 7) and *P. tentaculata*. Krober & Marwitz (ITS-clade 1) were found. *Phyotphthora* species were isolated from infected roots using selective media and axenic cultures were identified by sequence analysis of ITS regions of rDNA. *P. hedraiandra*, *P. niederhauserii* and *P. tentaculata* were recovered from potted plants of laurustinus (*Viburnum tinus* L.), *Banksia* sp. and *Origanum* sp., respectively. *P. hedraiandra* has been reported previously on laurustinus in various Italian regions. At present, only species of *Viburnum* and *Rhododendron* are known to be susceptible to this species of *Phytophthora*. *P. tentaculata* has been reported previously on several ornamental plants, including *Chrysanthemum*, *Delphinium* and *Verbena*. Recently, it was reported in Italy as a new pathogen of African Daisy (*Gerbera jamesonii* H. Bolus). However, to our knowledge, this is the first report of *P. tentaculata* on *Origanum*. *P. niederhauserii* was identified as a separate species within the ITS-clade 1 on the basis of DNA analysis, but it has not yet been described formally. The host range and distribution of this new species are not yet known. To our knowledge,this is both the first report of *P. niederhauserii* in Europe and the first report of this species on *Banksia*.

INDUCTION OF UV-MUTANTS OF *TRICHODERMA HARZIANUM* TOLERANT TO ANTIFUNGAL METABOLITES OF *FUSARIUM OXYSPORUM*. M. Marzano[1,2], M. Vurro[2] and C. Altomare[2]. *[1]Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso, Italy. [2]Istituto di Scienze delle Produzioni Alimentari del*

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The mycoherbicide *Fusarium oxysporum* strain FT2 is a pathogen of *Orobanche ramosa*, a destructive parasitic weed of vegetables in Mediterreanean countries. FT2 produces metabolites with antifungal activity, which may hamper its use in combination with other fungal biocontrol agents. The inhibitory effect of the organic extract of FT2 cultures was assayed towards the antagonistic strain *Trichoderma harzianum* CFA16, resulting in 82% inhibition of CFA16 colonies grown on PDA supplemented with 5% of FT2 extract. In order to improve the resistance of CFA16 to antifungal compounds of FT2 and ameliorate the compatibility between the two biocontrol agents, a UV-mutagenesis strategy was adopted. Conidia of CFA16 were exposed to UV-C rays for 120 seconds to obtain 80-85% of kill. Then, an aliquot of the irradiated suspension was seeded on water agar supplemented with FT2 extract. After 24 h, germinated conidia were isolated as presumptive tolerant mutants and transferred on PDA + 5% FT2 extract. The colony growth of mutants was measured daily and compared with that of the wild type (wt). 200 presumptive UV-C mutants were isolated and 25 mutants that exhibited up to 75% faster growth than the wt in the presence of 120 ppm of an FT2 antifungal metabolite were selected for further studies of biocontrol efficacy and ecological fitness. The development of strains with enhanced tolerance to toxic metabolites is expected to lead to an improvement of compatibility of CFA16 and biocontrol *F. oxysporum* strains, as well as to an increase of competitive capability of CFA16 towards phytopathogenic *F. oxysporum.* strains.

SANITARY SELECTION OF SWEET CHERRY. A. Materazzi, H. Bouyahia and E. Triolo. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: amatazzi@agr.unipi.it*

The research project "Tutela e valorizzazione della ciliegia di Lari", supported by the "Fondazione Cassa di Risparmio di Pisa", was launched for preserving the genetic resources of sweet cherry (*Prunus avium*) in Lari (Pisa, Italy). A multidisciplinary team was established and 12 autochthonous sweet cherry cultivars in danger of extinction were identified. The research was carried out on 99 thirty-year-old plants, distributed in 10 orchards. Composite samples of 15 leaves from each plant were collected in spring and tested by ELISA and RT-PCR for the presence of the following viruses: *Plum pox virus* (PPV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Cherry leaf roll virus* (CLRV), *Apple mosaic virus* (ApMV) and *Apple chlorotic leaf spot virus* (ACLSV). Laboratory tests showed that 76 of 99 plants (76.8%) were infected by at least one virus. The most frequent virus was PDV, accounting 98.7% of the infections (75 of 76 infected samples); PNRSV was detected in 2 samples and only in one case ACLSV was detected in mixed infection with PDV. PPV, CLRV and ApMV were not found. A subsequent analysis, showed that the sour cherry (*P. cerasus*) rootstock mother plants, largely used in the area as a source seeds for for seedlings, was 100% infected by PDV. As reported, PDV is transmitted to seeds with about 70% efficiency, so we can conclude that the high incidence of this virus (75,8%) can be mainly attributed to the use of infected rootstocks.

COMPARATIVE ANALYSIS OF PLUM BARK NECROSIS STEM PITTING-ASSOCIATED VIRUS BY IC-PCR AND ELISA DURING DIFFERENT SEASONS OF THE YEAR. S. Matic, M. Morelli and D. Boscia. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126, Bari, Italy. E-mail: d.boscia@ba.ivv.cnr.it*

Plum bark necrosis stem pitting-associated virus (PBNSPaV) was monitored during different seasons over the year by the comparative use of ELISA and immuno-capture (IC)-PCR. A field-grown tree of Japanese plum cv. Black Beaut, inoculated with an Italian PBNSPaV isolate (ASP), was sampled, testing 18 leaf samples in spring, summer and autumn and cortical tissues from dormant branches in winter. Virus was detectable during all seasons using both techniques. The highest virus detection was found in spring by both techniques (100%). During summer and autumn, IC-PCR was more successful in virus detection (100%), than ELISA which gave positive reactions with 83% and 56% of the samples, respectively. During winter, ELISA detected more samples (94%), compared to IC-PCR (89%). The virus was rather uniformly distributed in the tree canopy since it was detected in the apical, middle and basal part of the branches with slight differences. Taking into account the estimated infection values on the whole, both techniques gave satisfactory detections levels, for PBNSPaV was detected in 97% of the samples by IC-PCR and in 83% of the samples by ELISA. The use of both detection assays seems to afford an efficient identification of PBNSPaV the whole year round.

***WATERMELON MOSAIC VIRUS*: THE PREDOMINANT VIRUS INFECTING WINTER MELON IN SOUTHERN ITALY. M. Meneghini[1], C. Mennone[2], M.L. Palermo[3], G.F. Siddu[4] and L. Tomassoli[1].** *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]ALSIA, Regione Basilicata, Strada Statale Jonica 106, km 448,2, 75010 Metaponto (MT), Italy. [3]Regione Sicilia Assessorato Agricoltura e Foreste, U.O. 107, Via Toniolo 44b, 91026 Mazara del Vallo (TP), Italy. [4]Sardegna Agricoltura LAORE, Via Giovanni XXIII 99, 09096 Santa Giusta (OR), Italy. E-mail: l.tomassoli@ispave.it*

In Italy, *Watermelon mosaic virus* (WMV, genus *Potyvirus*, family *Potyviridae*) has been known since 1973 and it is one of the most common viruses affecting cucurbits in open-air crops together with *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV). However, WMV has been long considered a minor pathogen because it induces very mild symptoms on the laves and it seldom affects yield and quality of the fruits. In the last years, extensive outbreaks of a severe mosaic were observed on field-grown winter melons (*Cucumis melo* L. var. *inodorus*) in Southern Italy. Infected plants showed mosaic, vein-banding and malformation of the leaves and discolorations of the fruits which heavily reduced market quality. Samples from several infected fields in Sicily, Basilicata and Sardinia, the main producing regions of this crop, were analyzed by DAS-ELISA using commercial antisera against the most frequent mosaic-inducing viruses of cucurbits, mainly belonging to the genus *Potyvirus*. Two years of assays showed the WMV is the prevalent causal agent of mosaic of winter melon (infection rates: WMV, 75%; CMV, 15%; other viruses, 10%). WMV thus represents a real threat in those regions where winter melon is of major economic importance. In comparison with other cucurbit potyviruses, WMV occurs on a wide host range including species of other families and many weeds growing all year round. In addition several aphids species are vectors of this virus. Thus, it is necessary to implement appropriate sanitary measures, as insecticide treatments, culture practices, or providing resistant varieties to control WMV.

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EFFECT OF NATURAL PHENOLIC COMPOUNDS IN PEAR SHOOTS INOCULATED WITH *ERWINIA AMYLOVORA*. P. Minardi[1], S. Mucini[2] and U. Mazzucchi[2]. *[1]Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. [2]Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi, Via Fanin 4, 40127, Bologna, Italy. E-mail: paola.minardi@unibo.it*

In recent years, investigations of the antimicrobial and antioxidant properties of essential oils have shown that they can be used as natural additives to enhance the safety of fresh and processed fruits and vegetables. Most essential oils and their constituents are classified as GRAS (Generally Recognized As Safe) and their use is of great interest to the food industry. More specifically, phenolic components, such as thymol and carvacrol, are responsible for the antibacterial properties of many essential oils and appear to act as membrane permeabilizers. The antimicrobial activity of carvacrol and thymol towards the causal agent of fireblight disease, *Erwinia amylovora* (*Ea*), has been previously reported. The growth of this pathogen is completely inhibited by these compounds at the concentration of 50mM in double-layer agar plate tests, whereas, when the disc diffusion method is used, inhibition zones appear at the concentration of 35mM for both compounds. The minimum inhibitory concentration is 0.65 mM. From our previous studies *in vitro* we concluded that thymol has a bactericidal effect towards *Ea*. In order to test the hypothesis that carvacrol and thymol may induce resistance in pear shoots against *Ea*, both antimicrobials were sprayed at different concentrations on shoots of three-year-old pear plants and, after 7 days in the open, each shoot was inoculated with a bacterial suspension. The plants, kept for 7 days in a climatic chamber, were inspected for the presence of cortical cankers. Only thymol induced a slight protection against *Ea*. The procedures for the *in planta* experiments are discussed.

INDUCED SUPPRESSIVENESS TO *FUSARIUM OXYSPORUM* f.sp. *RADICIS LYCOPERSICI* IN RECYCLED SUBSTRATES IN CLOSED SOILLESS SYSTEMS. A. Minuto[1], F. Clematis[2], M.L. Gullino[1] and A. Garibaldi[1]. *[1]Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. [2]Centro di Competenza per l'Innovazione in Campo Agro-ambientale (AGROINNOVA), Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy. E-mail: andrea.minuto@unito.it*

Soilless tomatoes can be seriously damaged by *Fusarium oxysporum* Schlect f. sp. *radicis lycopersici* the agent of Fusarium crown and root rot (FCRR). FCRR suppression can be achieved through the use of chemicals, selected substrates, composts and artificially introduced. antagonistic microorganisms The aim of the current study was to evaluate the natural capacity of an used substrate (rockwool, perlite and perlite-peat mix) to suppress FCRR. New and used substrates, sampled from closed soilless systems, were either autoclaved or not, artificially inoculated with *Fusarium oxysporum* f. sp. *radicis lycopersici* or not and, finally, sown with tomato seeds cv Cuore di Bue. The effects of autoclaved/non autoclaved and used/new substrates on FCRR incidence were assessed by evaluating the symptoms of crown-rot on the root-stem transition zone of tomato seedlings. Non autoclaved and inoculated used rockwool, perlite and perlite-peat mix reduced FCRR incidence significantly when compared with non autoclaved and inoculated new substrate. Autoclaved and inoculated used rockwool and perlite-peat mix did not suppress FCRR, similarly to new and inoculated substrates. These results are in accordance with other research that, on cucumber/*Pythium* host/pathogen complex in a closed rockwool soilless system, showed the key role of resident microflora in suppressing root rot disease. By contrast, recycled perlite suppressed FCRR incidence and severity also when it was sterilized before inoculation with the pathogen.

SEED HEALTH AND BIOCONTROL PERSPECTIVES OF SEED-BORNE PATHOGENS IN THE VEGETABLE PRODUCTION CYCLE FOR QUALITY PRODUCTIONS. A. Mirotti, M. Sportelli and S. Gennai. *C.D.F. s.r.l. Via Amendola 40, 48022 Lugo (RA), Italy. E-mail: cdf@cdflugo.it*

Seed is the initial step of most vegetable crop productions. Italian legislation is not clear enough on the matter, as exhaustive sanitary standards on production of both conventional and biological seeds, have not yet been issued. Extant rules on the quality of nursery productions of vegetable species (D.M. April 14, 1997) require absence of specific pathogens in propagation materials, except for seeds. The aim of this study was to assess the sanitary condition of marketed seeds of tomato, cabbage, lettuce and melon and the relationship between their health status and disease incidence in an Emilia Romagna (northern Italy) nursery. We also evaluated the benefits of biocontrol agents available on the market. Soil compost was integrated during the productive cycle in greenhouse nursery with commercial formulations of *Trichoderma harzianum* (Rootshield® - Intrachem Bio Italia S.p.A.), *Streptomyces* (Mycostop® - Kemira Agro Oy), and mycorrhizae plus *Actinomycetes* (Micosat F Or®-CCS Aosta). Analyses showed that the percentage of seed samples positive for fungi, bacteria, and viruses were: (i) tomato: 5%, 10%, 2%; (ii) cabbage: 6%, 7%; (iii) lettuce: 2%, 3%, 3%; (iv) muskmelon: 9%, 2%, 1%, respectively. There was no significant difference between biological and conventional seed samples. Testing made during the productive cycle in the nursery showed the presence of diseases, particularly in plants coming from infected seed lots. The efficacy of biocontrol agents on plant growth was confirmed, for plants grew better in the nursery and had a longer productive cycle in the field. Mycorrhizal preparations with the addition of *Actinomycetes* showed a positive action on seeds germination. However, biocontrol of seed-borne plant pathogens was not enough to curb plant diseases in the nursery.

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DEVELOPMENT OF A PCR-BASED METHOD FOR DETECTION OF *BRENNERIA NIGRIFLUENS* IN PERSIAN WALNUT PLANTS. C. Moretti and R. Buonaurio. *Dipartimento Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: chiaraluce.moretti@unipg.it*

Shallow bark canker of Persian walnut (*Juglans regia* L.) incited by *Brenneria nigrifluens* is considered one of the most dangerous diseases for walnut timber production. Since a number of bacterial species are frequently isolated from cankers, we developed a PCR-based method for the specific detection of *B. nigrifluens*. Rep-PCR performed with REP primers on *B. nigrifluens*, on bacteria we found associated with bark cankers (Moretti *et al.*,

J. Plant Pathol. **89**: 211-218, 2007) and on *Agrobacterium tumefaciens, Brenneria rubrifaciens, Erwinia amylovora, Pectobacterium carotovorum* subsp. *carotovorum, Pectobacterium crysanthemi, Pseudomonas syringae* pv. *syringae and Xanthomonas arboricola* pv. *juglandis*, yielded an amplified product specific for *B. nigrifluens,* which was cloned and used for designing a pair of primers. The deduced aminoacidic sequence of the amplicon had an high level of identity with those of an exo-poly-α-D-galacturonosidase of *Yersinia enterocolitica* subsp. *enterocolitica* and *Pectobacterium chrysanthemi*. The primers permitted the specific amplification of a 310 bp amplicon when the genomic DNA of 11 *B. nigrifluens* strains, type strain included, was used as template. No PCR products were obtained from bacteria associated with bark cankers and from any of the above-mentioned phytopathogenic species. Validation of this PCR assay to detect *B. nigrifluens* in infected Persian walnut plants is under way

PARTIAL CHARACTERIZATION OF THE *FOW1* GENE IN *VERTICILLIUM DAHLIAE* ISOLATES FROM OLIVE TREES. **F. Nigro, I. Pentimone, H. Barham and A. Ligorio.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: nigrof@agr.uniba.it*

Verticillium dahliae Kleb., the causal agent of a vascular wilt disease, is an economically important plant pathogen with worldwide distribution, affecting severely young olive trees. To date, the molecular mechanisms of pathogenicity and symptom induction by *V. dahliae* in olive trees remain largely unexplored, although some pathogenicity genes of the fungus have been recently identified and characterized. Improving the knowledge on *V. dahliae* pathogenicity could be useful for epidemiological studies and for developing effective control strategies. Using primers (giFow1F-giFowR), designed on a conserved hypothetical protein of *Gibberella zeae* (XM381597), a 550 bp DNA fragment (VerFow1) was amplified from DNA extracted from several *V. dahliae* isolates, cloned and sequenced. Basic local alignment search tool (BLAST) of the fragment VerFow1 showed a considerable homology with a yeast and fungal hypothetical protein related to a mitochondrial carrier protein (MCPs). This protein is known for its role in communication between mitochondrial matrix and cytosol, being essential in eukaryotic metabolism. In particular, sequence analysis revealed a 96% identity with the corresponding region of *Fusarium oxysporum FOW1* gene (AB078975), encoding a mitochondrial protein that was identified and characterized as a virulence determinant, specifically required for the colonization of melon plants. Trials to determine the role of the fragment VerFow1 in the pathogenesis of *V. dahliae* on olive trees are in progress. To the best of our knowledge this is the first report on the characterization of a fungal hypothetical protein related to mitochondrial carriers in *V. dahliae*.

PRELIMINARY PATHOGENICITY TESTS OF *FUSARIUM* spp. ON ORNAMENTAL PALMS. **M. Nigro, F. Mannerucci and N. Luisi.** *Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via G. Amendola 165/A, 70126 Bari, Italy. E-mail: luisin@agr.uniba.it*

In southern Italy ornamental palms belonging to the genera *Chamaerops* and *Phoenix* showed wilting or general dieback in summer 2006. From fragments of leaves, roots and vascular tissues of symptomatic plants two *Fusarium* spp. were isolated, whose pathogenicity was tested on 2-year-old *Chamaerops humilis* L. and *Phoenix canariensis* Hort. seedlings. Inoculum of both strain was prepared by flooding with 10 ml of sterile distilled water (SDW) the surface of a Petri dish containing a fungal culture, then scraping and collecting the aerial mycelium with a spatula. The resulting suspension was diluted with SDW and the conidial concentration was assessed. Seedlings were inoculated with the suspension according to three methods: injection with a syringe, spraying, root immersion. Control seedlings were inoculated with SDW. Disease development was recorded by counting the number of symptomatic plants every 10 days. After 3 months symptoms, consisting of chlorosis of the leaves followed by necrosis and dieback, were detected only on *Phoenix* seedlings inoculated with both fungal isolates, while *Chamaerops* seedlings and controls showed no symptoms. Among methods of inoculation, root immersion proved to be the most effective based on of the number of infected seedlings. Positive re-isolations were obtained mostly from roots, thus suggesting that the two *Fusarium* isolates possess some host and tissue specificity.

INDOLE-3-ACETIC ACID PRODUCTION BY *FUSARIUM SOLANI* ISOLATED FROM KIWIFRUIT. **P. Nipoti[1], A. Prodi[1], C. Oliver[1], R. Baraldi[2], F. Rapparini[2] and A. Pisi[1].** *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Alma Mater Studiorum Università degli Studi, Via Fanin 42, 40127 Bologna, Italy. [2]Istituto di Biometeorologia del CNR, Via P.Gobetti 10, 40129 Bologna, Italy. E-mail: paola.nipoti@unibo.it*

Kiwifruit elephantiasis is characterized by hypertrophy of the trunk. *Fusarium solani* has been frequently isolated from affected plants and its involvement in the disease is being tested with pathogenicity trials underway. *F. solani* is known for its ability synthesize indole-3-acetic acid (IAA). This auxin is essential for cell growth, affecting both cell division and cellular expansion, and may promote axial elongation, lateral or isodiametric expansions at the tissue level. Monosporic cultures of 14 strains of *F. solani* were grown on agar-potato-dextrose (PDA) for determining their biological features (colonization ability and pigmentation) and the amount of IAA production. For *in vitro* tests, 3 cm long kiwifruit sprouts were placed on *F. solani*-colonized PDA and the extent of tissue necrosis was evaluated. Substrate pigmentation induced by the fungus was studied at 25°C. Determination of free IAA was done by a capillary gas-chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) using a Hewlett Packard 5890-5970 System. Half of the 14 isolates studied showed extensive tissue colonization, whereas most of them pigmented PDA with various levels of yellow/red tinge. IAA production was quite variable for all fungal strains. The high ability to colonize kiwifruit tissues by *F. solani* strains rarely corresponded to a high IAA production on PDA, except for two of the strains. These results call for further investigations on the relationships between host tissue colonization and IAA production by *F. solani* strains.

ACETIC AND PERACETIC ACID TREATMENT OF ORANGES TO CONTROL POSTHARVEST DECAY. **C. Oliveri, A. Bonaccorsi and V. Coco.** *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: c.oliveri@unict.it*

Post-harvest decay management has long relied on synthetic chemical fungicides. Recently, interest in 'natural' alternatives has been growing because they are effective against post-harvest pathogens of many fruits (pome and stone fruits, grapes, oranges,

plums, strawberries). In this study, the effects of acetic (AA) and peracetic (PAA) acid concentration on conidial germination of *Penicillium digitatum* and *Botrytis cinerea* and the application of AA and PAA for controlling decay of orange fruits in semi-commercial conditions were evaluated. AA (0.5%) reduced *B. cinerea* conidia germination below 15.6% after 3 h while at its highest concentration (3%) germination was totally inhibited after 3 and 6 h. Germination of *P. digitatum* conidia was reduced to 1.7% after 6 h treatment with 3% AA. A reduction of germ tube elongation was observed with all tested concentrations. PAA was much more active than AA for the lowest PAA concentration (0.1%) totally inhibited *P. digitatum* and *B. cinerea* conidia germination. Oranges were treated in the packinghouse as follows: (i) 3% AA spray; (ii) 3% AA dip; (iii) imazalil spray + wax (400ml/100Kg). After 2 weeks storage, 3% AA reduced decay incidence of sprayed fruits to 7.4%, compared with fungicide treated fruits. Since the fruits treated with AA showed peel browning, phytotoxic effects were evaluated in various ways. Dipping was effective for suppressing fungal decay but not browning. When cv. Tarocco fruits were sprayed with 2% AA and dried at 35°C, no browning was observed. Likewise, 2 and 3% PAA treatments induced no browning.

REAL-TIME PCR SYSTEMS BASED ON SYBR® GREEN I AND TAQMAN® TECHNOLOGIES FOR SPECIFIC QUANTITATIVE DETECTION OF *PHOMA TRACHEIPHILA* IN INFECTED CITRUS. M. Orrù[1], M. A. Demontis[1], S. O. Cacciola[2], V. Balmas[1], V. Chessa[1], F. Raudino[3], G. Magnano di San Lio[3] and Q. Migheli[1]. *[1]Dipartimento di Protezione delle Piante, Unità di Ricerca Istituto Nazionale Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. [2]Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche Agrarie e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. [3]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. E-mail: qmigheli@uniss.it*

Real-time PCR assays based on SYBR® Green I and TaqMan® technologies were developed for *in planta* detection and quantification of *Phoma tracheiphila*, the agent of citrus "mal secco". Primers and hybridization probe were designed on the internal transcribed spacer (ITS) region of the nuclear rRNA genes. Real-time PCR assays were compared with a classic isolation method in two separate experiments carried out on 6- and 24-month-old sour orange seedlings, artificially inoculated with a conidial suspension of the pathogen. Both technologies made it possible to follow the progression of infection by *P. tracheiphila*, enabling detection and quantification of the target fungus prior to the development of symptoms. Detection limit was 10 copies of the cloned target sequence and 15 pg of genomic DNA extracted from fungal spores. The presence of non-target fungal DNA had no effect on the specificity of the assay, but resulted in a 10-fold reduction of sensitivity. Total inhibition of the reaction occurred when conidia of the target pathogen were mixed with an organic soil substrate before extracting DNA by using the standard protocol, while an alternative purification kit resulted in a significant decrease in sensitivity. Compared to classic methods, real-time PCR proved faster and easier to perform and showed a higher sensitivity. These results suggest that real-time PCR has a great potential for early diagnosis of "mal secco" disease and for quantitative estimation of fungal growth within host tissue.

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NEW *PLUM POX VIRUS* FOCI IN CAMPANIA AND BASILICATA (SOUTHERN ITALY). R. Pacella[1], G. Massa[2], A. Fanigliulo[1], S. Comes[1] and A. Crescenzi[1]. *[1]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata. Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. [2]Bioagritest S.r.l., Zona PIP Lotto E2, 85010 Pignola (PZ), Italy. E-mail: aniello.crescenzi@unibas.it*

During the last decade stone fruits orchards in Southern Italy have suffered extended and severe outbreaks of Sharka disease mainly caused by *Plum pox virus* (PPV) D isolates. An extensive survey in several stone fruit orchards of Salerno (Campania) and Matera (Basilicata) provinces, was conducted aiming at the identification of new PPV foci over an area of 500 ha. Nearly 20,000 plants between apricot, plum, peach and nectarines were observed for the presence of symptoms. About 1000 plants that showed symptoms of viral infection were analysed by DAS-ELISA using PPV-specific polyclonal antibodies, 2% of which proved to be infected by PPV. Virus isolates responsible of the infection were characterised by biological indexing, by ELISA using monoclonal antibodies specific to the four strains and finally by RT-PCR, followed by *Alu*I and *Rsa*I RFLP analysis. All viral isolates belonged to the PPV-D subgroup. Two PPV-D isolates from different provinces, Salerno and Matera, selected for molecular characterisation, consisting in the amplification, cloning and sequencing of the CP gene, shared a similarity of about 100%, indicating that the identified foci were caused by the same PPV isolate. In May 2007, two new severe PPV foci were identified at Nocera Inferiore (SA) and on the Ionian coast of Basilicata, both on apricot cv. Ninfa. Virus isolates responsible for infection are under investigation.

NEW ADVANCES ON *IN VITRO* ANTIVIRAL CHEMOTHERAPY. A. Panattoni and E. Triolo. *Dipartimento Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: apanatto@agr.unipi.it*

The detrimental effects of virus infections on the quantity and quality of the yield, highlight the importance of virus-free propagation material and underline the need to produce virus-free plant material for the nuresry industry. Since 1992, we investigate the antiviral activity of several drugs for improving virus eradication, as chemotherapy offers wide alternative possibilities. At the beginning, our research was focused on the use of three well known synthetic nucleotide analogues: ribavirin, DHT and DHPA. Results were obtained in the control of *Plum pox yvirus* and *Prunus necrotic ringspot virus* on a selection of *Prunus cerasifera* explants. New antiviral groups characterized by different action mechanism were investigated more recently, i.e. inosine monophosphate dehydrogenase (IMPDH), S-adenosilhomocysteine hydrolase (SAH) and neuraminidase (NA) inhibitors. A first screening was conducted on the *in vitro* system *Nicotiana tabacum* cv Xanthi/*Cucumber mosaic virus* treated with drugs of these groups, obtaining positive results with all combinations (reported at the 13th Annual Meeting of SIPaV). New trials were therefore carried out for the sanitation of grapevines with poor sanitary condition. Treatments gave a good control of *Grapevine leafroll-associated virus* 3 infection on i*n vitro*-grown cv. Sangiovese explants, yielding 100% free explants when NA inhibitors were used. *In vitro*-grown cv. Sagrantino plantlets infected by *Grapevine virus A* were treated with the same compounds and successful virus eradication was obtained with the combination of IMPDH and SAH inhibitors.

EVALUATION OF PEAT MIXTURE SUPPRESSIVENESS AGAINST *RHIZOCTONIA SOLANI*. **C. Pane, C. Chiantese, G. Bonanomi, L. Cozzolino, V. Antignani, G. Puopolo, A. Zoina and F. Scala.** *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: catello.pane@libero.it.*

Sphagnum peats generally has no suppressive effects on soil-borne pathogens, although in few cases it was reported to reduce the incidence of seedling damping-off caused by *Rhizoctonia solani* and *Pythium ultimum*. Previous studies related the suppressive capability of peats to their content of carbohydrates and readily degradable organic compounds, which sustain the activity of antagonistic microorganisms. This hypothesis was corroborated by the observation that light peats, which are rich in carbohydrates, support a higher microbial activity compared to dark peats, that are consistently conducive. In this study four organic amendments (i.e. A= mixture of light and dark peats; B= peats + green plant residues; C and D= peats enriched with different micronutrients), were analyzed for a number of physical, chemical, and biological properties, including the suppression of damping-off caused by *R. solani*. Disease suppression varied largely among these four peat mixtures, according to the following ranking: B≥C>A>>D. Peat suppressiveness was positively related to its enzymatic activity (i.e. FDA, endochitinase, biase, glucanase and NAGase), pH, electrical conducivity, N organic, actinomycetes population density and phytotoxicity level. Only amendment B was found to be phytoxic using the *Lepidium sativum* test. The presence of undecomposed plant residues in B probably sustained the activity of antagonistic microbes but, at the same time, induced substrate phytotoxicity. By contrast, C suppressivity was related to the abundant presence in the mixture of *Trichoderma* species. Our results indicate that the combined application of peats and suppressive materials (e.g. crop residues at low dosages) is promising for container-produced plants.

CONTROL OF PLANT PATHOGENS BY USING A COMPOST TEA. **C. Pane[1], F. Valentini[2], G. Bonanomi[1], L. Cozzolino[1], V. Antignani[1], G. Puopolo[1], A. Zoina[1] and F. Scala[1].** *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Ge.Se.Nu. S.p.A., Via della Molinella 7, 06125 Ponte Rio (PG), Italy. E-mail: catello.pane@libero.it*

Compost tea is increasingly being used to control plant diseases. Aerated compost teas are highly concentrated aqueous microbial suspensions obtained from the decomposition of composts in water for a defined period of time. Effective disease control with compost teas has been obtained for several air-borne pathogens such as *Sphaerotheca* spp., *Venturia* spp. and *Alternaria* spp. In this study, a compost tea was analysed for the abilility to suppress the following diseases: grey mould of bean and tomato caused by *Botrytis cinerea*, bacterial speck of tomato by *Pseudomonas syringae* pv *tomato* (Pst), and damping-off of *Lepidium sativum* by *Rhizoctonia solani*. This tea was produced by the aerobic water decomposition of a compost obtained from a mixture of municipal organic waste, green pruning residues, and tobacco and aromatic plant refuses (6:4:2, respectively). Compost tea contained 2,6 x 10^9 c.f.u. ml^{-1} of culturable bacteria (fluorescent *Pseudomonads* were absent) and 1,15 x 10^5 c.f.u. ml^{-1} culturable filamentous fungi (almost exclusively present as *Aspergillus wentii*). Tea was not phytotoxic in soil and foliar applications; inhibited *B. cinerea* conidial germination and suppressed leaf lesion development on bean and tomato and significantly reduced severity of bacterial speck on tomato plants. In contrast, soil drenching applications were unable to control *Rhizoctonia* damping-off. From these results, it appears that the compost tea has a potential for useful applications in plant disease management.

FUNGAL CONTAMINATION OF AIR AND WHEAT GRAIN SAMPLES FROM FIELD AND STORAGE ENVIRONMENTS. **S. Panebianco, A. Bonaccorsi, A. Vitale, R. La Rosa and G. Cirvilleri.** *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: sapanebianco@yahoo.it*

Considering the risk that mycotoxin contaminations represent for human health, a study was undertaken to estimate the contamination of wheat (*Triticum durum* L.) grains and the air of storage warehouses by potential mycotoxin-producing fungi in Sicily. Samples of air and wheat grains were collected from the field, warehouses and farmer's cooperative storey-buildings and examined to estimate the total fungal population. *Fusarium, Alternaria, Aspergillus* and *Penicillium* spp. were isolated and identified. *Fusarium* and *Alternaria* spp. were predominant in the air and grain samples from the field, whereas *Penicillium* and *Aspergillus* spp. were predominant in the air and grain samples from warehouses and storey-buildings. Among *Aspergillus* spp., *A. ochraceus* and *A. niger* were the most common. Antagonism against *Aspergillus* isolates by different bacterial strains was investigated. Bacteria and their cell-free-filtrates strongly inhibited *A. ochraceus* and *A. flavus* isolates in *in vitro* screening. In *in vivo* screening of wheat grains, fungal growth was markedly inhibited during 15 days of storage. The *in vitro* and *in vivo* assays showed that *A. ochraceus* isolates were more sensitive than those of *A. flavus* to the antagonistic activity of bacterial strains. *Pseudomonas syringae* 48SR2 and *Burkholderia gladioli* DISTEF strains showed the strongest antagonistic activity in both assays. Results show that wheat contamination with potentially mycotoxigenic fungi is a risk present both in the open field and storage environments in Sicily. Moreover, biological control by antagonistic bacteria should be more deeply investigated.

A SEVERE OUTBREAK OF *TOMATO INFECTIOUS CHLOROSIS VIRUS* IN LETTUCE AND ESCAROLE IN SOUTHERN ITALY. **G. Parrella[1] and F. Filella[2].** *[1]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. [2]ARSSA, Agenzia Regionale per lo Sviluppo e per i Servizi in Agricoltura, 87027 Paola (CS), Italy. E-mail: parrella@ipp.cnr.it*

Tomato infectious cholorosis virus (TICV), a member of the genus *Crinivirus*, family *Closteroviridae*, is the causal agent of yellowing diseases in several vegetable crops grown under protected environments. It is a phloem-limited bipartite genome virus, transmitted by the greenhouse whitefly *Trialeurodes vaporariorum* in a semi-persistent manner. In 2005 and 2006, a severe disease of lettuce cv. Romana (*Lactuca sativa* var. *longifolia*) and escarole (*Cichorium endivia* var. *latifolium*) characterized by intervenial yellowing of the leaves was observed in Calabria (Southern Italy). The percentage of affected plants ranged between 70 and 80% for both crops. Leaf samples collected from symptomatic plants were positive in RT-PCR assays when degenerate primers for criniviruses were used. The identity of the virus was then assessed using TICV-specific primers and probe. Transmission tests with viruliferus *T. vaporariorum* collected form infected lettuce and escarole, reproduced the symptoms on healthy plants after about one month from exposure to the insects. Based on biological and

molecular data, TICV was for the first time detected in lettuce in Italy and for the first time in escarole, which could consequently represent a new concern for these crops, at least in Southern Italy. Although the distribution of TICV and its impact on lettuce and escarole production need to be further investigated in Italy, these data confirm that whitefly- transmitted viruses are increasing their importance worldwide, becoming a potential limiting factor for many crops, also in some previously unaffected temperate areas of the Mediterranean basin.

EFFECT OF *TRICHODERMA* sp. ON SPORULATION OF *STEMPHYLIUM VESICARIUM*, THE CAUSAL AGENT OF PEAR BROWN SPOT. E. Pattori, S. Cavagna, K. Righi and V. Rossi. *Istituto di Entomologia e Patologia vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it*

It is known that fallen pear and weed leaves support the production of conidia and ascospores of *Stemphylium vesicarium* and its teleomorph *Pleospora allii*, thus representing the inoculum source for infections during the season. It was also shown that biological control based on application of *Trichoderma* reduces the production of ascospores. In this work, the effect of *Trichoderma* formulations on suppression of conidial production was investigated under both environment-controlled and natural conditions. Pear and crabgrass (*Digitaria sanguinalis*) leaves were inoculated with *S. vesicarium* or *Trichoderma* or both fungi, and the production of conidia was determined microscopically for six weeks. BCAs were able to colonize both substrates and produced abundant *Trichoderma* spores, with significant differences between formulations. BCAs reduced spore production by *S. vesicarium*, by about 99% after 6 weeks. *Trichoderma* formulations were also applied in spring on a grassy area naturally converted into a wildflower meadow that was inoculated with *S. vesicarium*, and covered with an inoculated pear leaf litter. Conidia were continuously trapped from spring to autumn with spore traps placed above the soil. In one of two years, BCAs significantly reduced the amount of conidia trapped compared to the untreated control.

GENETIC CHARACTERIZATION BY AFLP OF *BACILLUS LICHENIFORMIS* STRAINS, POTENTIAL BIOCONTROL AGENT OF SOIL-BORNE PATHOGENS. I. Pentimone, M. Ferrara, A. Ligorio, A. Ippolito and F. Nigro. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: nigrof@agr.uniba.it*

Bacillus licheniformis, a Gram-positive, spore-forming soil bacterium, is used in the biotechnology industry to produce enzymes, antibiotics, and other biochemicals. The species synthesizes a range of extracellular products that may contribute to nutrient cycling in nature. Moreover, in recent years interest in biocontrol of soil-borne plant pathogens has been encouraged by trends in agriculture towards a greater sustainability. There are clear evidences that several bacteria and their products play a key role in the suppression of various soil-borne plant pathogens. The biocontrol agent *B. licheniformis,* strain MBBL1, proved to be effective in reducing the inoculum density of *Verticillium dahliae* in the rizosphere of olive trees. However, the introduction of antagonistic microbes in the soil calls for the necessity of monitoring their dispersal and interactions with the natural occurring rhizosphere microbes. Therefore, in this study the amplified fragment length polymorphism (AFLP) technique was applied and evaluated to discriminate strain MBBL1 from a group of highly related *Bacillus* spp. isolates from the olive rhizosphere. For AFLP analysis, the total purified genomic DNA from 100 isolates was digested with Eco*RI* and Mse*I* and ligated to constructed adapters. Restricted/ligated fragments were pre-amplified using primers complementary to the adapters, then selectively amplified using primers exceeding three nucleotides beyond the 3'-end of the adapters. Finally, amplified products were resolved with polyacrylamide gel electrophoresis and were silver stained. The method enabled the identification of the targeted strain even after soil treatment. Moreover, the AFLP method allowed a preliminary characterization of *B. licheniformis* strains at the intra-subspecific population level.

INFLUENCE OF CLIMATIC CONDITIONS AND PHENOLOGICAL STAGES ON AIR POLLUTION DAMAGES TO WHEAT VARIETIES. V. Picchi[1], S. Quaroni[2], M. Saracchi[2], P. Viola[3], M. Iriti[1,2] and F. Faoro[1,2]. *[1]Istituto di Virologia Vegetale del CNR, Sezione di Milano, Via Celoria 2, 20133 Milano, Italy. [2]Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. [3]Apsovsementi, 27058 Voghera, (PV), Italy. E-mail: franco.faoro@unimi.it*

A three year survey on wheat performance in the Po Valley (northern Italy) was carried out, correlating climatic conditions (temperature and rainfall) and tropospheric ozone levels with the evolution and severity of a peculiar symptomatology due to air pollution, very likely ozone, as assessed with open top chambers (OTC) facilities and cytochemical investigations. Symptoms consisted of small chlorotic spots, usually appearing in the last decade of April and evolving, in some varieties, in elliptical necrotic lesions. The surveys (March-June of each year) showed that symptom severity, besides being related to the sensitivity of each variety, depended also on the phenological stage in which critical ozone AOT40 (Accumulated dose Over a Threshold of 40 ppb) levels were reached. In 2007, a year characterized by a mild winter and heavy early spring rainfalls, enhancement of symptom severity was observed. In this year, even tolerant varieties, such as Guarni (bread wheat) and Dylan (durum wheat), showed chlorotic spots already in the first decade of April, even though AOT40 levels were similar to those recorded in the previous years. This could be related to an earlier occurrence of tillering and, in turn, to the enhanced pollutant uptake in this period of maximum growth rate. Crop yield data of six bread and two durum wheat varieties indicated that symptom severity is not strictly correlated with productivity, though trials with OTC demonstrated that filtered air led to 10-20% enhancement of productivity.

FUNGAL PATHOGENS OF WEEDS GROWN IN SOIL AMENDED WITH OLIVE MILL WASTE BY-PRODUCTS. A.M. Picco[1], M. Rodolfi[1], S. Chinaglia[1], L. Pecoraro[2], E. Salerni[2] and C. Perini[2]. *[1]Dipartimento di Ecologia del Territorio, Sezione di Micologia, Università degli Studi, Via S. Epifanio 14, 27100 Pavia, Italy. [2]Dipartimento di Scienze Ambientali "G. Sarfatti", Università degli Studi, Via P.A. Mattioli 4, 53100 Siena, Italy. E-mail: apicco@et.unipv.it*

Different soil amendments with olive mill by-products (olive husk and olive mill waste mixture arranged according to MATReFO procedure developed by ISAFoM-CNR) were compared in an experimental trial conduced in the Botanical Garden of the University of Siena, in April 2006. In the first year of the trial plots were analyzed for their bacterial and fungal population as

well as natural weed colonization; soil samples were also collected for evaluating carbon evolution. In the context of this agronomic trial, the present study reports the results of a phytosanitary investigation carried out on the spontaneous vegetation grown on amended soils. Some phytopathogenic species were detected among the different phylloplane fungi identified from randomly collected leaves with necrosis. During winter sampling, *Cercospora medicaginis*, the causal agent of "summer black stem and leaf spot", was isolated from *Trifolium pratense* grown on both amended and non amended soils. In the next sampling, (spring 2007), the disease appeared only in *T. pratense* plants grown in control plots. A similar picture was observed on the leaves of *Holcus lanatus*, colonized by the cosmopolitan pathogen *Bipolaris cynodontis*. The only fungal pathogen detected on plants grown on amended plots was *Ramularia pratensis*, which was frequently isolated from leaf spots of *Rumex crispus*. The frequent presence of *Trichoderma* strains in amended soils seems worth of further investigations to confirm the protective role observed.

SENSITIVITY AND SPECIFICITY EVALUATION OF REAL-TIME PCR PROTOCOLS IN THE DIAGNOSOS OF GRAPEVINE YELLOWS. L. Pivetti[1], C. Ratti[2] and E. Stefani[1]. *[1]Dipartimento di Scienze Agrarie e degli Alimenti, Università di Modena e Reggio Emilia, Via J.F. Kennedy 17, 42100 Reggio Emilia, Italy. [2]Dipartimento di Scienze e Tecnologie Agro-ambientali, Università degli Studi, Via G. Fanin 44, 40127 Bologna, Italy. E-mail: emilio.stefani@unimore.it*

In the last decade, grapevine yellows outbreaks became increasingly common in the vineyards of Emilia Romagna (northern Italy). At least two phytoplasmas have been associated with these outbreaks i.e. Aster yellows (16SrI) and Stolbur (16SrXII). Diagnosis of these pathogens is somewhat troublesome, due to their uneven distribution in the vines and to the nature of the samples for testing (plant tissues and insect vectors). In general it is recognised that nested PCR, which is a common diagnostic technique for grapevine yellows, might have a reduced sensitivity, whereas RealTime-PCR is more sensitive. This study aimed at comparing nested PCR with two RealTime-PCR protocols using a TaqMan probe and the Sybr-Green chemistry, respectively. Test samples were grapevine tissues and insect vectors. Results showed that nested PCR was less sensitive than RealTime-PCR techniques. Of these, the protocol based on the TaqMan probe had the highest specificity, whereas the SybrGreen chemistry protocol gave better results as to sensitivity. These results suggest the use of the RealTime-PCR protocol with the TaqMan probe for certification of nursery material and for checking symptomless plant material from the field.

THE COMBIMATRIX PLATFORM FOR MICROARRAY ANALYSIS: GENE EXPRESSION IN RESISTANT AND SUSCEPTIBLE GRAPE GENOTYPES DURING *PLASMOPARA VITICOLA* INFECTION. M. Polesani[1], D. Glissant[1], D. Dametto[1], A. Ferrarini[1], F. Desario[1], A. Kortekamp[2], M. Pezzotti[3], M. Delledonne[1] and A. Polverari[3]. *Dipartimento Scientifico e Tecnologico, Università degli Studi, Strada Le Grazie 15, 37134 Verona, Italy. [2]Institute of Special Crop Cultivation and Crop Physiology, University of Hohenheim, 70593 Stuttgart, Germany. [3]Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino, Villa Ottolini-Lebrecht, 37029 San Floriano di Valpolicella (VR), Italy. E-mail: annalisa.polverari@univr.it*

A comprehensive analysis of transcriptional changes associated with the infection process of *Plasmopara viticola* in susceptible (*Vitis vinifera* cv. Pinot Noir) and resistant (*Vitis riparia* cv. Gloire de Montpellier) grapevine genotypes has been undertaken by microarray analysis, at different times after infection. The analysis was performed on the newly developed Combimatrix platform at the University of Verona on a Grape chip carrying 24562 specific probes in triplicates from assembly of Tentative Consensus of the last TIGR *Vitis vinifera* Gene Index release 5.0, and from non reduntant genomic sequences produced by the genome annotation in the International Grape Genome Project. Combimatrix technology is characterized by an exclusive *in situ* oligo (up to 40 mers) synthesis driven by electrochemistry and by the reusability of the same microarray, all factors that confer high flexibility to the system and reduce drastically the costs of microarray analysis. Leaves of resistant and susceptible grape plants grown *in vitro* were infected with *P. viticola* or treated with distilled water as a control, and collected at 12, 24, 48 and 96 h post-inoculation. Hybridisations were carried out with samples deriving from three independent replicates. Differentially expressed genes were selected using the multi experiment Significance Analysis of Microarray test, and gene clustering was performed using Genesis software.

DIFFUSION OF THIELAVIOPSIS TRUNK ROT IN IMPORTED DATE PALM AND DETECTION OF HEART ROT BY RESISTOGRAPH AND TREE TOMOGRAPHY PROCEDURES. G. Polizzi[1], I. Castello[1], A. Vitale[1] and C. Fruscione[2]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, University of Catania, Via S. Sofia 100, 95123 Catania, Italy. [2]Studio Verde S.A.S. Via Cervino 42/C, 10155 Torino, Italy. E-mail: gpolizzi@unict.it*

During 2006 and 2007, a survey was conducted in nurseries, private and public gardens of eastern Sicily for assessing the distribution of heart rot caused by *Thielaviopsis paradoxa* on mature plants (5 to 8 meters in hight) of date palm (*Phoenix dactylifera*) from Egypt. Symptoms of heart rot were observed in several private and public gardens of the Catania and Ragusa provinces affecting a high percentage (40-80%) of the plants. Rotting of the upper half of the trunk caused a sudden collapse of the canopy, which had an apparently normal aspect. Infected plants died 2-3 years after transplanting. Symptoms were not detected on lignified and external fibres. Isolations from internal tissues adjacent to the rotten areas made on carrot agar with 500 µl streptomycin sulphate and on acidified (lactic acid; pH 3.6) potato dextrose agar, yielded frequently *Thielaviopsis paradoxa* colonies. Internal decay of symptomless palms was assessed by a drilling resistance-measuring procedure with resistograph (IML-RESI B 400) and by Tree Tomography (Arbotom® Rinnteck). Transverse cross-sections of plants with no external symptoms confirmed instrumental analysis and revealed a brown rot of non-lignified or lightly lignified tissues. These results show that instrumental analysis may be used for diagnosis of heart rot. In any case, it is recommended to avoid importation of mature date palms from known infected areas due to symptomless infections by *T. paradoxa*.

A NEW EMERGENCE IN SOILLESS TOMATO CULTURES IN SICILY: VASCULAR AND PITH DISCOLORATION CAUSED BY *PSEUDOMONAS FLUORESCENS* AND *P. PUTIDA*. G. Polizzi, M.A. Dimartino, S. Panebianco and G. Cirvilleri. *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: gpolizzi@unict.it*

During February-May 2007, a widespread whitering was ob-

served in seven tomato soilless cultures under plastichouses in the Catania, Siracusa and Ragusa provinces (eastern Sicily). Transverse sections of the stem of tomato cvs Piccolo, Tyty, Jessica, Shiren, Desiderio, and Clave revealed a yellow, pink or brown discoloration of the tissues, especially at the crown level. These symptoms, resembling those induced by *Fusarium oxysporum*, were detected on most of the plants (50-100%). Pith necrosis and brown spots on the stem surface were detected sporadically only in a plastic-house. *Fusarium oxysporum* f. sp. *radicis-lycopersici* was seldom isolated on potato dextrose agar (PDA) or acidified PDA (lactic acid; pH=3.6). By contrast, Gram negative bacteria were always isolated from the internal stem tissues of tomato plants on nutrient agar and King's medium B. One hundred bacteria isolates were purified and used for further studies. They showed the LOPAT characters of group Vb (++-+-) and group Va (-+-+-) and were identified as *Pseudomonas fluorescens* (biovar I) and *Pseudomonas putida* (biovar A) on the basis of morphological, physiological and biochemical tests. The identity of representative strains was confirmed by the nutritional profile obtained with Biolog analysis (Microbiolog TM System Release 4.2; Biolog, Inc., Hayward, CA, USA). Koch's postulates were fulfilled using cvs Tyty and Piccolo confirming the pathogenicity of the isolates. *P. fluorescens* (biovar F) or *P. corrugata* were isolated only occasionally from symptomatic stems. In our conditions, vascular and pith discoloration caused by *P. fluorescens* and *P. putida* can be confused with symptoms induced by *F. oxysporum*.

OCHRATOXIN A CONTAMINATION OF MUSTS AND WINES: OBSERVATIONS ON PREDISPOSING FACTORS IN THE VINEYARDS. S. Pollastro[1], C. Dongiovanni[2], C. Giampaolo[2], R.M. De Miccolis Angelini[1], F. Mingolla[1], P. Natale[2] and F. Faretra[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. [2]Centro di Ricerca e Sperimentazione in Agricoltura "Basile Caramia",Via Cisternino 281, 70010 Locorotondo (BA), Italy. E-mail: faretra@agr.uniba.it*

Ocratoxin A (OTA) is a common contaminant of several foods and beverages. *Aspergillus carbonarius* (Bainier) Thom is the main responsible of OTA contamination of grapes and wines in the Mediterranean area. Although fungicides (i.e. anylinopyrimidines) are available for controlling this fungus, IPM strategies are essential to limit contamination below the maximum tolerable limit of 2.0 μg kg^{-1} of OTA established for wine and other grape-juice derivatives [Reg. (CE) N. 123/2005 of 26.1.2005]. Investigations carried out in southern Italy showed that several factors, i.e. grape cultivars and clones, growing areas, training systems, fungal diseases and pests, influence the risk of contamination. 'Gaglioppo', 'Negroamaro', 'Primitivo' and 'Sangiovese' were more susceptible than 'Cabernet sauvignon' to contamination. Within each cultivar, the risk of contamination differs with the clone, being higher for clones with compact bunches and thin-skin berries (i.e. 'Primitivo' 36C, 9.1 ng OTA g^{-1} must) than clones with less compact bunches and thick-skin berries (i.e. 'Primitivo' 35I, 0.1 ng OTA g^{-1} must). Vineyards near the sea were generally at higher risk of OTA contamination than those in windy areas. Similarly, trellis- and arbor trained vineyards were at higher risk than the head-pruned (alberello pugliese) plantings. The level of contamination of musts by *A. carbonarius* and OTA was correlated (r^2=0.5-0.9) with the severity of damage to the bunches caused by fungal diseases (esca and powdery mildew) or pests (*Lobesia botrana*). Such findings indicate that a careful integrated management of cultural practices and crop protection of vineyards is essential for reducing the risk of OTA contamination.

CRITICAL POINTS FOR *PHAEOMONIELLA CHLAMYDOSPORA* INFECTIONS OF PROPAGATION MATERIALS IN GRAPEVINE NURSERIES. S. Pollastro, A. Pichierri, W. Habib, N. Masiello, C. Sebaaly and F. Faretra. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it*

Phaeomoniella chlamydospora (Gams, Crous, Wingf. *et* Mugnai) Crous *et* Gams, a fungus involved in brown streaking of rootstocks, Petri disease and esca disease of grapevine, is frequently detected in rootstocks ready to be planted. Hence, infected propagation material is supposed be its main cause for dissemination to new vineyards. Nested-PCR was used to detected *P. chlamydospora* in 374 grafts and cuttings before callusing, 373 graft-cuttings after callusing, 319 grafted rootstocks, 248 water samples from 80 tanks used for pre-grafting or pre-callusing hydration and 155 samples of plant debris from blades and benches of 40 grafting machines, collected in several nurseries in southern Italy. For plant materials, the type and severity of wood discoloration was assessed using an empirical scale with six classes. An increase in the frequency of pathogen detection was observed during the various stages of the process: *P. chlamydospora* was not detected in grafts and dormant cuttings, it was found in 7% of the graft-cuttings, and in 57% of grafted rootstocks. A similar trend was observed for wood discoloration which reached a McKinney's Index as high as 48% in grafted rootstocks. *P. chlamydospora* was detected in water samples collected from 30% of the tanks and in debris samples collected from 23% of grafting machines in all the nurseries. Such findings point out several critical points in the production of grapevine propagation materials that should be kept under control for preventing infections and the consequent spreading of the fungus in new vineyards.

SCREENING OF FRESH MARKET TOMATO FOR RESISTANCE TO *PYRENOCHAETA LYCOPERSICI*. N. Pucci[1], S. Voltattorni[2], A. Infantino[1] and N. Acciarri[2]. *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]CRA, Istituto Sperimentale per l'Orticoltura, Via Salaria 1, 63030 Monsampolo del Tronto (AP), Italy. E-mail: nicoletta.pucci@entecra.it*

Pyrenochaeta lycopersici is a soil-borne fungus causal agent of "corky root" of tomato (*Solanum lycopersici* L.) that has hampered the cultivation of several tomato ecotypes/varieties lacking the resistance gene *py-1*. The aim of the present work was the transfer of genetic resistance to *P. lycopersici* from resistant cvs. Moboglan and Mogeor in Cuore di Bue di Albenga, a traditional Italian cultivar, within the framework of the national project PROM (Progetto di Ricerca per potenziare la competitività di Orticole in aree Meridionali). One isolate of the CRA-ISPaVe collection (ISPaVe ER-1211), out of 20 screened, was choosen following artificial inoculation on the susceptible tomato cv. Monalbo and the resistant tomato cv. Moboglan. A total of 19 tomato breeding lines (two F2; ten F3; five F4 and two F2BC1) segregating for *py-1* were screened in the greenhouse by growing tomato seedling (36 plants for each line) into a soil artificially infested with the fungus. Disease evaluation was done after 40 days using a 0-4 empirical scale based on the percentage of necrotized roots. As a result, 6 lines were considered resistant, 4 susceptible and 9 intermediate. In order to validate the efficiency of the molecular marker for *py-1*, apical leaves of each plant were used for DNA extraction and PCR amplification. Preliminary results showed a positive correlation between disease scoring and marker profiling. If confirmed on the other lines tested, this marker

will be used in marker-assisted selection procedures, allowing the rapid large-scale screening of tomato lines for corky root resistance.

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CHARACTERIZATION OF LIPOXYGENASE GENES AND OXYLIPIN PROFILE IN *ASPERGILLUS OCHRACEUS*. **F. Punelli[1], M. Reverberi[1], H. Velez[3], M. Scarpari[1], S. Zjalic[1], A. Ricelli[2], A. Dobson[3], C. Fanelli[1] and A.A. Fabbri[1].** *[1]Dipartimento di Biologia Vegetale, Università "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. [2]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. [3]Microbiology Departement, University College, College Road, Cork, Ireland***.** E-*mail*: *federico.punelli@uniroma1.it*

In previous works the lipoxygenase (lox) profile of *Aspergillus ochraceus* (wt, OTA producer), was characterized. A lox fragment of 673 bp - DQ087531 (47-78% homology with other fungal lipoxygenase sequences), was found and studied. This fragment was used to generate a Δlox mutant which showed delayed conidia formation and increase of sclerotia production. In addition, low lipoperoxide formation in the mycelium, reduced lipoxygenase activity and strong reduction of OTA biosynthesis were observed in the mutant in comparison with wt. The use of lipoxygenase inhibitors (SHAM, salicylhydroxamic acid and resveratrol) allowed to further study the role played by lipoperoxides alteration in OTA synthesis by *A. ochraceus*. Resveratrol showed antioxidant properties and inhibition effect on lipoxygenase and cicloxygenase in humans. In *A. ochraceus* wt the use of this LOX-inhibitors led to a delay of lipoxygenase activity and oxylipin profile in comparison with Δlox showing a marked reduction of lipoperoxides and OTA formation. Supplementation of culture media with several fatty acids was also investigated. The complete *lox* gene sequence was obtained with several techniques such as Genome Walker Library, Reverse PCR and 5'-3' RACE. In conclusion, the results obtained with both wt and Δlox indicate that lipoperoxidation plays a major role in controlling OTA biosynthesis in *A. ochraceus*.

FIRST INSIGHT ON THE POSSIBLE CORRELATION BETWEEN BACTERIOLYTIC ACTIVITY AND BIOFILM FORMATION IN A *LYSOBACTER* sp. STRAIN. **G. Puopolo[1], A. Raio[2] and A. Zoina[1].** *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. E-mail: puopolo@unina.it*

Most members of the genus *Lysobacter* possess high bacteriolityc activity against both Gram-negative and Gram-positive bacteria. *Lysobacter* sp. strain PG4 was evaluated on different growth media for its antagonistic activity towards 16 strains belonging to different bacterial species. The highest bacteriolytic activity was observed against bacterial species belonging to the Gram-positive group such as *Clavibacter michiganensis* subsp. *michiganensis*, *Rhodococcus fascians* and *Listeria monocytogenes* while no activity was scored against Gram-negative bacteria. The bacteriolytic activity was affected by growth media. King's B Medium (KBM) was the only one that affected negatively the antibiotic capacity of strain PG4. Interestingly, the addition of $FeCl_3$ to this medium restored PG4 bacteriolytic activity indicating that Fe^{3+} ions are involved in this activity. Some of the growth media used in bacteriolytic tests were also used in adhesion assays performed in 96 wells polystyrene plates, a common test that allows to evaluate the production of biofilm by bacteria. The ability of *Lysobacter* sp. strain PG4 to form biofilm was correlated to the entry in the stationary phase, except when the bacterium was cultivated in KBM. By contrast, strain PG4 was unable to form biofilm at all. These results represent a first insight on the correlation between biofilm formation and bacteriolytic activity in a member of the genus *Lysobacter.*

TOBACCO-*ERYSIPHE CICHORACEARUM*: A POSSIBLE ROLE OF PLANT SEMIOCHEMICALS IN INDUCED RESISTANCE. **M. Quaglia, C. Zadra, M. Fabrizi, D. Volpe and A. Zazzerini.** *Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: quagliamara@hotmail.com*

Plants release a large number of volatile molecules (including ethylene, terpenoids, lipoxygenase-derived volatiles) and the emission profiles change after damaging. Several studies have demonstrated that semiochemicals play an important role in plant defence against biotic stresses, for example, some plants may emit molecules when attacked by herbivores to attract their natural enemies. Moreover, exogenous application of these molecules can induce a subset of defence responses against fungal and bacterial pathogens. In order to evaluate the role of semiochemicals in tobacco (*Nicotiana tabacum* cv Havana 425) resistance against *Erysiphe cichoracearum* (causal agent of powdery mildew), analyses were conducted to identify and quantify the emission of volatile compounds in uninfected and infected plants. Plants were analysed at different time intervals after infection done by spraying an aqueous suspension containing 10^5 fresh conidia/ml. Volatile compounds emitted from plants were collected by headspace-solid phase micro extraction (HS-SPME) and analysed by gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC-MS). Significant differences in the emission of volatile compounds by infected plants were observed with time with respect to uninfected control. An increase in terpenoids (E-β-ocimene and Z-β-ocimene) was observed 24h after infection, whereas cis-jasmone and methyl-jasmonate showed highest concentrations at 48 and 72h after infection, respectively. The volatile compounds reported above will be further tested for their ability to induce resistance in tobacco against powdery mildew.

AETIOLOGY OF BARK CANKERS ON TIMBER *JUGLANS* TREES: FURTHER RESULTS. **F. Ravaioli, A. Fabi, L. Varvaro and N. Anselmi.** *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail varvaro@unitus.it*

Bark canker is one of the most noxious diseases of walnut trees (*Juglans regia*) for timber production as it causes serious wood deterioration. During the last years several studies have been carried out in Italy to determine the etiology of the disease. Isolations from wood at the border of the lesions showed the presence of five pathogens, i.e. the bacterium *Brenneria nigrifluens* and the fungi *Botryosphaeria* sp., *Fusarium solani, Paraphaeosphaeria* sp., and *Phomopsis* sp. Fungi were identified morphologically and *B. nigrifluens* molecularly and by atty acid profiling. *Paraphaeosphaeria* and *Phomopsis* were occasionally present in the lesions while *Botryosphaeria* and *F. solani* were always

associated with cankers. Artificial inoculations made on young scions of *J. regia*, *J. nigra* and their hybrid NG23 with the above mentioned three main pathogens always produced necrosis, with a maximum colonizing speed for *Botryosphaeria* sp., followed by *B. nigrifluens* and *F. solani*. These tests have shown that *J. regia* proved more susceptible than *J, nigra* whereas the hybrid NG23 had an intermediate behaviour.

NEW *DIAPORTHE/PHOMOPSIS* SPECIES ON SOYBEAN. L. Riccioni[1] and K. Vrandecic[2]. *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]Faculty of Agriculture, University of J.J. Strossmayer, P.O. Box 71, 31000 Osijek, Croatia. E-mail: luca.riccioni@ispave.it.*

Diaporthe/Phomopsis (D/P) isolates obtained from thirty seed samples of soybean were grouped by PCR-RFLP method and morphological characteristics in three groups. Two group with uniform RFLP-PCR profile included *Phomopsis longicolla* and *Diaporthe phaseolorum* var. *caulivora* isolates; respectively, whereas the third group, characterized by 5 different RFLP-PCR profiles and by producing alfa and beta conidia, included isolates previously identified as *D. phaseolorum* var. *soyae*. To clarify the dubious identification of the isolates of the third group, the ITS rDNA regions of 29 representative isolates were sequenced. A phylogenetic analysis of ITS data distinguished four well supported clades. Based on this study, only 50% of the isolates can be identified as *D. phaseolorum* var. *soyae*, all other isolates belong to a putative new D/P species on soybean.

ACCUMULATION OF *CITRUS VIROID III* IN THREE DIFFERENT ROOTSTOCKS AND EXPRESSION OF SOME GENES. S. Rizza[1], G. Catara[3], C. Capasso[3], V. De Luca[3], G. Nobile[2], V. Carginale[3] and A. Catara[1,2]. *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Stradale G. Agnelli angolo V. Lancia, 95030 Catania, Italy. [3]Istituto di Biochimica delle Proteine del CNR, Via P. Castellino 111, 80131 Napoli, Italy. E-mail: srizza@unict.it*

Gene expression in symptomless sour orange, Troyer citrange and alemow seedlings inoculated with a *Citrus viroid III* (CVd-II-Ib) source was compared with Etrog citron showing the typical symptoms induced by the viroid. To detect the viroid infection signal in inoculated seedlings, green bark samples were processed through a rapid and sensitive RT-SYBR Green I-based real time PCR assay for CVd-III quantification. A proper signal was detected ten weeks after inoculation in the three rootstocks without significant differences in viroid titre among them. Among the up- and down-regulated genes previously identified by DDRT-PCR in Etrog citron following CVd-IIIb infection, metallothionein (MT), alcohol-dehydrogenase (ADH), ethylene-responsive binding protein (EREBP), regulator of gene silencing (RGS), aminoacid permease (AP), peroxidase (PRX), and CONSTANS-like protein were chosen to verify if their expression pattern was different in other citrus species. Northern blot analysis showed that Etrog citron, Troyer citrange, sour orange and alemow exhibited the same expression pattern for almost all the genes following CVd-III infection. However, AP expression levels did not vary in Troyer citrange, and PRX was down regulated in alemow.

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF TWO ADDITIONAL *CITRUS TRISTEZA VIRUS* ISOLATES ASSOCIATED WITH SOUR ORANGE INVERSE PITTING. S. Rizza[1], A. Lombardo[2], G. Nobile[2] and A. Catara[1,2]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123, Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia, Stradale G. Agnelli angolo V. Lancia, 95030 Catania, Italy. E-mail: srizza@unict.it*

In recent years, *Citrus tristeza virus* (CTV) has often been reported in Italy as detected by DAS-ELISA and immunoprinting, but information on the biological, molecular and pathogenic properties of the different isolates is still limited. In 2005, during a citrus orchard survey in the province of Catania (Sicily), some cv. Sanguinello sweet orange trees in a 30-year-old grove showed slight but clear inverse pitting on sour orange rootstock. Since this was the first report of symptoms of CTV on sour orange rootstock in Italy, a study was undertaken to characterize the isolate of CTV infecting two representative trees of the orchard through ELISA, indexing on indicator plants, and nucleotide sequence analysis. Mexican lime seedlings inoculated by leaf punch showed typical vein clearing after only ten days, whereas sour orange, sweet orange and grapefruit seedling bark inoculated with two sanguinello sources showed different degrees of stunting, yellowing, small and/or cupped leaves and vein corking. The gene coding for p23 was amplified and the nucleotide sequences were determined in both directions. BLAST analysis showed a nucleotide identity of 99% with seedling yellow strains like BaraoB, Val-CB and C271-2. Since it is clear that the isolates were different from those previously reported in spite of the proximity of the grove to that investigated by others, we assume that more than an introduction of CTV has occurred in recent years in Sicily.

SOFT ROGUING INDUCES RECOVERY IN BOIS NOIR INFECTED GRAPEVINES. G. Romanazzi[1], S. Murolo[1], L. Landi[1] and S. Virgili[2]. *[1]Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Via Brecce Bianche 10, 60131 Ancona, Italy. [2]Agenzia Servizi Settore Agroalimentare delle Marche, Via Alpi 21, 60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it*

Bois Noir (BN), the main grapevine yellows in Central Italy, has been spreading through several European countries over the last years. This disease, caused by "*Candidatus* Phytoplasma solani", can result in severe crop losses and death of the vines. At present, there are no known effective control methods for controlling BN or other phytoplasmas. Roguing and transplanting of the vines infected by phytoplasmas can induce the phenomenon known as "recovery", whereby disease symptoms disappear from the canopy. In the Marche region, at the beginning of spring 2006, grapevines infected by BN were exposed to soft roguing, obtained by disrupting part of the root system using a small excavator. Plants of cvs Chardonnay, Sangiovese, and Verdicchio, grafted on Kober 5BB and trained as low cordon, were partially rogued and left in their position. Soft rouging induced recovery in all 5 plants of cvs Chardonnay and Verdicchio and in 4 out of 5 of cv. Sangiovese. The same experiment, carried out on grapevines of cv. Chardonnay grafted on 420A and trained as high cordon (single curtain), proved to be less effective. Such preliminary results show possible interactions between the type of rootstock and/or the training system and the effectiveness of soft roguing in the induction of recovery in BN-infected vines. An increased expression of the phenylalanine ammonia-lyase gene was observed in recovered plants.

ORGANIC PLANT PROTECTION STRATEGIES AGAINST BACTERIAL PATHOGENS OF KIWIFRUIT. A. Rossetti[1], A. Quattrucci[1], L. Fratarcangeli[1], M. Benuzzi[2], G.M. Balestra[1]. *[1]Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. [2]Intrachem Bio Italia S.p.A., Via Calcinaro 2085/7, 47023 Cesena (FO), Italy. E-mail: balestra@unitus.it*

During the last 15 years, in Italy and in other kiwifruit-growing areas of the world frequent attacks by *Pseudomonas syringae* pv. *syringae* (Pss) and *Pseudomonas viridiflava* (Pv) have been recorded. Pss and Pv populations resulted able to survive on aerial kiwifruit organs during the different seasons. Both pathogens induce heavy damage to the foliage and reduce the yield. Moreover, by their ice nucleation activity (INA), they result particularly dangerous to cause/favour frost damages to different organs. Control of Pss and Pv largely depends on treatments with copper compounds, their frequency and timing of application. Recently, different attempts have been made to improve the level of protection of kiwifruit plantings from both micro-organisms. To reduce the environmental impact of copper, especially in organic kiwifruit stands, studies were carried out to optimize its utilisation and to evaluate the use and efficacy of natural antagonists.

DYNAMICS OF THE PRIMARY INOCULUM OF *VENTURIA PIRINA*, THE CAUSAL AGENT OF PEAR SCAB. V. Rossi[1], E. Pattori[1], F. Salinari[1], R. Bugiani[2] and S. Giosuè[1]. *[1]Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. [2]Servizio Fitosanitario Regionale, Via di Saliceto 81, 40128 Bologna, Italy. E-mail: vittorio.rossi@unicatt.it*

A 5-year study was carried out in two pear orchards of northern Italy, by trapping ascospores of *Venturia pirina* with volumetric spore samplers operated continuously during the ascospore season, with the aim of better defining weather conditions favouring ascospore discharge. Characteristic of ascospore discharge and conditions of temperature, rainfall and leaf wetness were observed during each event, in order to discriminate between weather conditions favouring or not ascospore release. Differences in the length of the trapping season (from 32 to 75 days) and number of ascospores trapped (from a minimum of 135 to a maximum of 3833 spores m^3 air/season) were observed. Ascospores of *V. pirina* showed a diurnal periodicity similarly to those of *V. inaequalis*, as most of them (92%) were trapped between 6 am and 8 pm. In aggregate 185 ascospore discharge events were observed. Rainfall triggered 37% of trapping events and accounted for the highest percentage of trapped spores (44%). Trappings associated with wet periods without rain were 55%, while 8% occurred in dry conditions. A method to estimate the dynamic of the primary inoculum was elaborated using a logistic equation to estimate the onset of the ascospore season, and an asymptotic model to estimate the subsequent dynamics of primary inoculum. This method yielded satisfactory results showing a significant correlation between observed and estimated data ($r=0.90$) and proved able to forecast both early and late ascospore releases.

EFFECT OF *CRYPHONECTRIA PARASITICA VIRUS 1* INFECTION IN *CRYPHONECTRIA PARASITICA* ISOLATES FROM PIEDMONT. L. Rostagno[1], G. Crivelli[1], M. Turina[1] and G. Tamietti[2]. *[1]Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. [2]Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: l.rostagno @ivv.cnr.it*

Cryphonectria parasitica is the etiological agent of chestnut blight, a fungal disease endemic in Northern Italy. A collection of *C. parasitica* isolates from Pellice, Gesso, Tanaro and Mala valleys in Piedmont was evaluated for the presence or absence of dsRNA and, in particular, for the presence of *Cryphonectria hypovirus 1* (CHV-1), a virus known to cause hypovirulence when present in the cytoplasm of its fungal host. Five isolates carrying CHV-1 in their cytoplasm, and showing phenotypic variability when cultured on PDAmb plates, were single-spored. Virus-free isogenic isolates were obtained from each CHV-1 infected isolate through screening of conidial progeny. The effect of the virus on fungal virulence was evaluated through inoculation of 3-year-old European chestnuts (*Castanea sativa*). Surprisingly, when measuring canker area of virus-infected and isogenic virus-free *C. parasitica* isolates, no statistically significant advantage due to the presence of CHV-1 was observed in some of the fungal isolates studied. We are currently evaluating the molecular diversity of the CHV-1 and *C. parasitica* isolates object of our investigation. Furthermore, we report on the phenotype of *C. parasitica* strains over-expressing Cpkk1, a MAPKK isolated from *C. parasitica*, previously shown to be infected by CHV-1

PRODUCTION OF ENNIATINS BY *FUSARIUM TRICINCTUM* AND THEIR EFFECTS ON *DIPLODIA CORTICOLA*. A. Ruscelli[1], G. Campanile[1], A. Moretti[2], S. Somma[2], A. Ritieni[3] and N. Luisi[1]. *[1]Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. [2]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. [3]Dipartimento di Scienze degli Alimenti. Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: luisin@agr.uniba.it*

Diplodia corticola A.J.L. Phillips, Alves & Luque is the causal agent of cankers, vascular necrosis and dieback in various oak species. Scanty information is available on the biological control of this fungus. However, in a recent study, a strain of *Fusarium tricinctum* (Corda) Sacc., isolated from the buds of *Quercus pubescens* Willd. in declined oak woods, showed maximum inhibition of *D. corticola* growth both *in vitro* and *in planta.* The *F. tricinctum* strain has been evaluated for its capability to produce possible toxic metabolites toward *D. corticola*. Fungal extracts from an *in vitro* rice kernel culture of *F. tricinctum* were analyzed by High Performance Liquid Chromatography (HPLC), showing that this strain was able to produce high levels of enniatin B, enniatin B1, enniatin A1 and enniatin A (5050 µg/g; 4750 µg/g; 1860 µg/g, and 240 µg/g, respectively). These metabolites were inoculated in seedlings of *Q. cerris* L. and *Q. pubescens* to investigate possible phytotoxic properties and their ability to reduce the growth of *D. corticola* was evaluated with *in vitro* tests. The results obtained showed that the enniatins produced by *F. tricinctum* were biologically active towards *D. corticola* and were not phytotoxic to the seedlings. The potential use of these metabolites for the biological control of *D. corticola* on oaks deserves further study.

EVALUATION OF A QUANTITATIVE REAL-TIME PCR ASSAY TO MONITOR *PHOMA TRACHEIPHILA* COLONIZATION IN DIFFERENT CITRUS HOSTS. M. Russo[1], F. Grasso[1], G. Licciardello[1,2] and V. Catara[1]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia,*

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Seedlings of lemon ('Femminello', and 'Lunario'), Mexican lime, 'Hamlin' sweet orange, Troyer citrange, *Poncirus trifoliata* and sour orange were inoculated with *Phoma tracheiphila* phialoconidia to evaluate colonization rates in relation to symptom development. Moreover, fungal survival in soil inoculated with phialoconidia was also evaluated. *P. tracheiphila* DNA was quantified by a real-time PCR assay previously developed with slight modification to the Taq-Man probe labeling. Seedlings of the above-mentioned species were inoculated in the leaves with a water suspension containing 10^6 phialoconidia ml^{-1}. Disease development was monitored weekly using an arbitrary scale based on the intensity of leaf symptoms. Fourteen days after inoculation, leaf disks were removed from the inoculation points with a cork borer and DNA was recovered with a commercial DNA extraction kit. *P. tracheiphila* was quantified by real-time PCR assay in the leaf disks. Threshold cycle values from the assay with unknown samples were plotted against the standard curve and the inferred concentration of the fungus were calculated. Fungal DNA was also detected in the leaves of the citrus species that had not yet developed symptoms. The method was also applied successfully to soil samples.

EVALUATION OF FIVE FLUORESCENT PSEUDOMONADS FOR THE PREVENTION OF TOMATO CROWN ROOT ROT AND TOMATO WILT DISEASE CAUSED BY *FUSARIUM OXYSPORUM* f. sp. *RADICIS LYCOPERSICI* AND *FUSARIUM OXYSPORUM* f. sp. *LYCOPERSICI*. **A. Russo[1], G. Puopolo[1], A. Raio[2] and A. Zoina[1].** *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Istituto per la Protezione delle Piante del CNR, Via Università, 133, 80055 Portici (NA), Italy. E-mail: puopolo@unina.it*

Fusarium oxysporum f. sp. *radicis lycopersici* and *Fusarium oxysporum* f. sp. *lycopersici* represent a high threat for tomato crops. Banning of methyl bromide fumigation associated with the increasing demand for safer crop production led to consider new management strategies for the control of these two phytopathogenic fungi. One of these strategies relies on the selection and exploitation of antagonistic bacteria able to protect the plants against these fungal pathogens. The genus *Pseudomonas* represents a reservoir of potential antagonistic agents against several phytopathogenic *Fusarium* species. In this work, five fluorescent pseudomonad strains were characterized for their antagonistic attitudes by scoring siderophore, protease and antibiotic production and by evaluating the inhibition of mycelial growth of both *Fusarium* species *in vitro*. Moreover the production of quorum sensing signals by *Pseudomonas* strains was assessed by using the two biosensor strains *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NTL4. Finally the five strains were evaluated for the control of both phytopathogenic fungi on tomato plantlets with an useful and predictive *in vivo* approach. Results showed that *P. chlororaphis* strain M71 was the only pseudomonad able to produce siderophores, proteases, antibiotics and quorum sensing signals. Furthermore, this bacterial strain resulted to be significantly effective in the reduction of disease gravity in both cases while the other pseudomonad strains, belonging to *P. fluorescens* group failed to protect tomato plantlets.

EVALUATION OF LATE BLIGHT RESISTANCE IN POTATO BREEDING CLONES SUITABLE FOR ORGANIC FARMING. **E. Sala, P. L. Burzi, S. Galletti, S. Marinello and C. Cerato.** *CRA, Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, 40129 Bologna, Italy. E-mail: e.sala@isci.it*

Phytophthora infestans causes late blight on a range of solanaceous plant species. The most frequently used management strategy against this disease relies on repeated fungicide applications and use of partially resistant cultivars. Eighteen new Italian breeding clones of potato were evaluated for their resistance to late blight under controlled and field conditions in absence of chemical treatments. Foliage resistance tests were conducted inoculating 60-day-old potato plants with a *P. infestans* isolate phenotipically characterized for mating type (A1) and avirulence genes (R1, R2, R3, R4, R6, R7, R8, R 10, R11). The pathogen isolate was also used to inoculate tubers of the same clones following the shallow wounds method. One clone showed high resistance to foliage late blight while other two clones showed very high resistance on tuber assay. Field trials were carried out in 2006 and 2007 under natural inoculum pressure in Sicily. In 2006, pathogen pressure was very low, preventing the detection of significant differences among the clones. On the contrary in 2007, under high pathogen inoculum pressure, one clone that had shown good resistance levels in greenhouse tests was found very tolerant. Our results did not show significant correlation between tuber and foliage resistance of the same genotype as reported in the literature. Moreover, these results suggest that preliminary greenhouse screenings could partially predict field resistance levels helping breeder's work.

MOLECULAR ANALYSIS OF PHLOMIS MOTTLE VIRUS. **P. Saldarelli, D. Boscia and C. Vovlas.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: vovlas@agr.uniba.it*

A virus provisionally called Phlomis mottle virus (PhMV), isolated in 2006 from *Phlomis fructicosa* (family *Lamiacee*) by mechanical transmission to *Nicotiana occidentalis,* has flexuous particles 800-850 nm long, with an outward aspect recalling those of some members of the family *Flexiviridae*. Sequencing of a 363 nt fragment of the RNA dependent RNA polymerase (RdRp) gene had disclosed homology with the comparable protein of Banana virus X (BanVX), an unassigned member to the family *Flexiviridae*. Further sequencing of a 3035 nt fragment of the viral genome, spanning from the RdRp gene to the 3' terminal poly(A) tract, has now shown a structural organization comparable to that of species of the genus *Trichovirus*. The 5' proximal open reading frame (ORF1) encoding the RdRp protein, is followed by two ORFs showing homologies with movement proteins of the p30 superfamily (ORF2) and the coat proteins of flexivirids (ORF3). The expression products of all three ORFs have a substantial level of identity with the corresponding proteins of members of the genus *Trichovirus*, a result confirmed by phylogenetic analysis. A further 3'most ORF, encoding a RNA binding protein, was identified which, as in the case of some trichoviruses, lacks the canonical AUG initiation codon. The molecular analysis of the PhMV genome, though incomplete, strongly suggests that this virus is a member of the genus *Trichovirus.*

CHARACTERIZATION OF *FUSARIUM LANGSETHIAE* ISOLATED FROM WHEAT KERNELS IN ITALY. A. Santori[1], G. Aureli[2], M.G. D'Egidio[2] and A. Infantino[1]. *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]CRA, Istituto Sperimentale per la Cerealicoltura, Via Cassia 176, 00101 Roma, Italy. E-mail:alberto.santori@entecra.it*

Among seed-borne fungi present on wheat kernels (*Triticum durum* Desf. and *T. aestivum* L.), several *Fusarium* species are of interest due to their ability to produce mycotoxins. *Fusarium langsethiae*, a species producing type A trichotecenes (T2 and HT2 toxins) harmful to humans and cattle, has been recently isolated from durum and common wheat kernels coltivated in central and southern Italy. Toxin production of seven *F. langsethiae* isolates grown on autoclaved wheat kernels was measured with the ELISA Ridascreen T2 kit (Biopharm, Glasgow, UK). Some isolates were positive for T2 production. The pathogenicity of one isolate (ISPaVe ER-1409) was evaluated in the open field by spraying plants of cv. Simeto at the 10.5.2 Feeke's scale growth stage with a suspension of 1×10^5 spores ml^{-1} of the fungus. *F. graminearum* (ITEM 1852) and *F. culmorum* (ITEM 1851) were inoculated at the same concentration of *F. langsethiae* for symptom comparison. Controls were sprayed only with water. For each species, 0.8 m^2 plots with three replicate were inoculated. To maintain high humidity levels, plants within each plot were covered with plastic bags for 48 h. Typical Fusarium head blight symptoms developed after 15 days only on spikes inoculated with *F. graminearum* and *F. culmorum* with a McKinney severity of 87.2 and 89.8, respectively while no differences with the control were observed on plants inoculated with *F. langsethiae*. Epidemiological studies are in progress to assess the distribution and the incidence of this fungus in Italy.

IDENTIFICATION OF APPLE GENES DIFFERENTIALLY EXPRESSED IN RESPONSE TO QUERCETIN APPLICATION. S.M. Sanzani[1], L. Schena[2], A. De Girolamo[3], A. Ippolito[1] and L. Gonzalez Candelas[4]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126, Bari, Italy. [2]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. [3]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. [4]Instituto de Agroquimica y Tecnologia de Alimentos (IATA), Consejo Superior de Investigaciones Científicas, Apartado 73, 46100 Burjassot, Valencia, Spain. E-mail: simona.sanzani@agr.uniba.it*

Penicillium expansum Link infections of apples result in economic losses in all producing countries and have potential public health significance, since may cause accumulation of the mycotoxin patulin. Control of this postharvest pathogen is commonly done with fungicides; however the appearance of resistant strains and consumer concern about food and environmental safety are leading to an increasing demand for alternative treatments. In previous trials the phytoalexin quercetin was effective in reducing both disease severity and toxin accumulation. Since quercetin was more effective in *in vivo* than in *in vitro* trials, it was believed to enhance host resistance. To verify this hypothesis the method "Suppression Subtractive Hybridization" (SSH), was used to construct a library of cDNA differentially expressed after quercetin application. A set of cDNA clones was obtained, out of which 150 were randomly selected, analysed for the presence of a single insert and sequenced. Most sequences revealed high similarities with those available in GenBank databases. In particular, a correspondence was obtained with different classes of pathogenesis-related proteins, such as RNase-like PR10 and PR8 protein, and with proteins expressed under stress conditions. In addition, several transcripts showed similarity to genes coding proteins having a role in host-pathogen recognition and in signalling pathways. Similarity was also found with genes coding for proteins whose role in defence mechanisms is still unknown. Further studies are in progress to quantify accurately the expression of these genes and to correlate them with the *Penicillium* control exerted by quercetin application to apples.

REAL-TIME RT-PCR ASSAY FOR DETECTION AND DIFFERENTIATION OF *CITRUS TRISTEZA VIRUS* ISOLATES. M. Saponari[1] and R. K. Yokomi[2]. *[1]Istituto di Virologia Vegetale del CNR, Sezione di Bari e Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola, 165/A, 70126 Bari, Italy. [2]USDA, ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA 93648, USA. E-mail: m.saponari@ba.ivv.cnr.it*

For universal detection of *Citrus tristeza virus* (CTV) strains by real time RT-PCR, a protocol was developed based on a set of primers and a Cy5-labeled TaqMan probe. This test included primers and a TET-labeled TaqMan probe selected on the mitochondrial *nad5* gene for the simultaneous detection of RNA as an internal control. This protocol was tested on total RNA extracts from fresh, frozen, and desiccated leaf petioles from CTV-infected plants with 80 isolates from a worldwide collection of CTV isolates maintained *in planta* in Beltsville, MD and Tulare and Parlier, CA, USA. A dilution series of an *in vitro* synthesized transcript containing the target sequence showed that the protocol detected less than 2 fg of viral template. A multiplex, one-step real-time RT-PCR assay was also developed for the detection and differentiation of CTV strains. Based on multiple alignments of the nucleotide sequences of the CTV minor and major coat proteins, a set of primers and FAM-labeled TaqMan probe were developed to detect stem pitting and seedling yellows CTV strains (VT and T3 genotypes). This assay was accurate when the two primers and TaqMan sets were combined and allowed the simultaneous detection of CTV and differentiation of mild and severe strains of the virus. This test successfully distinguished all 25 severe from 22 mild isolates from an *ad hoc* group of CTV isolates from the different collections.

COMPARISON OF FUNGAL DNA ESTRACTION PROTOCOLS FROM DECAYED WOODY TISSUES. M. Saracchi and F. Rocchi. *Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: marco.saracchi@unimi.it*

Rapid identification of wood-rotting fungi in absence of fruiting bodies is one of the keystones in the diagnosis of these diseases and the management of urban and ornamental green spaces The aim of this research was the comparison of fungal DNA extraction protocols, directly from different decayed woods, in order to extract the nucleic acids of the pathogens suitable to be amplified and sequenced. No information is apparently available on the availability of a single protocol for analysing woody tissues from a wide range of species. Considering the chemical composition of the wood, particular attention was paid to protocols able to remove high quantity of polysaccharides and polyphenolic compounds that commonly reduce or hamper PCR reactions. Two different commercial kits and two protocols described in the literature (Ziegenhagen *et al.*, *Plant Mol. Biol. Reptr.* **11**: 117-121, 1993; Kelly *et al., Physiol. Mol. Plant Pathol.* **52**: 392-409, 1998) for DNA extraction from leaves and shoots of many herbaceous and

woody species, were compared. Further steps were added to the latter of the two protocols, to improve its capacity to remove inhibiting compounds. The comparison was carried out on specimens collected from some woody plant species. The results, based on PCR products quality and sequencing data, point out the suitability of DNAs extracted by means of both commercial kits and the modified protocol, based on the use of hot CTAB buffer treatments. Further trials need to be conducted to test the suitability of these protocols for a higher number of woody plant species.

STUDIES ON THE *CARPINUS BETULUS* DECLINE IN THE HISTORICAL GARDEN OF "VILLA ARESE-BORROMEO" AT CESANO MADERNO. M. Saracchi, F. Rocchi and M. Vaghi. *Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: marco.saracchi@unimi.it*

Villa Arese-Borromeo, a historical complex dating back to the seventeenth century, includes a garden characterized by more than 650 European hornbeam (*Carpinus betulus*), from 10 to 90 years old, planted in rows. This is one of the largest hornbeam stands in Europe. On the bark, along the trunks and the biggest branches, large lesions were observed with oustanding red clups, similar to resin, gathered in more or less numerous groups (red type cankers). Moreover, on the same cortical surfaces, small globose fruiting bodies were present, from which, during humid weather, long and yellowish cirri came out (yellow type cankers). The involved branches parch in few years and, if the main trunk is affected, the entire tree could die. Detailed and repeated inspections on each tree allowed to determine the distribution and severity of the disease. 251 out of 652 trees were symptomatic (38.5%): 92 showed only the yellow type canker, 84 only the red type, and on 75 plants both types of cankers. The lenght of cankers varied from few centimetres up to 2 m, generally those of the yellow type were the longest. Analyses of red and yellow type cankers ascertained the presence of two different fungal forms that can be referred, on the basis of their morphocultural and biomolecular characterization, to the genera *Naemospora* and *Endothiella,* respectively. Their taxonomic position at the specific level has not yet ben established. Experimental infection trials confirmed that both fungal forms are phytopathogenic.

DOES DEOXYNIVALENOL AFFECT THE ABILITY OF FILAMENTOUS FUNGI TO COMPETE FOR WHEAT STRAW? S. Sarrocco, F. Matarese and G. Vannacci. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124, Pisa, Italy. sarrocco@agr.unipi.it*

Cereals are among the most affected crops by mycotoxins production. *Fusarium graminearum* and *F. culmorum*, the predominant pathogenic species associated with *Fusarium* head blight (FHB), are producers of the mycotoxin deoxynivalenol (DON) that is frequently found in cereals. Because it is known that decomposing plant material, as wheat straw, serves as inoculum source for subsequent crops, the aim of this work was to evaluate the role of DON in the competition for straw colonization among soil-borne filamentous fungi. Wheat straw treated with DON, was buried in three natural soils, all with a previous history of wheat cropping. Mycobiota associated with the treated crop debris was evaluated and compared with that obtained from untreated straw. A general rule for reducing the risk of ear infection of wheat by pathogenic *Fusarium* spp. is to limit residues of infected crops in susceptible crop fields, by suppressing saprophytic colonisation or sporulation of toxigenic strains. Among fungi included in the mycobiota associated with treated crop debris, some *Pythium* spp. and a *Trichoderma* sp. isolates were collected. These latter fungi were tested for the ability to reduce sporulation and growth of *Fusarium* strains. Positive results emerged from these preliminary experiments represent the starting point for further investigations aimed at selecting potential antagonists for biocontrol of FHB, as a strategy to prevent production and accumulation of mycotoxins in cereals.

RNA SILENCING OF THE TRICHOTHECENE BIOSYNTHESIS GENE *TRI6* IN *FUSARIUM CULMORUM*. B. Scherm[1], M. Orrù[1], V. Balmas[1], T.M. Hammond[2], N.P. Keller[2] and Q. Migheli[1]. *[1]Dipartimento di Protezione delle Piante, Centro interdisciplinare di eccellenza per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. [2]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, USA. E-mail: qmigheli@uniss.it*

Post-transcriptional regulation of eukaryotic genes through interception and degradation of mRNA is known as RNA silencing. This mechanism is activated by an RNAse III enzyme, which digests double-stranded RNA (dsRNA) molecules into 21- to 25-bp fragments. These fragments (siRNAs) are incorporated into a complex of proteins, the "RNA-induced silencing complex" (RISC), which uses the incorporated siRNAs to target and degrade mRNA with complementary sequences. It was recently demonstrated that inverted repeat transgenes (IRT) are efficient activators of RNA silencing in fungal species. The aim of this study was to evaluate whether RNA silencing could be applied to suppress mycotoxin production in the plant pathogen *F. culmorum* (W.G. Smith) Sacc., incitant of crown and foot rot on wheat. Transformation of a highly virulent strain of *F. culmorum* with IRT-containing sequences corresponding to the trichothecene biosynthesis gene *tri6* was achieved by using the hygromycin B resistance gene *hph* as selectable marker in PEG-mediated co-transformation of fungal protoplasts. The pattern of integration indicates that most transformants underwent homologous recombination events with partial deletion of the endogenous *tri6* gene. A subset of transformants possessing both the endogenous gene and the *tri6*-specific IRT construct were selected for further studies. The *tri6*-specific IRT did not alter physiological characteristics, such as spore production, pigmentation, and growth rate on solid media. Pathogenicity assays are being carried to evaluate whether impairment in deoxynivalenol production in the *tri6*-IRT strains correlates with a loss of virulence.

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DIAGNOSIS OF LATENT INFECTION CAUSED BY *MONILINIA LAXA* ON SWEET CHERRIES BY TRADITIONAL AND MOLECULAR METHODS. S. Sharrawi[1], I. Pentimone[1], T. Yaseen[2], L. Schena[3], A. Ligorio[1], A. Ippolito[1] and F. Nigro[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126, Bari, Italy. [2]Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy. [3]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. E-mail: nigrof@agr.uniba.it*

Monilinia laxa is the causal agent of brown rot, one of the

most important diseases of sweet cherries in southern Italy. The pathogen can elicit latent infections whose detection is a critical factor for developing an effective control strategy, as well as appropriate postharvest handling procedures. Usually, latent infections are diagnosed with traditional techniques, i.e. fungal isolation on agarized media from superficially sterilized tissues, freezing or dipping surface-sterilized fruits in a paraquat solution. In addition, retailers often ask for guarantees on fruit quality concerning possible development of storage rots. Some traditional procedures (surface-sterilization, paraquat solution and freezing), compared for two seasons (2006 and 2007), clearly demonstrated that, under favourable conditions, the incidence of latent infections can reach 100%. Beside the inefficiency, these procedures are time consuming and require specialized personnel for fungal identification. A molecular method was thus developed for the rapid and early detection of *M. laxa* latent infections. IGS region of the fungus was amplified, cloned, sequenced, and used to design species-specific primers. These primers enabled the specific detection of *M. laxa* DNA among DNAs from a wide range of different fungal species. Moreover, this method detected early brown rot infection in buds, flowers, and fruits of sweet cherries cvs Bigarreau and Ferrovia. IGS sequencing for *M. frucigena* and *M. fructicola* is in progress, in order to develop specific primer sets for more rapid and single-step PCR identification in different host species.

EVALUATION OF SOME CHEMICALS AS INDUCERS OF RESISTANCE IN TOMATO PLANTS AGAINST *PSEUDOMONAS SYRINGAE* pv. *TOMATO*. **S. Silvestri, C. Moretti, P. Ferrante and R. Buonaurio**. *Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: simonaaidasilvestri@hotmail.com*

Potassium monophosphate, potassium diphosphate, potassium permanganate, calcium oxide and potassium phophate buffer (pH 7) as well as acibenzolar-S-methyl were tested for their capacity to suppress tomato bacterial speck caused by *Pseudomonas syringae* pv. *tomato*. Basal leaves of tomato plants (cv. PS1296) were sprayed with the above mentioned chemicals and the upper leaves were inoculated with a suspension of the bacterium at 10^6 cfu ml^{-1}, three days after the treatments. Among the chemicals used, potassium phosphate buffer treatment significantly and systemically protected tomato plants from the disease though to at lesser extent respect to the acibenzolar-S-methyl. The protective effect was expressed through the reduction of the leaf surface infected, number of bacterial spots per leaflet, spot diameter and bacterial growth *in planta*. Further experiments are in progress to establish whether the resistance is mediated by salicylic acid.

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DIFFUSION OF TWO DIFFERENT ISOLATES OF *CITRUS TRISTEZA VIRUS* IN SICILY. **G. Sorrentino[1], S. Davino[2], M. Guardo[1], M. Davino[2] and A. Caruso[1].** *[1]CRA, Istituto Sperimentale per l'Agrumicoltura, Corso Savoia 190, 95024 Acireale (CT), Italy. [2]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: guido.sorrentino@entecra.it*

In the area where the first large outbreak of *Citrus Tristeza Virus* (CTV) occurred in the province of Catania (Sicily), a severe CTV isolate (CTV-DS2) was discovered in 2002. In the years that followed the area around the focus was monitored to determine the spreading of this isolate. Monitoring was carried out by DAS-ELISA and DTBIA and characterization and sequencing by molecular analysis (SSCP) of the P20 and P23 genes. During monitoring a mild CTV-isolate (CTV-DS1) was detected in a small plot at the borders of the original focus. CTV-DS1 occurred in a small area, in 13-14-years old sweet oranges cv. Tarocco O.L. grafted on sour orange, bordering a plot with 100% CTV-infected trees of mandarin cv. Fortune grafted on sour orange. Field observation aimed at estimating the movement and rate of spread of both viral isolates and at identifying possible aereas of coexitance and interactions of these strains. The severe isolate present in the original focus showed a fast spreading inside the plots infected since 2002 and in the bordering groves, so as to enlarge considerably its distribution. By contrast, the mild isolate moved slowly, remaining practically restricted to the area in which it was originally found. At the borders of the two foci both isolates isolates occurred in some sweet orange cv. Navelina OL grafted on sour orange. These doubly infected trees declined rapidily and died.

***HIBISCUS MOSCHEUTOS* subsp. *PALUSTRIS*, NEW HOST OF *VERTICILLIUM DAHLIAE*.** **V.M. Stravato[1], G. Carannante[1], M. Quaglia[2] and C. Cappelli[2].** *[1]Genista S.r.L., Strada Statale Flacca, 04022 Fondi (LT), Italy. [2]Dipartimento di Scienze Agrarie e Ambientali. Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: sumas@tiscali.it*

In the last three years near the lake of Fondi (Latina, central Italy), severe symptoms of stunting and wilting were observed on some plants of *Hibiscus moscheutos* subsp. *palustris.* an ecologically relevant, very rare wild species producing appreciated beautiful flowers, recorded in Tuscany and Latium, and constituting important example of biodiversity in the marsh areas. The severity of the symptoms prompted more detailed investigations to understand the etiology of the disease. The results of our investigations, based on isolations from infected stems and artificial inoculations of healthy plants, demonstrated that the fungus *Verticillium dahliae* Kleb., that was consistently isolated from infected tissues, is the causal agent of the disease. To our knowledge, this is the first report of *V. dahliae* on *H. moscheutos* subsp. *palustris*. The pathogen is a polyphagus and ubiquitous fungus recorded in Italy from a wide range of plant species, including another hibiscus (*Hibiscus cannabinus* L.). Infection of *H. moscheutos* subsp. *palustris* could origiante from inoculum spreading from cultivated infected fields. In the province of Latina different susceptible vegetables and fruits trees are widely grown, so that superficial water could play an important role in the dissemination of *V. dahliae* towards the lake of Fondi.

SURVEY OF VIRUS DISEASES OF TABLE GRAPE IN SICILY. **M. Tessitori[1], E. Buonocore[2] and R. La Rosa[1].** *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. [2]Regione Siciliana, Servizio Fitosanitario, U.O. 54-O.M.P. di Acireale, Contrada Fanello, 97019 Vittoria (RG), Italy. mtessitori@ unict.it*

Sicily ranks second in Italy for table grape production. Because information on the sanitary conditions of this crop in Sicily are scanty, a survey for virus diseases was conducted in 10 commercial vineyards (in greenhouse or arbor) located in different provinces of the island. In autumn 2006 mature canes were collected from a total of 53 individual vines that showed virus disease symptoms (60% of the samples) or were apparently healthy (40%). Different symptoms were observed, i.e. leafroll, leaf mo-

saic, fanleaf, fasciations of shoots and bunches, and short internodes. ELISA tests on phloem scrapings were made using commercial kits (Agritest, Valenzano, Italy) for *Grapevine virus A* (GVA), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 3* (GLRaV-3) and *Grapevine fleck virus* (GFkV). Results showed that 99% of the tested vines were infected even if only 60% of them was symptomatic in the field. Of the positive samples, 85% had mixed infections with different virus combinations, whereas 15% was infected only by GFkV. GLRaV-3 and GFkV were the most widespread viruses (90.5% and 79.2%, respectively) whereas GFLV was the least represented (16.9%). RT-PCR analyses are in progress to confirm ELISA results and to check a larger number of samples.

PARTIAL NUCLEOTIDE SEQUENCE OF *CAPER LATENT VIRUS*. **A. Tiberini and L. Tomassoli.** *CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: l.tomassoli@ispave.it*

Virus origin, evolution and genetic variability represent a current and interesting issue in plant virology. In recent times, many papers reported on these aspects to better understand the process involved in plant virus evolution. In this context, a molecular study was recently initiated of *Caper latent virus* (CapLV), a member of the genus *Carlavirus*, that infects latently caper in nature and occurs in all the minor islands of Sicily where caper is grown for commercial purposed. Since CapLV has probably coexisted with caper for long time in very closed ecosystems, it represents an interesting virus to be characterized molecularly and compared to members of the genus *Carlavirus*. In this study, ORF1, encoding the replicase gene of CapLV was amplified (Superscript One-Step RT-PCR for Long Templates, Invitrogen, Carlsbad, CA, USA) using specific primers designed on two partial short sequences (1000 bp each) previously obtained in the 5' and 3' portion, respectively, of the gene in question. The resulting 5,500 bp amplicon was cloned (TA Cloning kit, Invitrogen) and sequenced. Results from nucleotide analysis (BLAST and FASTA) demonstrated that ORF1 of CapLV shares 60% to 75% identity with that of six other carlaviruses for which sequence information is available in GenBank. In particular, CapLV was closest to *Helenium virus S* (HelVS) and *Garlic latent virus* (GaLV) with a homology of 75% and 73%, respectively. Further, a total of fifteen CapLV isolates from different islands of Sicilian Archipelagoes were characterized and analyzed for a preliminary molecular phylogenetic study of the genus *Carlavirus*.

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PRELIMINARY INVESTIGATION ON ASPARAGUS DISEASES IN SICILY. **L. Tomassoli[1], A. Zaccaria[1], D. Valentino[2] and G. Tamietti[2].** *[1]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Patologia Vegetale, Università degli Studi di Torino, Via Leonardo da Vinci, 44, 10095 Grugliasco (TO), Italy. E-mail: l.tomassoli@ispave.it*

The green-quality asparagus (*Asparagus officinalis* L.) is a promising crop in Sicily because of the favourable soil and climatic conditions along the coasts, mainly in the provinces of Messina, Trapani, Siracusa and Agrigento. In these areas, asparagus is grown in semi-forced culture under tunnel and in the open field, its season usually running from January to April. Since asparagus is a recent crop, no information is available on diseases and relative causal agents. Therefore, the aim of our work was to survey asparagus fields to assess the occurrence of diseases caused either by pathogens that normally occur in this crop in other Italian regions or, being indigenous, are new to this host. As ascertained by a two-year survey, the sanitary status of the cultivations appeared generally good. However, plants showing severe stunting, decline, and root rot were observed in a plastic-house in early 2007 at Mazara del Vallo. *Fusarium proliferatum* (Matsushima) Nirenberg was associated with this condition. We suppose that a thermal stress due to a prolonged closure of the tunnel may have predisposed the crop to the disease. Spears and ferns from symptomless plants were also analyzed for the presence of viruses. *Asparagus virus 1* (AV-1) and *Asparagus virus 2* (AV-2) were found in two different areas with a significantly different incidence. These viruses are widely spread both in Italy and in the world. Until now, no other viruses reported from asparagus have been detected.

CHEMOTYPING OF *FUSARIUM GRAMINEARUM* FROM DURUM WHEAT IN AN AREA OF EMILIA ROMAGNA. **S. Tonti[1], A. Prodi[1], S. Sandalo[1], D. Pancaldi[2], L. Flamini[3] and A. Pisi[1].** *[1]Dipartimento di Scienze e Tecnologie Agroambientali, Alma Mater Studiorum, Università degli Studi, Via Fanin 42, 40127 Bologna, Italy. [2]Dipartimento di Protezione e Valorizzazione Agroalimentare, Alma Mater Studiorm, Università degli Studi, Via Fanin 44-46, 40127 Bologna, Italy. [2]ASSAM, Agenzia Servizi Settore Agroalimentare Marche, Servizio Fitosanitario Regionale, Via Alpi 21, 60131 Ancona, Italy. E-mail:aprodi@agrsci.unibo.it*

Fusarium graminearum is one of the main causal agents of fusarium head blight (FHB) of wheat. *F. graminearum* population can be divided into two chemotypes based on the production of the trichothecenes deoxynivalenol (DON) and nivalenol (NIV). DON-producing isolates can be further distinguished on the basis of the predominant acetyl DON derivative that produces 3-acetyl DON (3-AcDON) or 15-acetyl DON (15-AcDON). In this investigation, fungal isolates collected between 2006 and 2007 from symptomatic spikes of durum wheat in several fields, around Bologna (Emilia-Romagna region, northern Italy), were tested with a multiplex version (Starkey *et al.*,, *Fungal Genet. Biol.*, doi: 10.1016/j.fgb.2007.03.001, 2007) of the chemotype-specific PCR proposed by Ward *et al.* (*Natl. Acad. Sci. USA* **99**: 9278-9283, 2002). All the three fungal chemotypes were found. In particular, of the 66 isolates tested, 79% belonged to the 15-AcDON chemotype, 15% to the 3-AcDON and 6% to NIV. The 15-AcDON chemotype was the most frequent. This is the first time that a population of *F. graminearum,* exclusively isolated from durum wheat from the area under investigation, was examined for chemotypes. Taking into consideration the variability of the Italian environmental characteristics, it would be desirable to monitor wheat fields from all over the country, especially where durum wheat prevails, to better understand the distribution of *F. graminearum* chemotypes in this country.

RADIO FREQUENCY IDENTIFICATION TECHNOLOGY FOR IMPROVING TRACEABILITY IN THE GRAPEVINE NURSERY SECTOR. **E. Triolo[1], A. Luvisi[1], R. Bandinelli[2] E. Rinaldelli[2] and M. Pagano[2].** *[1]Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vege-*

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Radio frequency identification (RFID) is an innovating system for developing traceability programs in agriculture, as widely exemplified by other fields of applications, i.e. logistics, animal identification and environmental monitoring. In the grapevine nursery sector, this technology could be useful for increasing consumers' confidence, thanks to the possibility of checking the "history" of grapevines used for producing a given wine, besides assisting the logistics of the production phase of the farming process. The aim of our trial is the evaluation of the the effects on growth following the implantation of microchips into grapevines, and the development of an effective procedure for online managing of information associated to chips. The trial was initiated in 2007 in the Vivai New Plants, Cenaia (Pisa, central Italy) using five different grape clones supplied by the Associazione Toscana Costitutori Viticoli (TOS.CO.VIT., San Piero a Grado, Pisa, Italy; www.toscovit.it). Clones (Sangiovese *I-SS-F9-A5-48*, Prugnolo gentile *I-Bruscello*, Colorino *I-US-FI-PI-10,* Trebbiano toscano *I-S. Lucia 12* and Vernaccia di S. Gimignano *I-VP6*) were grafted on 1103P, inserting the microchips (Transponder Glass TAG) with two different procedures, encompassing TAG insertion into the medulla or under the bark, with the assistance of a specific machine. Each inserted TAG is identified by a number readable by a Palm-PC, linked with a simple identification file. To consult a complete grapevine identification file with attached technical information, users (farmers, consumers) will have access to a website, actually under construction, and enter an online database, using the identification number supplied by the Palm-PC.

OVER-EXPRESSION OF CHITINASE AND ß-1,3-GLUCANASE GENES IN ORANGE FRUIT TREATED WITH *AUREOBASIDIUM PULLULANS* AND CHITOSAN. M.C. Trullo[1], L. Schena[2], I. Pentimone[1], A. Ligorio[1], F. Nigro[1] and A. Ippolito[1]. [1]*Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy.* [2]*Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. E-mail: ippolito@agr.uniba.it*

Chitosan and *Aureobasidium pullulans*, strains L47 and 547, are known for their activity in controlling a number of post-harvest diseases of fruits and vegetables. The mechanisms by which these alternative control means exert their activity are not fully elucidated, although it has been inferred that, among other mechanisms, they are able to induce resistance in the host. To confirm this hypothesis at a molecular level, the expression of two genes, known to be involved in the host resistance (chitinase and β-1,3-glucanase), was evaluated by real-time PCR in oranges cv. Valencia late at different level of maturity (veraison and full-ripening). ATP-synthase was utilised as a housekeeping non-regulated reference gene. At the beginning of ripening the relative transcript level of chitinase was up-regulated in all treated fruits by 518- (strain L47), 726- (strain 547) and 1790-fold (chitosan). In the same fruits β-1,3-glucanase expression was increased by L47 and 547 (55- and 20-fold, respectively) and to a minor extent by chitosan (5.4 fold). Full-ripen fruits were less reactive compared to those in the veraison stage. In particular, chitinase expression was up-regulated by 20, 83 and 259 fold in fruits treated with 547, L47 and chitosan, respectively. Very limited increases of expression were shown by the β-1,3-glucanase gene. Our data demonstrate that L47, 547 and chitosan are able to induce resistance in orange tissues; however their action is strongly influenced by the ripening stage. Further studies are in progress to identify other genes involved in the resistance induced by strain 547 and chitosan in oranges.

EFFECT OF DRY MILLING ON TOXIGENIC FUNGI CONTAMINATION OF CORN. G. Venturini, M. Moretti, G. Assante and A. Vercesi. *Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: giovanni.venturini@unimi.it*

Mycotoxin contamination occurs with increasing frequency in corn produced in Northern Italy. In order to evaluate the effects of dry milling on toxigenic fungi associated with corn, one batch of cv. Costanza was sampled in five subsequent processing stages: kernels, flaking grits, coarse grits, cornmeal and flour. The high contamination level on the kernel surface (2.5×10^5 CFU/g fresh weight), was mainly due to *Fusarium verticillioides*. Other fungi, namely *Aspergillus flavus* and *A. niger* and several *Penicillium* species, were less frequently found. Dry milling induced a strong decrease in fungal contamination, mostly caused by the reduction of *F. verticillioides* CFUs. Inner seed tissues and flour were still contaminated mainly by *F. verticillioides*, while contamination by *A. flavus* and *A. niger* decreased following germ removal, but increased in the final product. Only the 17.6% of the *A. flavus* strains isolated were able to produce aflatoxins. No toxigenic *Penicillium* species were found. These results show that the major risk for mycotoxin contamination in the examined samples is due to *F. verticillioides* and to a lesser extent, to *A. flavus.* Further studies are needed in order to investigate the distribution of *F. verticillioides* in the corn-growing areas in northern Italy and the toxigenic capability of the extant strains.

DETECTION AND PHYSIOLOGICAL CHARACTERIZATION OF *FUSARIUM SOLANI* f. sp. *CUCURBITAE* RACE 1 FROM SHELTERED ZUCCHINI CULTIVATIONS IN EMILIA ROMAGNA. A. Veronesi, C. Sigala, G. Alberoni and R. Roberti. *Dipartimento di Protezione e Valorizzazione Agroalimentare, Alma Mater Studiorum, Università degli Studi, Via Fanin 46, 40127, Bologna, Italy. E-mail: roberta.roberti@unibo.it*

Crown and foot rot of zucchini has been observed in five sheltered cultivations in Emilia Romagna (northern Italy) from 2005 to 2007. The objective of this research was the identification of the casual agent of the disease and the study of the virulence and physiological variability of its monosporic cultures. On the basis of host specificity and morphological characteristics, the pathogen was identify as *F. solani* f. sp. *cucurbiate* W.C. Snyder & H.N. Hansen. Isolations from necrotic tissues gave 100 single-spore-cultures. The DNA transcriber elongation factor (TEF) from 20 selected monosporic cultures was amplified, sequenced and placed in a *Fusarium* database. All isolates were confirmed as *F. solani* f.sp. *cucurbitae* race 1. The 20 monosporic cultures inoculated on zucchini under controlled conditions showed different degrees of virulence, infecting 61-97% of inoculated plants. They were also able to produce different amounts of pathogenesis-related enzymes, such as cellulase, pectin lyase, pectinase, polygalacturonase and protease, whose activity was tested in fungal crude protein extracts, using the agarose diffusion assay method. Monosporic cultures plated on potato dextrose agar with the addition of carnation leaf powder, exhibited different growth rates at 17, 23 and 28°C in the dark or in the light.

DIFFERENCES IN THE MICROFUNGAL COMMUNITY BETWEEN SPRING AND WINTER COMPOSTS. A.M. Vettraino, S. Franceschini and A. Vannini. *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: vettrain@unitus.it*

Composting is a general treatment method used to recycle different sources of materials such as municipal, yard and other kind of organic wastes. The high organic matter content as well as the biological activity make composts useful for a variety of applications (fertilization, soil erosion, bioremediation). The biomass ratio of fungi to prokaryotes in compost is 2:1 highlighting the fundamental role of fungi in polymer degradation and their importance as part of the compost microflora. Focusing on the microfungal community, we assessed some biological differences between two different green composts produced with materials collected in winter and spring 2006. The total microbial activity was measured through enzyme activity using the FDA hydrolysis assay. Isolation of fungi from compost was performed on PDA amended with streptomycin at 20°C. Fungal species were identified according to their morphological traits. For each sample the total fungal load, their concentration and the statistic index of richness, dominance and evenness were determined. The estimated enzymatic activity was comparable to that reported in the literature for other composts and soils. As expected, spring compost had a higher fungal load and a wider diversity consequent to the influence of the season on the microfungal community composition.

ANTIBIOTIC AND PLANT GROWTH PROMOTION ACTIVITY OF *TRICHODERMA* KONINGININS. F. Vinale[1], G. Chiessa[4], K. Sivasithamparam[2], E.L. Ghisalberti[2], R. Marra[1], P. Conte[5], A. Piccolo[5], V. Aloj[1], D. Turrà[1], S. Lanzuise[1], M. Ruocco[3], S.L. Woo[1] and M. Lorito[1]. *[1]Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]University of Western Australia, 35 Stirling Highway, Crawley, Perth, WA. Australia. [3]Istituto di Protezione delle Piante del CNR, Via Università 133, Portici (NA), Italy. [4]Instituto Nacional de Tecnología Agropecuaria, Aristizabal y De Los Reseros, Hurlingham, Argentina. [5]Centro Interdipartimentale di Spettroscopia di Risonanza Magnetica Nucleare (CERMANU), Università di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: frvinale@unina.it*

The production of *Trichoderma* secondary metabolites with antibiotic activity is a well documented phenomenon. However, only a few reports deal with the ability of antagonistic fungal strains to produce compounds acting as plant growth promoting factors. Some *Trichoderma* secondary metabolites significantly inhibit the growth of etiolated wheat coleoptiles at a relatively high concentration (10^{-3} M), but no effect is registered at lower doses (range from 10^{-4} to 10^{-6} M). Such compounds may act as plant hormones, which typically have an optimum activity between 10^{-5} and 10^{-6} M, while having an inhibitory effect at higher concentrations. In this work, we isolated koninginin A (2-Hexyl-3,10-dioxa-tricyclo[6.2.2.$0^{4,9}$]dodec-8-en-7-one), koninginin B (8-Hydroxy-2-(1-hydroxy-heptyl)-2,3,4,6,7,8-hexahydro-chromen-5-one) and its diastereoisomer koninginin E, produced by a biocontrol strain of *T. koningii*. Antifungal activity tests with koninginin B and E revealed a weak inhibitory activity on *Rhizoctonia solani* and *Pythium ultimum*. In contrast, complete inhibition of both pathogens was achieved with koninginin A at 10 and 100 µg, respectively. The plant growth promotion effect of koninginins was evaluated by monitoring the germination rate of tomato seeds and the length of seedlings grown in the presence of these secondary metabolites as applied at different concentrations. Koninginin A, that showed the highest antibiotic activity, did not improve significantly seedling growth. On the contrary, koninginin B and E produced a clear-cut growth promotion effect when applied at 10^{-5} and 10^{-6} M.

CHARACTERIZATION AND IDENTIFICATION OF NEW *TRICHODERMA* SPECIES COMPROMISING THE COMMERCIAL PRODUCTION OF *PLEUROTUS* IN ITALY. S.L. Woo[1], M. Ruocco[2], F. Vinale[1], R. Marra[1], S. Lanzuise[1], D. Turrà[1], V. Aloj[1], P. Marinelli[1], C.P. Kubicek[3], I. Druzhinina[3] and M. Lorito[1]. *[1]Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. [2]Istituto di Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. [3]Microbial Biochemistry and Gene Technology Department, Institute for Chemical Engineering, Technical University of Vienna, Getreidemarkt 9/166-5, 1060 Vienna, Austria. E-mail: woo@unina.it*

Trichoderma spp. attacks to *Pleurotus ostreatus* (oyster mushroom) cutivations are relatively recent, and new to Italy. Previously, *Trichoderma aggressivum* forms have caused severe green mould epidemics in *Agaricus bisporus* (champignon) in North America and Europe. It is unknown if *Trichoderma* spp. compromising the production of oyster mushrooms are the same as those causing disease to champignons. Samples were collected from all phases of *P. ostreatus* production; isolated by dilution plating, and pure *Trichoderma* cultures were obtained. Fungal species, including *Trichoderma,* were recovered in the initial compost stages (CFU's 9.2×10^4 to 2.9×10^8 per g of compost), were not detected in post-pasteurization or grain spawn inoculation, but were present in subsequent stages of incubation and fructification (CFU's 5.2×10^6 to 4.1×10^8 per g). The phenotypic, genetic and biological characters of all isolates were analyzed. *Trichoderma* isolates problematic to *P. ostreatus* differed in mycelial growth and sporulation from isolates used in biological control and from the forms pathogenic to *A. bisporus*, and they were not mycoparasitic to *P. ostreatus*. Genetic markers specific to the two *T. aggressivum* forms attacking *Agaricus* were not detected in any of the *Trichoderma* spp. associated with *P. ostreatus*. Identity analysis using ITS sequences, *Trich*Okey (www.isth.info) and NCBI BLAST indicated that many of the *Trichoderma* isolates associated with *P. ostreatus* were not similar to any existing species. These isolates are taxonomically distinct and have been identified as two new species, *T. fulvum* sp. nov. and *T. pleurotophilum* sp. nov.

POTENTIAL USE OF ESSENTIAL OILS ALONE OR IN COMBINATION WITH ANTAGONISTIC BACTERIA FOR SOIL STERILIZATION. M. Zaccardelli and F. Campanile. *CRA, Istituto Sperimentale per le Colture Industriali., Strada Statale 18 n. 204, 84091, Battipaglia (SA), Italy. E-mail: massimo.zaccardelli@entecra.it*

A large number of essential oils, extracted from medicinal plants, have antimicrobial activity. Against soil-borne pathogens as *Fusarium* spp., *Rhizoctonia solani*, *Sclerotinia* spp., essential oils extracted from *Carum carvi* L., *Melissa officinalis* L., *Origanum vulgare* L., *Rosmarinus officinalis* L., *Thymus vulgaris* L. and *Verbena officinalis* L., are among the most active. Moreover, these essential oils strongly inhibit germination of weeds. For these characteristics, these six essential oils are potential candidate for soil sterilization. To investigate their impact on the soil, each oil was separately added to pots containing natural soil. After irrigation

with an aqueous solution at 1% concentration, each pot was sealed in a plastic bag at 25°C to simulate a chemical soil treatment. Pots treated only with water were used as control. After three days, plastic bags were removed and hydrolase activity, one of the biological indexes that most rapidly respond to soil treatments, was measured on soil samples. Respect to control soil, hydrolase activity was lower, especially in soils treated with oils extracted from *Thymus vulgaris* L. and *Verbena officinalis* L. To investigate the possibility to integrate essential oils treatments with antagonistic bacteria, the effects of these oils on thirteen genetically different *Bacillus* spp., that showed antibiosis activity, were tested. All the essential oils were not toxic for any of the bacterial strains used; only two strains showed some inhibition by oils from rosemary and verbena. Further treatments on soils artificially contaminated with soil-borne pathogen are in progress.

IN VITRO CULTURE OF _ASTER SEDIFOLIUS_ AND _SOLANUM LYCOPERSICON_ FOR THE PRODUCTION OF FUNGITOXIC SAPONINS. **M. Zaccardelli[1], I. Caruso[2], F. Campanile[1] and A. Errico[2].** *[1]CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy. [2]Dipartimento di Scienze del Suolo, delle Piante e dell'Ambiente, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. E-mail: massimo.zaccardelli@entecra.it*

Saponins are glycosides with antimicrobial effects commonly found in plants. In particular, *Aster sedifolius* and *Solanum lycopersicum* produce, respectively, high level of triterpenoid and steroidal saponin with fungitoxic effects. For example, a mix of astersedifoliosides A, B and C, inhibit the growth of the phytopathogenic fungi *Fusarium solani*, *Rhizoctonia solani*, *Sclerotinia* sp. and *Sclerotium rolfsii*; α-tomatine inhibits the growth of *F. semitectum*, *F. solani*, *S. rolfsii* and, expecially, of *R. solani, F. oxysporum* and *Botrytis* sp. In this work, the production was evaluated of these fungitoxic coumpounds by *in vitro* cultures of *Aster caucasicus*, *A sedifolius* and *S. lycopersicum. In vitro* cultures were made in different conditions, i.e. use of different plant organs for callus tissue production, growth of the callus tissue in the dark or in the light, different concentration of hormones in the substrate. Fresh or lyophilized callus tissue were added to PDA and the level of saponins was measured using *Trichoderma viride* bioassays. The highest growth inhibition of *T. viride* was obtained from callus tissue of *S. lycopersicum* with respect to *A. caucasicus* and *A. sedifolius*. The most active were fresh or lyophilized callus tissue obtained from tomato roots and grown in the light or in the dark, but similar effects were observed with other tomato callus tissue. Among *Aster* spp., the best results were obtained with fresh callus tissue from leaves of *A. sedifolius*, grown in the light with the highest concentration of hormones. Results suggest that it is possible to produce fungitoxic saponins by *in vitro* culture.

SUSCEPTIBILITY OF DIFFERENT GENOTYPES OF CHICKPEA TO _ASCOCHYTA RABIEI_ IN THE OPEN FIELD. **M. Zaccardelli[1], F. Lupo[2] and A. Infantino[3].** *[1]CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy. [2]Dipartimeno di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy. [3]CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: massimo.zaccardelli@entecra.it*

Ascochyta rabiei is the most important pathogen of chickpea in the Mediterranean basin. Damages are particularly important especially when chickpea (*Cicer arietinum* L.) is sown in autumn or winter. During the warm and humid winter of 2006/2007, heavy attacks of *A. rabiei* were observed in an experimental field in the Sele valley (Campania, Southern Italy). Eight chickpea genotypes showed symptoms but differences in their incidence and severity were observed. Disease incidence, expressed as number of killed plants, was assessed in April 2007. The totality of the plants of ecotypes Maglianico, Spinazzola and Sassano was killed (100% incidence), whereas incidence levels of 96%, 68%, 61,5% and 52% were scored for the ecotype Cicerale, the cv. Visir, the ecotype Guardia dei Lombardi and the cv. Asia, respectively. The lowest figure (17,5%) was recorded for the genotype C9112 VT. These results show that local Italian chickpea genotypes are highly susceptible and that the use of resistant genotypes is of paramount importance in southern Italy for crops sown in autumn or winter. Moreover, the high susceptibility of the resistant cv. Visir, indicates that *A. rabiei* can overcome rapidly resistance genes of chickpea. Therefore, breeding programs are necessary to introduce in chickpea horizontal rather than monogenic or oligogenic resistance.

IDENTIFICATION OF BIO-ACTIVE COMPOUNDS IN ESSENTIAL OILS OF MEDICINAL PLANTS TOXIC FOR PHYTOPATHOGENIC FUNGI AND BACTERIA. **M. Zaccardelli[1], E. Mancini[2], F. Campanile[1], E. De Feo[2] and E. De Falco[2].** *[1]CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy. [2]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy. E-mail: massimo.zaccardelli@entecra.it*

Medicinal plants are of much interest for the production of natural and potentially eco-compatible essential oils that, beside other biological properties, are able of controlling dangerous phytopathogenic organisms. Previous studies has showon that essential oils extracted from *Carum carvi* L., *Foeniculum vulgare* Mill., *Hyssopus officinalis* L., *Lavandula angustifolia* Mill., *Majorana hortensis* L., *Melissa officinalis* L., *Ocimum basilicum* L., *Origanum vulgare* L., *Pimpinella anisum* L., *Salvia officinalis* L., *Thymus vulgaris* L. and *Verbena officinalis* L., are very toxic to several phytopathogenic fungi and bacteria, when used at 1% concentration. A total of 23 monoterpenes, representative of the main components of essential oils extracted from the above cited plants, were tested against eight phytopatogenic fungi and four bacteria. Limonene inhibited totally *Fusarium sambucinum* and *F. semitectum*; linalool inhibited totally *Rhizoctonia solani*; geraniol inhibited totally *Alternaria* sp., *Botrytis* sp., *F. sambucinum*, *F. semitectum* and *Sclerotinia* sp.; thymol inhibited totally *Botrytis* sp., *F. sambucinum*, *F. solani* and *Sclerotinia* sp.; carvacrol inhibited totally all eight phytopatogenic fungi; citronellal and β-citronellol inhibited *Botrytis* sp.; α-terpineol inhibited totally *Botrytis* sp., *F. oxysporum* and *R. solani*; carvone inhibited totally *R. solani*; citrale inhibited totally *Alternaria* sp., *Botrytis* sp., *F. sambucinum*, *F. semitectum*, *F. solani* and *Sclerotinia* sp. Camphor, thymol and carvacrol, inhibited totally the four phytopathogenic bacteria tested. Linalool, citronellal, β-citronellol, menthone, α-terpineol and carvone, inhibited totally *Xanthomonas campestris* pv. *campestris*, *Xanthomonas axonopodis* pv. *alfalfa* and *Erwinia carotovora*. Further studies are in progress.

INCIDENCE OF SOFT ROT OF FENNEL FERTILIZED WITH DIFFERENT DOSES OF NITROGEN AND CULTIVATED AFTER THREE GRAIN LEGUMES AND WHEAT. **M. Zaccardelli, D. Perrone, B. D'Onofrio, A. Del Galdo and F. Campanile.**

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In this work the effects were evaluated of four levels of nitrogen fertilization (0, 60, 120 and 180 kg ha^{-1}) on the incidence of soft rot of fennel grown after three grain legumes (pea, faba bean and chickpea) and wheat. The experimental field was in the Sele Valley (Campania, southern Italy). Soft rot incidence, evaluated at harvesting, increased with nitrogen fertilization (mean 5.1%) with respect to no fertilization (mean 0.8%). The highest incidence was registered in fennels fertilized with the highest nitrogen dose and grown after chickpea (mean 7.35%). Samples of symptomatic plants were collected to isolate pectinolytic agents using semi-selective media and the isolates obtained were purified and tested for pectinolytic activity on potato tuber plugs. Pectinolytic isolates were analysed by PCR using selective primers designed on pectate liase encoding gene (*pel*) of *Erwinia carotovora.* Different isolates gave the expected fragment of 434 bp.

ROLE OF THE NEUROTOXIN ODAP TO CONTROL FUNGAL INFECTIONS IN GRASS PEA (*LATHYRUS SATIVUS* L.). **M. Zaccardelli[1], A. Piergiovanni[2], F. Campanile[1] and F. Lupo[3].** *[1]CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy. [2]Istituto di Genetica Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. [3]Dipartimeno di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: massimo.zaccardelli@entecra.it*

ODAP (β-N-Oxalyl-α, β-diaminopropanoic acid) is a neurotoxin that causes to animals and humans severe neurological disorders (lathyrism) involving oxidative stress The content of this neurotoxin in grass pea (*Lathyrus sativus* L.), that ranges from 0.1% to 2.5%, is related to environmental factors and abiotic stresses. The toxin precursor, β-(isoxazolin-5-on-2-yl)-L-alanine (BIA), is exuded by the roots of some leguminous plants and has toxic effects on mammalian cells, yeasts, phytopathogenic fungi, unicellular green algae and higher plants. Its broad antifungal activity suggests that BIA might play a role as allelochemical. *L. sativus* has a high resistance to biotic and abiotic stresses, supposedly because of these neurotoxic compounds. To establish if ODAP has antifungal activity as BIA, crude extracts of the neurotoxin were assayed *in vitro* against different phytopathogenic fungi. Moreover, the content of ODAP was determined in seeds of 14 different genotypes of grass pea, cultivated in two different environments (a plane area near the sea and an internal hilly area), to establish the influence of genotype and environment on its concentration. First results of *in vitro* tests showed no effect of ODAP on *Ascochyta* sp., *Alternaria* sp. *Botrytis* sp., *Fusarium solani*, *F. oxysporum, Rhizoctonia solani, Sclerotinia* sp. ODAP content was up to three times higher in the seeds of plants grown in the hills than on the plane for all genotypes, confirming the higher effect of the environment with respect to the genotype.

EFFECTS ON PHYTOPATHOGENIC FUNGI AND BACTERIA OF ESSENTIAL OILS EXTRACTED FROM DIFFERENT SPECIES OF SAGE. **M. Zaccardelli[1], G. Roscigno[2], F. Campanile[1], E. De Falco[2] and V. De Feo[2].** *[1]CRA, Istituto per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy. [2]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy. E-mail: massimo.zaccardelli@entecra.it*

The goal of this work was the evaluation of the effects of essential oils, extracted from six different species of sage, on the growth of phytopathogenic fungi and bacteria. The species of sage were *Salvia greggii* A. Gray, *Salvia mellifera* Greene, *Salvia africana* L., *Salvia rutilans* Carrière, *Salvia officinalis* L. 'Icterina' and *Salvia munzii* Epling; the fungi tested were *Alternaria* sp., *Botrytis* sp., *Fusarium oxysporum*, *F. sambucinum*, *F. semitectum*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia* sp.; the bacteria tested were *Erwinia carotovora* and *Xanthomonas campestris* pv. *campestris*. Essential oil extracted from *S. rutilans* was partially active against *Alternaria* sp. and *Botrytis* sp. and totally active (100% inhibition) against *Sclerotinia* sp. and both bacteria; essential oil extracted from *S. munzii* totally inhibited the growth of *F. semitectum* and *R. solani*; essential oil extracted from *S. greggii* inhibited partially *Botrytis* sp. and totally *Alternaria* sp., *Sclerotinia* sp. and *R. solani*. Essential oils extracted from *S. africana*, *S. icterina* and *S. mellifera* did not inhibit or only partially inhibited some fungi. Chemical analyses of these six essential oils showed that the main common components of *S. rutilans*, *S. munzii* and *S. greggii* are *cis* and *trans* thujones, camphor, δ-cadinene and geraniol. Assays on phytopathogenic fungi and bacteria with these chemical compounds, alone or in combination, are in progress.

INFLUENCE OF A CRUDE POLYSACCHARIDIC EXTRACT FROM *LENTINULA EDODES* ON PATULIN SYNTHESIS BY *PENICILLIUM EXPANSUM* AND ON THE ACTIVITY OF TWO BIOCONTROL YEASTS. **S. Zjalic[1], R. Castoria[2], M. Reverberi[1], A. Ricelli[3], V. Tolaini[1], A.A. Fabbri[1] and C. Fanelli[1].** *[1]Dipartimento di Biologia Vegetale, Università degli Studi "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. [2]Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Via De Santis, 86100 Campobasso, Italy. [3]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. E-mail: slazjl@libero.it*

The basidiomycetous edible fungus *Lentinula edodes* is very popular in Asia for its nutritional and medical properties. It produces lentinans, which are β-glucans displaying antioxidant, immuno-stimulating and anticancer effects. These polysaccharides have been reported to decrease the synthesis of mycotoxins by *Aspergillus* and *Penicillium* spp, possibly through the lentinan-mediated stimulation of the antioxidant system of the toxigenic fungi. Wounds of stored apples are the main sites of penetration of *Penicillium expansum*, which synthesizes patulin in rotting fruits. Wounding of fruit tissues generates the reactive oxygen species superoxide anion (O_2) and hydrogen peroxide (H_2O_2), which can cause oxidative stress to the yeasts used as biocontrol agents of postharvest diseases of fruits, thus hindering their timely colonization of wounds and their protection of apples from fungal pathogens. In this preliminary work, we report that the crude lentinan extracts from *L. edodes* strain CF23 caused an evident decrease in *in vitro* patulin synthesis by *P. expansum* and, in lab-scale experiments, enhanced the antagonistic activity of the biocontrol yeasts *Cryptococcus laurentii* LS28 and *Rhodotorula glutinis* LS11 against the same fungus on apple fruits. The influence of crude lentinans on ROS production by apple tissues and on the resistance of the biocontrol yeasts to oxidative stress is under assessment.

ACQUISITION OF *GRAPEVINE LEAFROLL ASSOCIATED VIRUS 1* BY *PLANOCOCCUS FICUS*. **A. Zorloni, M. Molino Lova, S. Prati and G. Belli**. *Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: anna.zorloni@unimi.it*

It is known that the mealybug *Planococcus ficus* Signoret can transmit the vitiviruses GVA (*Grapevine virus A*) and GVB (*Grapevine virus B*), and the ampelovirus GLRaV-3 (*Grapevine leafroll-associated virus 3*). Whether this mealybug species could transmit also GLRaV-1 was investigated in the present work. Therefore, individuals of *Pl. ficus* were allowed to feed on GLRaV-1 infected vines (singularly or in mixed infection with GLRaV-3 and GVA) for different acquisition access periods (AAP): 1h, 2h, 4h, 6h, 1day, 4dd, 7dd, 10dd, 15dd, 21dd, or for undetermined periods. 902 insects, collected from infected plants after the acquisition access period, were analysed singularly or in groups of 2 to 10 individuals; 540 samples were tested by DAS-ELISA using a GLRaV-1 specific polyclonal antiserum. Positive results were obtained in 61 cases; GLRaV-1 was detected in 57 single insects and in samples made up of 3, 5, 7 and 10 individuals. In our experiments, the minimum AAP required to acquire GLRaV-1 was 2 h, but the virus was detected in insects also after 21 days feeding period. These results show that *Pl. ficus* can acquire GLRaV-1. Further work is in progress in order to test its capacity to transmit this virus from infected to healthy vines.