CHARACTERIZATION OF *COLLETOTRICHUM ACUTATUM* ISOLATES CAUSING ANTHRACNOSE OF STRAWBERRY IN CALABRIA. G.E. Agosteo[1], C. Macrì[1] and S.O. Cacciola[2]. *[1]Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 4, 89061 Reggio Calabria, Italy. [2]Dipartimento S.En.Fi.Mi.Zo., Università di Palermo, Viale delle Scienze 2, 90129 Palermo, Italy. Fax: +39.091.423238; E-mail: cacciola@unipa.it.*

Strawberry (*Fragaria* x *ananassa* Duchesne) is a crop of major economic importance in the Lametia Terme plains (Calabria, southern Italy). Cold-stored and fresh strawberry plantlets are imported from Belarus, California, and Spain, and are transplanted from late September through early November. Fruits are harvested from January through early June. In the last 10 years, outbreaks of fruit anthracnose, commonly known as blackspot, have been observed after late spring rains. 'Camarosa', the prevalent cultivar in this area, is moderately susceptible to this disease. A collection of 120 single-conidium strawberry isolates of *Colletotrichum* from Calabria and other regions of southern, central, and northern Italy were characterized by morphological and cultural traits, isozyme (ACP, ALADH, G_6PD, EST, MDH, SOD, XDH and GPI) electrophoresis, RAPD analysis of DNA, with 16 decamer primers, and vegetative compatibility tests, with nitrate non-utilizing (*nit*) mutants. All isolates were identified as *C. acutatum* J.H. Simmonds (anamorph of *Glomerella acutata* Gueber & Correll) and showed very limited genetic diversity. Their isozyme and RAPD patterns were very similar or almost identical to the patterns of strawberry reference-isolates of *C. acutatum* from California and Spain. Moreover, all isolates, including reference-isolates from California and Spain, were assigned to a single vegetative compatibility group, with the only exception of 35 isolates that resulted to be self-incompatible. These results suggest that all isolates belong to a clonal population and that spread of *C. acutatum* has occurred between distant geographic areas via infected strawberry material, notwithstanding the fact that this fungus is a quarantine pathogen in the EPPO region.

SUSCEPTIBILITY OF OLIVE CV ITRANA TO ANTHRACNOSE. G.E. Agosteo[1], C. Macrì[1] and P. Taccone[2]. *[1]Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, 89061 Gallina, Reggio Calabria, Italy. [2]Farm "Acton", Cannavà, Rizziconi (RC) Italy. Fax: +39.0965.689049; E-mail: geagosteo@unirc.it.*

Anthracnose, caused by a *Colletotrichum* species formerly referred to as *Gloeosporium olivarum*, is a major disease of olive in the Gioia Tauro area of Calabria (southern Italy). It attacks fruits, leaves and shoots. A rot of ripening drupes is the most typical symptom of the disease. 'Itrana', a variety with medium to high-sized drupes, introduced recently in this area, has showed a high susceptibility to this disease. In autumn, an internal brown rot of the pulp and a brown discoloration of the pit can be observed on apparently healthy olives. Initially, only the internal tissues around the pit of green drupes are affected. Subsequently, circular water-soaked spots with pink masses of conidia in the centre appear on the outer surface of ripening drupes. The *Colletotrichum* species responsible for olive anthracnose has consistently been isolated from the pit, but never from the internal pulp with symptoms of brown rot of apparently healthy drupes either green or in véraison. Furthermore, positive isolations of the fungus were obtained from the internal tissues of non-symptomatic green drupes collected in June and July, before pit hardening, suggesting an endophytic behaviour of the causal agent of olive anthracnose.

BIOLOGICAL CONTROL OF APPLE AND TOMATO POST-HARVEST DISEASES CAUSED BY *BOTRYTIS CINEREA* AND *ALTERNARIA ALTERNATA* BY USING CULTURE FILTRATES OF *TRICHODERMA HARZIANUM* T22. P. Ambrosino, R. Prisco, M. Ruocco, S. Lanzuise, A. Ritieni, S.L. Woo, F. Scala and M. Lorito. *Dipartimento Ar.Bo.Pa.Ve, Sezione di Patologia Vegetale, Università degli Studi di Napoli, CNR-IPP, Via Università 100, Portici (NA) 80055 Italy. Fax: +39.081.2539339; E-mail: lorito@.unina.it.*

Fungal diseases cause major losses of fruits and vegetables in post-harvest. Pre and/or post-harvest applications of synthetic fungicides imply serious risks. For this reason alternative or integrative means to these compounds are being intensively investigated. In this study, we report and discuss the results of investigations regarding the inhibitory effects of culture filtrates of *T. harzianum* T22 towards fungal pathogens. *In vitro* and *in vivo* tests were performed on apple and tomato artificially inoculated with a spore suspensions of *Botrytis cinerea, Alternaria alternata* and *Penicillium expansum*. The degree of inhibition was dependent on carbon and nitrogen sources used as growing media; the best results were obtained by growing the antagonistic fungus in presence of barley fibers. The diameter of the disease lesions was significantly reduced five days after inoculation and treatment with the culture filtrate of *T. harzianum*. The development of *B. cinerea* was reduced up to 52%, while that of *A. alternata* and *P. expansum* up to 66% and 23%, respectively. Treatment of apple fruits with *T. harzianum* 20 min before the inoculation with phytopathogenic fungi provided a better protection than simultaneous or delayed applications. The efficiency of *T. harzianum* culture filtrate may be partly explained by the presence in it of strong chitinolytic, glucanolytic, cellulolytic and xylanolytic activities. A proteomic analysis is in progress to characterize the molecules involved in the postharvest control of phytopathogenic fungi.

MANAGEMENT OF INSECT-BORNE VIRUSES IN TOMATO BY UV-REFLECTING MULCH. T. Amenduni[1], V. Gentile[1], F. Lops[1], L. Colatruglio[1] and D. Gallitelli[2]. *[1]Dipartimento di Scienze Agro-Ambientali, Chimica e Difesa delle Piante, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. [2]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.0881.589321; E-mail: t.amenduni@unifg.it.*

Tomato spotted wilt virus (TSWV) vectored by thrips and *Cucumber mosaic virus* (CMV) vectored by aphids are highly detrimental to tomato production in Apulia (southern Italy). Insecticides applied on a calendar schedule for vector control are not effective in preventing diseases induced by the two viruses and tomato varieties tolerant to TSWV were recently attacked by the resistance-breaking (RB) strains of the virus. In field experiments carried out in USA, UV-reflecting plastic mulches proved effective in reducing primary infections of TSWV. For unknown reasons, this type of mulch is not used by Italian farmers. Therefore we have set up two experimental tomato fields in different areas of the province of Foggia (Apulia, southern Italy) to evaluate the effects of this type of mulching on the spread and incidence of CMV and TSWV. Five types of mulch, i.e. UV-reflecting, UV-reflecting and black, black, transparent and biodegradable, were compared. Each experiment consisted of three replicates of twenty-five plants. Preliminary results suggest that UV-reflecting mulches can significantly reduce primary and secondary infections.

EFFECT OF *THYMUS CAPITATUS* OIL, ACETALDEHYDE, CITRAL, AND IMAZALIL ON MANDARIN LIKE FRUITS CV FAIRCHILD. G. Arras[1], G. D'hallewin[2] and M. Agabbio[2]. *[1]C.N.R. - Istituto di Scienze delle Produzioni Alimentari, Sezione Sassari, Via dei Mille 48, Sassari, Italy. [2]Dipartimento di Scienze Ambientali e Biotecnologie Agroalimentari, Università degli Studi di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.232047; E-mail: giovanni.arras@ispa.cnr.it.*

Thymus capitatus essential oil, acetaldehyde (AA), citral and imazalil (IMZ) were comparatively tested for effectiveness in controlling decay of mandarin like fruits cv Fairchild sprayed with a conidial suspension (10^5 ml^{-1}) of *Penicillium digitatum* and placed in 15-l desiccators, each containing 30 fruits. Two containers were dipped in an IMZ water suspension at 100 and 1,000 ppm. Thyme oil, citral and AA, all at 100 ppm (v/v), were heated to complete vaporization either on fruits previously dipped in 100 ppm IMZ or on fruits sprayed with conidia. Then, a 0.2 bar pressure was applied inside the desiccators. Fruits treated with IMZ only were exposed to the same subatmospheric pressure after treatment. Three control containers were placed at 0.2 bar and atmospheric pressure, respectively. Viability of fungal spores was checked by plating rinsing water of three fruits from each container on potato-dextrose agar dishes. Colony counts were done daily. All treatments significantly reduced the viability of the pathogen, IMZ 1000 resulting the best. The volatile compounds showed a higher control of the pathogen than IMZ at 100 ppm. Thus, *T. capitatus* oil, citral and AA appear to be of great interest for controlling postharvest diseases of citrus fruits.

COMPLEXATION OF TEBUCONAZOLE WITH β-CYCLODEXTRIN FOR CONTROLLING FOOT AND CROWN ROT OF DURUM WHEAT INCITED BY *FUSARIUM CULMORUM*. V. Balmas[1], G. Delogu[2], S. Sposito[1,2], D. Rau[3] 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 di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. [2]CNR- Istituto di Chimica Biomolecolare, Sezione di Sassari, Traversa La Crucca, 3, Località Baldinca, Li Punti, I-07040 Sassari, Italy. [3]Dipartimento di Scienze degli Alimenti, Università Politecnica delle Marche, Via Brecce Bianche, I-60131 Ancona, Italy. Fax: +39.079.229316;E-mail: balmas@uniss.it.*

Fusarium culmorum is the most common incitant of crown and foot rot of wheat in Italy. Seed treatment with fungicides represents an efficient means of control, but it is usually limited to the early stages of the wheat cycle, since the fungicides do not maintain their efficiency for a longer period. Thus, regardless of the systemic properties of the fungicide, methods are needed to improve the slow release of the delivered compound, so that the plant organs may be protected for longer. The molecular inclusion of pesticides offers several advantages over conventional agrochemical formulations: (i) release of a constant level of the active ingredient, providing enhanced efficacy; (ii) reduced mammalian toxicity and phytotoxicity; and (iii) increased solubility of water-insoluble compounds. A methodology for the inclusion of tebuconazole in β-cyclodextrin, spectroscopic characterization of the inclusion complex and its activity for the control of a major soilborne disease of wheat caused by *Fusarium culmorum* are reported. Controlled release measured by chemical shift of the diagnostic proton H_3 and H_5 of β-cyclodextrin confirmed the stability of the complex at the solid state and in aqueous solution. Greenhouse and field experiments were conducted on durum wheat (*Triticum durum*, cv Prometeo) sown in substrate or in soil artificially infested with a virulent strain of *F. culmorum*. The inclusion complex β-cyclodextrin - tebuconazole, applied as seed dressing in combination or not with carboxymethylcellulose, reduced disease incidence caused by *F. culmorum*, improved grain yield, which did not differ significantly from that of healthy control.

DIAGNOSIS OF MAL NERO DISEASE OF CITRUS BY CONVENTIONAL METHODS AND PCR. V. Balmas[1], M.A. Demontis[1], V. Lo Giudice[2], F. Raudino[3], Q. Migheli[1] and S.O. Cacciola[4]. *[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 di Sassari, Via E. De Nicola 9, I - 07100 Sassari, Italy. [2]Private address: Via Calvario 45, 95030 Mascalucia (CT). [3]Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 2, 89061 Reggio Calabria, Italy. [4]Dipartimento S.En.Fi.Mi.Zo., Università di Palermo, Viale delle Scienze 2, 90128 Palermo, Italy. Fax: +39.091.7028855; E-mail: cacciola@unipa.it.*

Mal nero is a form of mal secco disease of citrus incited by chronic root infection by *Phoma tracheiphila* (Petri) L.A. Kantsch. & Gikaschivili. Initially, the pathogen invades the inner xylem without causing apparent symptoms on the tree. Eventually, it colonises the outer wood and causes vein leaf chlorosis followed by shedding of the leaves and a sudden wilting of either the whole tree or some limbs. The most typical symptom of mal nero is a dark brown discolouration of the inner wood. In late spring 2003, symptoms resembling mal nero were observed in eastern Sicily in numerous 15- to 18-year-old trees of 'Fortune' mandarin (*Citrus clementina* Hort. *ex* Tanaka x *C. reticulata* Blanco 'Dancy') and 'Tacle'[*C. sinensis* (L.) Osbeck 'Nucellar Tarocco' x *C. clementina* Hort. *ex* Tanaka 'Monreal'), grafted on alemow (*C. macrophilla* Wester). Up to 30% of the trees were affected in a single orchard. Surprisingly, orchards with symptomatic trees were located in an orange-growing area. The diagnosis was confirmed by isolation of the pathogen on potato-dextrose-agar and by a specific PCR assay recently developed (Balmas *et al*., 2005, *Eur. J. Pl. Pathol.* 111: 235). The results obtained by PCR correlated with isolation from symptomatic wood. However, *P. tracheiphila* was also detected by PCR in symptomless tissues of infected trees. Alemow has been previously reported to be very susceptible to mal secco. The high incidence of mal nero on 'Fortune' and 'Tacle' trees grafted on this rootstock was correlated with the soil management system based on non-tillage with herbicide treatment.

ALTERATION OF ANTIOXIDANT ENZYME GENE EXPRESSION DURING REFRIGERATED STORAGE OF THE SCALD RESISTANT 'BELFORT' AND THE SCALD SUSCEPTIBLE 'GRANNY SMITH' APPLES. E. Baraldi and P. Zubini. *DIPROVAL, Facoltà di Agraria, Università di Bologna, Via Fanin 46, 40127, Bologna, Italy. Fax: +39.051.2096539; E-mail: ebaraldi@agrsci.unibo.it.*

The scald resistant 'Belfort' and the scald susceptible 'Granny Smith' apple cultivars were used to investigate the hypothesis that expression of antioxidant enzyme genes was correlated with scald susceptibility. Apples were harvested, kept in refrigerated storage, and periodically warmed at 20°C for two days. Samples were collected at harvest time and after 13, 20, and 26 weeks of storage. The α-farnesene, conjugated trienes (CT281 and CT258), and H_2O_2 content were determined. The α-farnesene content was not correlated with scald incidence, whereas CT281 were positively and CT258 were negatively correlated with scald incidence.

The H_2O_2 content in 'Belfort' apple peels did not vary during storage, whereas in 'Granny Smith' this value increased significantly. Total RNA was extracted from the peels of these apples, retro-transcribed into cDNA, and used in Real Time PCR to quantify expression of genes encoding Mn superoxide dismutase (MnSOD), catalase (CAT), ascorbate peroxidase (APX), monodehydoascorbate (MDHAR), and glutathione reductase (GR). In both apple cultivars the expression level of the MnSOD gene decreased from harvest during storage time, whereas that of APX did not vary. In 'Belfort' apples the expression of the other antioxidant enzyme genes did not change from harvest to subsequent storage surveys. Conversely, in 'Granny Smith' apples, expression of the CAT gene increased during the last storage period, and expression of MDHAR and GR genes increased during the first 13 weeks of storage. Therefore, scald resistance is not directly related to a higher expression of antioxidant enzyme genes.

FIRST REPORT OF CHERRY VIRUS A IN ITALY. M. Barone and A. Ragozzino. *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli, Facoltà di Agraria, Via Università 100, 80055 Portici (NA), Italy. Fax: +39.081.2539367; E-mail: marbaron@unina.it.*

Cherry Virus A (CVA), a member of the genus *Capillovirus*, has been reported to naturally infect sweet and sour cherry, apricot, peach and, recently, Mirabelle plum. The virus has not been associated to any specific symptom. In 2004, during a survey for the presence of trichoviruses, capilloviruses, and foveaviruses in Campania (Southern Italy), carried out using a polyvalent nested RT-PCR assay, CVA was detected in three local apricots (cvs Quattova, Zì Luisa, Paolona) and one local plum (*Prunus domestica*, cv. Turcona) present in a *Prunus* spp. germplasm collection. No specific symptoms were observed in infected apricots and plum. Sequence analysis of the four CVA sources showed a nucleotide and amino acid identity ranging from 87 to 99% and from 90 to 99%, respectively, when compared to CVA sequences from GenBank (Accession No. NC_003689, X82547, AY944064, AY944065, AY944066). To our knowledge, this is the first record of CVA from Italy.

CHARACTERIZATION OF *COLLETOTRICHUM* SPECIES CAUSING ANTHRACNOSE OF ORNAMENTALS AND HORTICULTURAL CROPS IN ITALY. S.O. Cacciola[1], A. Minuto[2], G.E. Agosteo[3], R. Faedda[4] and D. Bertetti[2]. *[1]Dipartimento S.En.Fi.Mi.Zo., Viale delle Scienze 2, Università di Palermo, 90128 Palermo, Italy. [2]AGROINNOVA, Università di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy. [3]Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 2, 89061 Reggio Calabria, Italy. [4]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Via S. Sofia 100, 95125 Catania, Italy. Fax: +39.091.7028855; E-mail: cacciola@unipa.it.*

Over 250 *Colletotrichum* isolates from a wide range of hosts and geographical origins were examined for morphological, cultural and physiological characters as well as for electrophoretic banding patterns of eight mycelial isozymes and RAPD profiles obtained with 16 decamer primers. Most of the isolates had been previously identified as either *C. gloeosporioides* (Penz.) Penz. & Sacc. or *C. acutatum* Simmonds. Cluster analysis of RAPD and isozyme profiles was performed with the UPGMA algorithm and was supported by bootstrap analysis. RAPD and electrophoretic profiles identified the same discrete groups. All isolates from strawberry produced fusiform conidia, grew slowly on agar-media, showed an optimum growth temperature of about 24°C, were benomyl-resistant (MIC $\geq 10^2$ µg ml^{-1}) and formed a distinct molecular group (*C. acutatum sensu strictu*). A group, identified as *C. gloeosporioides sensu stricto*, comprised isolates from diverse hosts, including isolates of the causal agent of pepper anthracnose from southern Italy. Isolates from camellia shared some morphological and physiological characters with *C. gloeosporioides sensu strictu* (i.e. cylindrical conidia, fast growth, optimum growth temperature ≥27°C, benomyl MIC ≤ 1 µg ml^{-1}), but were genetically distinct from this group. Isolates from sweet basil, oleander and rhododendron were in three separate molecular groups, genetically distinct from both *C. acutatum* and *C. gloeosporioides*. Each of these three groups represented very likely a distinct species. Surprisingly, rhododendron isolates, previously identified as *C. acutatum* (Vinnere *et al*., 2002, *Mycol. Res.* 106: 60), clustered together with the isolates of the causal agent of olive anthracnose from southern Italy, which was recently shown recently to be a new *Colletotrichum* species.

***PHYTOPHTHORA PSEUDOSYRINGAE* THE CAUSAL AGENT OF BLEEDING CANKERS OF BEECH IN CENTRAL ITALY. S.O. Cacciola[1], E. Motta[2], F. Raudino[3], A. Chimento[1], A. Pane[4] and G. Magnano di San Lio[3].** *[1]Department S.En.Fi.Mi.Zo., University of Palermo, Viale delle Scienze 2, 90128 Palermo, Italy. [2] Plant Pathology Research Institute, C.R.A., Via C.G. Bertero 22, 00156 Rome, Italy. [3]Department of Agrochemistry and Agrobiology, Mediterranean University of Reggio Calabria, Piazza S. Francesco di Sales 2, 89061 Reggio Calabria, Italy. [4]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, 95125 Catania, Italy. Fax: +39. 0965.689015; E-mail: gmagnano@unirc.it.*

In 2002, a disease of beech (*Fagus sylvatica*) caused by *Phytophthora pseudosyringae* was reported for the first time in central Italy on a single tree showing decline and bleeding cankers at the base of the trunk (Motta *et al.*, 2003, *Pl. Dis.* 87: 1005). *P. pseudosyringae*, a homothallic species with semipapillate sporangia, closely related to *P. ilicis*, was reported from beech and alnus in Germany and was isolated from the rhizosphere of European turkey oak in Tuscany. In summer 2003, mature trees with the same disease were found in a pure beech forest in the Abruzzo National Park. Symptomatic trees were scattered in thinned forest stands or alongside paths, thus suggesting an anthropogenic effect on disease development. Nineteen *P. pseudosyringae* isolates, from three regions, Abruzzo, Latium and Tuscany, showed very similar morphological features and identical electrophoretic phenotypes (total mycelial proteins and esterase zymograms). Colonies grew slowly on V8A and very slowly on PDA, with optimum and maximum growth temperatures of 15 and 25°C, respectively. This low growth rate temperature has ecological implications as, for example, in Abruzzo *P. pseudosyringae* was found at an altitude higher than 1,200 meters a.s.l.. All isolates were equally pathogenic on 2-year-old beech seedlings but their pathogenicity on apples varied greatly. The amplified rDNA sequences of three Italian isolates, from Tuscany (UdF Ph 24), Latium (IMI 390500) and Abruzzo (IMI 391716), respectively, were identical to the sequences of two reference-isolates of *P. pseudosyringae*, (GenBank accession numbers AY 366462 and AY 366463).

***PHYTOPHTHORA* SPECIES ASSOCIATED WITH ROOT ROT OF OLIVE IN SICILY. S.O. Cacciola[1], G. Scarito[1], A. Salamone[1], A.S. Fodale[2], R. Mulè[2], G. Pirajno[1], G. Sammarco[1] and D. Spica[1].** *[1]Dipartimento S.En.Fi.Mi.Zo., Università di Palermo, Viale delle Scienze 2, 90128 Palermo, Italy. [2]C.R.A. - Experimental Institute for Olive Growing, Palermo, Italy. Fax: +39.091.7028855; E-mail: cacciola@unipa.it.*

Phytophthora root rot of olive trees has been recognized as an

emerging problem in many olive-growing areas in the Mediterranean region, probably consequent to the increasing use of irrigation. A survey aimed at determining the diffusion of this disease in commercial orchards is being carried out in Sicily. *Phytophthora* species were identified by traditional morphological, biochemical (polyacrylamide gel electrophoresis of mycelial proteins and isozymes), and molecular (ITS sequences of rDNA) criteria. The following species were recovered from both young and mature (10- to 12-year-old) olive trees with symptoms of chlorosis, defoliation, and wilting: *P. inundata*, *P. megasperma*, *P. nicotianae*, and *P. palmivora*. The last two species were found associated with root rot of rootlets in both nursery plants and mature trees originated from rooted cuttings. Surprisingly, *P. palmivora* was more common than *P. nicotianae*. There are other recent reports of the occurrence of this tropical species in olive in southern Italy and Spain. *P. megasperma*, which was previously reported from other olive-growing countries including Greece and Spain, was recovered from roots and basal stem cankers of young plants. All Sicilian isolates of this latter species were referred to the BHR (Broad Host Range) group on the basis of DNA sequencing. *P. inundata,* a species formally described only recently, was associated with root rot in trees subjected to flooding. This is the first report of *P. inundata* in olive in Italy, where it has already been recovered from roots of ornamental palms and peach.

VERTICILLIUM WILT OF *XANTHIUM ITALICUM* AND *SOLANUM AETHIOPICUM* IN ITALY. I. Camele[1], C. Marcone[1], A. Caponero[2], S. Frisullo[3] and G.L. Rana[1]. *[1]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale Ateneo Lucano 10, 85100 Potenza, Italy. [2]A.L.S.I.A. - Agenzia Lucana di Sviluppo ed Innovazione in Agricoltura, Via Carlo Levi, 75100, Matera, Italy. [3]Dipartimento di Scienze Agro-Ambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. Fax: +39.*0881.589218; *E-mail: s.frisullo@unifg.it.*

A wilt disease of Italian cocklebur (*Xanthium italicum* Mor.) and African eggplant (*Solanum aethiopicum* L.) was observed in the Basilicata region (southern Italy) in the last two years. The most characteristic symptoms present on both plant species included yellowing, stunting, gradual wilting, and browning of vascular tissues. *Verticillium dahliae* was consistently identified morphologically from colonies on PDA. Polymerase chain reaction (PCR) assays, with the primer pair ITS5/ITS4, directed to nuclear ribosomal DNA (rDNA) repeat sequences, gave an amplification product of about 560 bp, using total DNA extracted from diseased Italian cocklebur and African eggplant xylem tissues, as well as from fresh mycelium of the corresponding pure culture isolates. Sequence analysis of the ITS5/ITS4 amplicons revealed no differences in their nucleotide positions. Sequences of all southern Italian isolates of *V. dahliae* were identical to that of a Greek strain of the same fungus (GenBank accession No. AF104926). To fulfill Koch's postulates, ten healthy seedlings of Italian cocklebur and African eggplant were inoculated by dipping their trimmed roots in conidial suspensions from 10-day-old colonies of *V. dahliae* pure cultures of Italian cocklebur and African eggplant isolates, respectively, containing $1.5 \cdot 10^6$ CFU ml^{-1}. All inoculated seedlings showed symptoms identical to those of naturally infected plants three weeks post inoculation. *V. dahliae* was consistently reisolated from artificially infected plants. This is the first report of Verticillium wilt of *X. italicum* and *S. aethiopicum*.

HPLC PROFILE OF SECONDARY METABOLITES OF *PENICILLIUM CANESCENS* AND *PENICILLIUM JANCZEWSKII*. A. Carella and R. Nicoletti. *C.R.A. - Istituto Sperimentale per il Tabacco, Via Vitiello 108, I-84018 Scafati, Salerno, Italy. Fax: +39.081.8506206; E-mail: ronic61@libero.it.*

Penicillium canescens and *Penicillium janczewskii* are two closely related species belonging to the section *Divaricatum* of the *Penicillium* subgenus *Furcatum*, which are primarily soil fungi. Although the discrimination between the two species is based on a number of distinctive morphological characters, strains showing intermediate features can occasionally be recovered, whose taxonomic position may be questionable. In the past few years, chemotaxonomy has been increasingly regarded as a fundamental criterion for classifying *Penicillium* species. This is especially for the terverticillate species belonging to the subgenus *Penicillium*, while available data concerning species in the subgenus *Furcatum* are less substantial. During investigations on the biological activity against plant pathogenic fungi by culture filtrates of both species recovered from soil samples collected in Apulia and Umbria, we have extracted and identified a number of secondary metabolites, such as griseofulvin, dechlorogriseofulvin, fumagillin, curvulic acid, and a new metabolite (PL-322), whose molecular structure is under investigation. The HPLC retention index of these metabolites was calculated according to Frisvad and Thrane (1987), and their production by all available strains evaluated. Griseofulvin was produced by all fungal strains, while the remaining compounds were detected in a restricted number of isolates, without any specificity for one species or the other. Therefore, their use as taxonomic markers for the identification of single isolates as *P. canescens* or *P. janczewskii* does not seem to be completely reliable.

A NEW METHOD FOR THE MONITORING OF *PHYTOPHTHORA* DIVERSITY IN SOIL AND WATER. A. Chimento[1], S. Scibetta[2], L. Schena[3], S.O. Cacciola[1] and D.E.L. Cooke[4]. *[1]Dipartimento S.En.Fi.Mi.Zo., Università degli Studi di Palermo, Viale delle Scienze, 90128, Palermo, Italy. [2]Department of Agrochemistry and Agrobiology, Mediterranean University of Reggio Calabria, Piazza S. Francesco di Sales 4, 89061 Reggio Calabria, Italy. [3]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Italy. [4]Scottish Crop Research Institute, Dundee, Invergowrie, Dundee, DD2 5DA, Scotland, UK. Fax: +39.091. 423238; E-mail: cacciola@unipa.it.*

A new molecular method for the generic identification of *Phytophthora* species was developed to monitor species diversity in natural ecosystems. A PCR assay based on *Phytophthora* genus-specific primers in the rDNA repeat regions was developed which amplifies the entire internal transcribed spacer (ITS) region. Primers were tested against over 30 *Phytophthora* species representing the known diversity in the genus. These primers did not amplify any of 13 *Pythium* species tested. The protocol for soil and water testing was based on a nested PCR assay and the cloning of the second round PCR product. Database comparisons of the DNA sequence derived from these cloned fragments were used for species identification. A survey was conducted in which soil and water samples were collected from a range of Scottish natural and planted woodland. Multiple samples were collected from six different sites and *Phytophthora* was detected in at least one sample per site. The most frequently detected ITS sequences were related to members of ITS-clade 6 (i.e. *P. gonapodyides*-like) or identical to *P. citricola*. The range and type of species present varied from sample to sample and in one single 500 g soil sample four different *Phytophthora* species were detected. A novel unreported clade 6 ITS sequence was also identified, suggesting the presence of a new species. A key advance from previously published methods was the lack of cross-reaction with the ubiquitous *Pythium* species. Further optimisation to improve sensitivity and develop filtration methods for water sampling is underway.

THE DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE DETECTION OF *PHYTOPHTHORA* IN ASPARAGUS. A. Chimento[1], S. Scibetta[2], L. Schena[3], S.O. Cacciola[1], K.R. Green[4] and D.E.L. Cooke[5]. *[1]Dipartimento S.En.Fi.Mi.Zo., Università degli Studi di Palermo, Viale delle Scienze, 90128, Palermo, Italy. [2]Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 4, 89061 Reggio Calabria, Italy. [3]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Bari, Italy. [4]ADAS Arthur Rickwood, Cambridgeshire, UK. [5]Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK. Fax: +39.091.423238; E-mail: cacciola@unipa.it.*

Phytophthora rot is a significant threat to asparagus production worldwide, causing severe damage to the developing spears particularly under cool wet conditions. Although the disease was described some time ago, little is known about the responsible pathogen. In this study, several *Phytophthora* isolates from asparagus and agaves were examined and compared with a wide panel of *Phytophthora* species to develop new sensitive molecular methods of diagnosis based on conventional and Real-Time PCR assays. The isolates of *Phytophthora* from asparagus and agaves showed morphological and genetic features that clearly discriminated them from any of the known *Phytophthora* species, so that they may be representative of a new species for which the name of *Phytophthora asparagi* is proposed. Sequence variation within *Phytophthora* Internal Transcribed Spacer (ITS) regions is generally appropriate for species discrimination. The ITS sequences of *P. asparagi* isolates differed markedly from that of related clade 6 taxa and, in particular, from *P. megasperma*, a related taxon previously identified as the cause of the disease of asparagus. We developed and tested ITS-based nested and Real-Time PCR assays for the detection of *Phytophthora* sp. (*P. asparagi*). The specificity to *P. asparagi* was confirmed by testing against 36 *Phytophthora* spp. and 5 *Pythium* spp. as out groups. Infected asparagus spears were tested with a nested PCR assay using DC6-ITS4 primers for the first amplification and specific primers for the second. Real Time primers were also designed to improve the assay throughout and then tested against infected spears and roots and infested soil samples from naturally infected sites.

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON VERTICILLIUM WILT OF ARTICHOKE. F. Ciccarese[1], O. Longo[1], C. Paciolla[1], D. Schiavone[1] and F.I. Morone[2]. *[1]Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. [2]Dipartimento di Scienze delle Produzioni Vegetali, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442906; E-mail: fciccare@agr.uniba.it.*

Arbuscular mycorrhizal fungi (AMF) are known to have beneficial effects on the growth and development of plants and to increase resistance or tolerance to pathogens. Positive effects on plant disease by AMF have been reported in various pathosystems, the most important mechanism of AMF effects on plant disease being the change in the biochemical constituents of plant tissues. The results are reported of studies on the effect of mycorrhizal artichoke offshoot with an isolate of *Glomus viscosum* Nicolson towards Verticillium wilt, and on changes of the ascorbate system. The severity of external symptoms was significantly lower in mycorrhizal than in non-mycorrhizal artichokes grown in infected soil. No effect was observed on vascular discolouration. Beneficial effects of mycorrhizal plants were evident on the growth, earliness, and marketable yield of artichoke. In healthy mycorrhizal plants there was an increase in ascorbate content with respect to the control, the same as in infected mycorrhizal artichokes as compared with infected non-mycorrhizal. The activity of ascorbate peroxidase, the most efficient scavenger of hydrogen peroxide, showed the same trend as ascorbate. As to dehydroascorbate reductase, the enzyme involved in ascorbate regeneration from its oxidized form, its activity increased equally well in healthy as in infected mycorrhizal plants. These results seem to indicate that, in mycorrhizal artichoke, there is an involvement of the ascorbate system in the defence response to Verticillum wilt.

GLUCOSE OXIDASE GENE EXPRESSION IN *TRICHODERMA HARZIANUM* T22 ENHANCES ITS BIOCONTROL ACTIVITY. R. Ciliento, M. Ruocco, F. Scala, S.L. Woo and M. Lorito. *Dipartimento Ar.Bo.Pa.Ve., Sezione di Patologia Vegetale, Università di Napoli, and CNR-IPP, Via Università 100, 80055 Portici (NA), Italy. Fax: +39.081.2539339; E-mail: lorito@.unina.it.*

Trichoderma-based biofungicides are a reality in agriculture, although they represent only a niche market in comparison to that of chemicals. *Trichoderma* spp. are able to control phytopathogenic fungi and nematodes, and to induce SAR and ISR in plants. Numerous genes and promoters involved in mycoparasitism have been cloned and characterized, and their role in the biocontrol processes demonstrated. In this work some biocontrol-related inducible promoters have been used in a reporter system based on the *Aspergillus niger* glucose oxidase gene (*GOX*) to monitor and increase biocontrol activity of *T. harzianum* T22. Glucose oxidase catalyzes the oxygen-dependent oxidation of D-glucose to D-glucono-1,5-lactone with the production of hydrogen peroxide. In plants, this latter compound is known to have an antifungal effect and to activate the defence cascade, thus increasing plant resistance to the attack of pathogens. *T. harzianum* T22 transformants with different promoters/gox constructs were obtained by protoplast co-transformation. The presence of the *GOX* gene was confirmed in mitotically stable transformants by PCR and Southern blots while its expression was verified by Northern analysis. Enzyme production and GOX activity were also found in culture filtrates of transformants. The transformants were tested *in vivo* to determine the effect of GOX on biocontrol activity and ISR induction. Bean seeds coated with *Trichoderma* spores were planted in soil infested with the soil-borne fungal pathogens *Rhizoctonia spp*. and *Pythium spp*. The presence on the roots of T22-GOX mutants reduced significatively disease symptoms caused by the pathogens in comparison with the wild type *Trichoderma* T22.

DIVERSITY AND POPULATION DYNAMICS OF *PENICILLIUM* SPP. ON CITRUS FRUITS IN POSTHARVEST ENVIRONMENTS. V. Coco, R. La Rosa and G. Cirvilleri. *Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi di Catania, Via S. Sofia 102, 95100 Catania, Italy. Fax: +39.095.292730; E-mail: vcoco@tiscalinet.it.*

Isolates of *Penicillium* spp. were collected from the surface of citrus fruits in postharvest and after two weeks in the storage room, and from the atmosphere and surfaces of warehouses and storage rooms. *Penicillium* spp. were detected on the surface of cvs Tarocco and Valencia in postharvest, spore density ranging between 0 to 44 propagules cm^{-2} on the fruits of both cultivars. However, these figures were reduced after processing (washing, drying and waxing) and storage for two weeks at 4°C, as they did non exceed 10 spores cm^{-2}. The level of *Penicillium* spp. on non-waxed fruits of both cultivars increased after 2 weeks storage up to 22-40 spores cm^{-2}. The level of airborne *Penicillium* was evaluated in warehouse environ-

ments prior to fruit storage and again after two weeks in storage rooms. Just before fruit storage, the density of airborne *Penicillium* in warehouse atmosphere ranged between 368 and 601 spores m^{-3}, whereas lower density levels were found in refrigerated storage rooms, where an average of only of 38 spores m^{-3} was recorded. The level of *Penicillium* on the surfaces of warehouse was generally low, as the density did not exceeded 80 spore m^{-3} in all points tested. *P. digitatum* and *P. italicum* were the most prevalent species detected on citrus fruits, in warehouse and storage rooms.

This work was partially supported by PRIN 2002 prot. 2002075322 "*Pseudomonas syringae* in lotta biologica: meccanismi d'azione e valutazione dei rischi".

CHARACTERIZATION OF A POTATO VIRUS Y ISOLATE FROM *CAPSICUM CHINENSE* CV RED HABANERO. S. Comes[1], A. Fanigliulo[1], R. Pacella[1], G. Parrella [2] and A. Crescenzi[1]. *[1]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, Campus Macchia Romana 3A310, 85100 Potenza, Italy. [2]Istituto per la Protezione delle Piante - CNR, Via Università 133, 80055 Portici (NA), Italy. Fax: +39.097.1205700; E-mail: crescenzi@unibas.it.*

Plants of chili pepper, *Capsicum chinense* Jacquin cv Red Habanero, with leaf mosaic, necrotic rings on the fruits, and stem necrosis were observed in June 2003 in a private garden of Naples (southern Italy). Preliminary serological characterisation showed that these symptoms were associated with the presence of *Potato virus Y* (PVY). This virus was isolated in *Nicotiana tabacum* cv Xanthi NC and characterised biologically and molecularly in its coat protein (CP) gene. Symptoms produced in indicators were generally consistent with those described for PVY, i.e leaf deformation and veinal necrosis in *N. occidentalis*, mosaic in *N. tabacun* cv Xanthi and *Capsicum annuum*, mosaic and epinasty in *Lycopersicon esculentum.* By contrast, the virus induced local symptoms of chlorotic mottling in *Chenopodium* spp.. When the viral isolate was exposed to monoclonal antibodies (Mabs) specific to PVY^N, PVY^C and PVY^O, it reacted only with PVY^C Mabs. RT-PCR was done on viral RNA extracted from purified virions using a couple of primers specifically designed on the CP coding region of PVY^{NP} (*non potato*) isolates. PCR product was cloned in a pCRII-TOPO vector using TOPO-TA cloning kit (Invitrogen) and sequenced. Both similarity and phylogenetic analysis made on the 801 bp long coat CP gene sequence showed that the isolate under study is more closely related to other PVY *non potato* isolates than to those of the PVY^O and PVY^N group. The highest percentage of similarity was detected with PVY isolates from *Solanum nigrum* (PVY-*SON41*), *Capsicum annuum* (PVY-*NNP*) and *Lycopersicon esculentum* (PVY-*LYE84.2*).

BIOCONTROL ACTIVITY OF *BACILLUS* SPP. STRAINS ISOLATED FROM THE RHIZOSPHERE OF VEGETABLE CROPS. L. Cozzolino[1], A. Rufolo[2], A. Zoina[2], A. Popolo[1] and A. Raio[1]. *[1]Istituto per la Protezione delle Piante - CNR, Via Università, 133, 80055 Portici, Italy. [2]ARBOPAVE – Università di Napoli "Federico II", Via Università 100, 80055 Portici, Italy. Fax: +39.081.7755320; E-mail: zoina@unina.it.*

Thirty-nine gram-positive bacteria isolated from the rhizosphere of broccoli, pepper, rosemary, and tomato were tested *in vitro* for their antagonistic activity by the dual culture method against the phytopathogenic fungi *Botrytis cinerea*, *Fusarium oxysporum* f.sp. *lycopersici*, *Pythium ultimum*, and *Rhizoctonia solani.* Sixteen strains that were able to inhibit the radial growth of fungal colonies were spore-forming bacteria. Identification of these bacteria was done with BIOLOG system and PCR-RFLP analysis. A fragment of 749 bp located in the region of the RAPD marker SG-850 (EMBL accession n. AF0361405) specific for *Bacillus cereus* group, was amplified by PCR and digested with *Alu*I restriction endonuclease. Eleven strains were identified as *Bacillus cereus* and one as *Bacillus mycoides* by both identification techniques, while the remaining four strains were identified as *Bacillus badius* (two strains), *B. pumilis* and *B. subtilis* by BIOLOG system, while they were not amplified by PCR. Most of the strains that showed antagonistic activity against the phytopathogenic fungi were able to produce chitinases and diffusible antibiotics and to form biofilm in *in vitro* tests. No siderophore and volatile antibiotic production was detected. A greenhouse trial was made to determine the biocontrol activity of *B. cereus* strain M123 (highly effective *in vitro*) and other putative antagonists belonging to *Enterobacter aerogenes*, *Pseudomonas aureofaciens* and *Pseudomonas fluorescens* species. Antagonists were used as a single strain or as a mixture against *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani*. The *B. cereus* strain was effective in reducing the incidence of infection by both pathogens.

OCCURENCE OF *CITRUS VARIEGATION VIRUS* IN CAMPANIA. A. Damiano[1], M. Malfitano[1], N. Duran-Vila[2] and D. Alioto[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli, Facoltà di Agraria, Via Università 100, 80055 Portici, Napoli, Italy. [2]Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain. Fax: +39.081.2539367*; *E-mail: malfitan@unina.it.*

In spring 2004, severe crinkling of the leaves was observed in the totality of lemon trees (cvs Ovale di Sorrento, Zagara, and Sfusato amalfitano) growing in two orchards of the Sorrentina peninsula (Campania, southern Italy). Symptoms resembled those caused by *Citrus variegation virus* (CVV), a member of the genus *Ilarvirus*, family *Bromoviridae*. Leaves from ten symptomatic trees (eight 'Ovale di Sorrento', one 'Sfusato amalfitano', and one 'Zagara') were collected and analysed by indexing on Arizona 861-S1 "Etrog" citron (*C. medica* L.), DAS-ELISA using a polyclonal antiserum to CVV provided by Drs. S. Garnsey and M. Hilf (USDA-ARS-USHRL), and by RT-PCR with the specific primers CVVa and CVV4 (Bennani *et al*., 2002). All tested samples were CVV-positive with all techniques. To evaluate the distribution of CVV in Campania, groves of other areas (Eboli, Portici, Sorrento, Massa Lubrense) were surveyed and 86 samples from symptomatic and symptomless trees of different citrus species and cultivars were collected and analysed. CVV was detected in one sour orange, three sweet oranges (cvs Valencia and Moro) and five lemons (cvs Zagara, Ovale di Sorrento and Monachello). All mandarins, clementines and grapefruits analysed were CVV free.

CUCUMBER MOSAIC VIRUS INFECTING *THEVETIA NEREIFOLIA* AND *NANDINA DOMESTICA*. S. Davino[1,2], A. Lombardo [1,2], M. Davino[1], A. Bertaccini[3] and M.G. Bellardi[3]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie (DISTEF), Sezione di Patologia Vegetale, Università di Catania, Via S. Sofia 100, 95123 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia, Viale Lancia, 95030 Catania. [3]Dipartimento di Scienze e Tecnologie Agroambientali (DiSTA), Patologia Vegetale, Alma Mater Studiorum-Università di Bologna, Viale G. Fanin 40, 40127 Bologna, Italy. Fax: +39.95.7147379; E-mail: davinowalter@libero.it.*

In June 2004, virus-like symptoms were observed in the foliage of yellow oleander (*Thevetia nereifolia* Juss. *ex* Steud.; family *Apoc-*

ynaceae) and common nandina (*Nandina domestica* Thunb.; family *Nandinaceae*) growing in the Botanical Garden of the University of Bologna (Italy). Yellow oleander showed distortion, malformation and chlorotic mottling of younger leaves, stunting, and reduction in the number of flowers; common nandina exhibited narrowed leaves. Symptomatic leaves from both ornamental shrubs were collected and used for mechanical inoculations to herbaceous hosts. Systemic symptoms induced in *Nicotiana tabacum* "Samsun", *N. bethamiana* and *Capsicum annuum* suggested a possible infection by *Cucumber mosaic virus* (CMV). The presence of this virus was verified by PAS-ELISA using the polyclonal antisera PVAS-30 and PVAS-242a. RT-PCR using primers for CMV movement protein (MP) gene confirmed its presence in yellow oleander and common nandina plants. Both sequences were compared by SSCP assay, and the MP gene was cloned and sequenced. The sequences obtained were compared with those of 16 CMV strains retrieved from GeneBank. Results showed that the two CMV isolates were molecularly similar to each other and closely related to isolate B2 from *Musa sapientum*. Both belonged to CMV subgroup II (95% nucleotide identity). No records of virus infection to *T. nereifolia* apparently exist, thus it appears that this ornamental shrub is a new natural host for CMV. As to *N. domestica*, this is the first record of a natural virus infection of this species in Italy.

MOLECULAR CHARACTERIZATION OF MYCOVIRUSES FROM *BOTRYOTINIA FUCKELIANA*. M.A. De Guido, A. Minafra, A. Santomauro, S. Pollastro, R.M. De Miccolis Angelini and F. Faretra. *Dipartimento di Protezione delle Piante e Microbiologia Applicata Università degli Studi di Bari and Istituto di Virologia Vegetale del CNR, Via Amendola 165/a, 70126 Bari. Fax: +39.080.5442911*; *E-mail: faretra@agr.uniba.it.*

Virus-like particles (VLPs) and double-stranded RNAs (dsRNAs) are commonly detected in *Botryotinia fuckeliana* (de Bary) Whetz. (*Botrytis cinerea* Pers.), the causal agent of grey mould on many cultivated and spontaneous plants. The potential role of such foreign elements in the variation and biology of the fungus is unknown. Previous work showed that strain WS276 of the fungus carries at least four dsRNAs associated with isometric VLPs (40 nm). By using various molecular approaches (RT-PCR, RACE, DOP), the nucleotide sequence of four dsRNA molecules was determined. The largest dsRNA was 5,223 bp in length and showed high similarity with sequences of mycoviruses of the family *Totiviridae* (i.e., 60% with *Helmintosporium victoriae* 190S virus). Computer-assisted analysis disclosed the presence in this dsRNA of two open-reading frames (ORFs), of which the 5'-proximal ORF coded for a putative coat protein (CP), and the 3'-proximal ORF coded for a putative RNA-dependent RNA polymerase (RdRp). Molecular sizes of the other dsRNAs were 1,792 bp (dsRNA1), 1,566 bp (dsRNA2) and 1,382 bp (dsRNA3). dsRNA1 and dsRNA2 had a high similarity level with sequences of mycoviruses of the family *Partitiviridae* (i.e., 75 and 62%, respectively, with *Discula destructiva* virus 1). Each dsRNA contained a single ORF. The dsRNA1 ORF coded for a putative RdRp and dsRNA2 ORF for a putative CP. The dsRNA3 sequence was almost identical to that of dsRNA2 except for a 210-bp deletion inside the coding region. These results suggest that two distinct coexisting viruses occur in the strain WS276 of *B. fuckeliana*.

ESSENTIAL OIL ACTIVITY AGAINST POST HARVEST PATHOGENS. G. Farina, M. Moretti, B. Ratti, M. Saracchi and P. Sardi. *Istituto di Patologia Vegetale, Facoltà di Agraria Università degli Studi di Milano, Via Celoria 2, 20133 Milano. Fax: +39.02.50316781*; *E-mail: marco.saracchi@unimi.it.*

Many plants and their extracts are known to have antifungal activity, thus they are potentially alternative to synthetic fungicides. In this study, the antifungal activity of eight essential oils, extracted from plants belonging to *Geraniaceae*, *Graminaceae*, *Lamiaceae*, *Lauraceae* and *Mirtaceae*, was investigated. Oils were tested against *Botrytis cinerea* and *Penicillium* sp. strains, isolated from strawberry and apple showing postharvest rots. The protocol proposed by Raboso *et al*. (1995) to assess fungicide activity was employed, which allows to test many compounds and concentrations at the same time. The results indicated that all tested compounds inhibited the growth of *B. cinerea* and *Penicillium sp*. The most effective were oils extracted from *Cymbopogon martinii* L. (palmarosa), *Pimenta dioica* L. (allspice), and *Syzygium aromaticum* L. (clove). To verify the actual effect of essential oils on the development of *B. cinerea* and *Penicillium* hyphae, observations with the scanning electron microscope were made on colonies of the pathogens, grown on polycarbonate membranes in the presence of different concentrations of oils. Most of the tested oils caused morphological alterations, both in *Botrytis* and in *Penicillium.* Hyphae appeared generally collapsed, frequently flexuous, and showed surface alterations. Modifications affected mostly hyphal tips that were frequently swollen and malformed. In some cases, an evident increase of branching was observed in proximity of the hyphal tips. The essential oil of palmarosa had a peculiar effect on *B. cinerea* hyphae, which showed heavy swelling and a typical bean-shaped apex.

RELATIONSHIP BETWEEN ENVIRONMENTAL CONDITIONS AND CYPRESS CANKER DISEASE IN TUSCANY BY USING GIS TECHNOLOGY. M. Feducci[1], G. Masi[2] and P. Capretti[1]. *[1]Università degli Studi di Firenze, Dipartimento Biotecnologie Agrarie, Sezione Patologia Vegetale, Piazzale delle Cascine 28, Firenze, Italy. [2]Università degli Studi di Firenze, Dipartimento di Ingegneria Agraria e Forestale, Piazzale delle Cascine 28, Firenze, Italy. Fax: Fax: +39.055.3288273*; *E-mail: paolo.capretti@unifi.it.*

The relationship among climatic and spatial data and the occurrence of cypress canker by *Seiridium cardinale* were recently studied in Tuscany to produce disease-risk maps. To this aim, a survey was conduced near Florence, following a transect of about 60 km along national roads, from Greve in Chianti, to Firenzuola in Mugello. During the survey a total of 6936 cypress trees were inspected. Using GIS technology, maps of canker disease were produced displaying exposure, elevation, and slope by Arcview software. Infected trees were 201. In 48% of the cases old cankers were present in the higher parts of the canopy, probably due to old infections. The highest number of damaged trees occurred on "sunny exposures" (S-E and S-W) at an elevation of about 400 to 500 m a.s.l., and on "colder exposure" (E-NW) under 300 m of elevation. At a multiple regression analysis, these relationships (elevation/exposure and plants/exposure/solar) proved to be statistically significant ($P<0.05$). These data, which show fungal preference for temperate areas, may represent an important tool for the management of diseased areas.

PROTEOME ANALYSIS OF THE INTERACTION AMONG *TRICHODERMA*, LETTUCE AND PATHOGENIC FUNGI. S. Ferraioli, P. Ambrosino, I. Soriente, M. Ruocco, S. Woo, F. Scala and M. Lorito. *Dep. Ar.Bo.Pa.Ve. - Plant Pathology, University of Naples, CNR-IPP, Via Università 100, Portici, Napoli, 80055 Italy. Fax: +39.081.2539339; E-mail: lorito@.unina.it.*

Fungi of the genus *Trichoderma* are among the most effective bio-pesticides used for controlling diseases caused by pathogenic fungi to vegetable crops. Molecular activation of specific genes in

the antagonist occurs during its interaction with pathogen and plants. The spectrum of enzymes involved in biological control activity includes glucanases, chitinases, lipases and proteases. In order to identify and characterize new biocontrol proteins, we produced proteomic maps of the *in vivo* interaction among *T. harzianum* (strain T22), *Lactuca sativa* and the pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. To extract intracellular proteins (ICs) from the antagonist's mycelium during the three-component interaction, *T. harzianum* was grown in the presence of lettuce seedlings immersed in water agar and by separating the plants from the mycelium with a cellophane membrane; the fungal pathogens were also included in the system. Extracellular proteins (ECs) were extracted from culture filtrates obtained by co-culturing plant, pathogen and antagonist in a two phase (solid-liquid) system. ICs and ECs proteins were analysed by two-dimensional electrophoresis (2-DE) and qualitative and quantitative differences were detected. Spots corresponding to proteins differentially expressed were identified by matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry (MS).

ENHANCERS OF THE BIOCONTROL ACTIVITY OF *TRICHODERMA* SPP.. S. Ferraioli[1], I. Soriente[1], V. Fogliano[2], M. Ruocco[1], S. Woo[1], F. Scala[1] and M. Lorito[1]. *[1]Dep. Ar.Bo.Pa.Ve. - Plant Pathology, University of Naples, CNR-IPP, Via Università 100, 80055 Portici (NA), Italy. [2]University of Naples "Federico II", Dept. Food Science, Via Università Parco Gussone, 80055 Portici, Italy. Fax: +390812539339; E-mail: lorito@.unina.it.*

Fungi of the genus *Trichoderma* are biological control agents able to parasitize pathogenic fungi and are commonly used against a variety of plant diseases. During the interaction among antagonist, plant and pathogen, molecules released by the pathogen and the plant are detected by the antagonist and may induce the biocontrol gene expression cascade. To isolate and characterize these molecules acting as 'biocontrol inducers', we obtained different culture filtrates (CFs) from co-cultures of *T. atroviride*, strain P1, and different plant or pathogen tissues. Fractions of these CFs with a molecular weight lower than 3 kDa were tested on *Trichoderma* mutants containing the GFP gene and the gene coding for a glucose oxidase under the control of biocontrol-related promoters (*ech42* and *nag1*). The best induction of endochitinase and exochitinase enzymes was given by the fractions obtained from CFs of *T. atroviride* grown in salt medium added with *R. solani* cell walls, tomato plant extracts and tomato live plants. The inducing molecules obtained under these conditions, when added to *T. atroviride* cultures, stimulated the production of antibiotics and metabolites that reduced *Botrytis cinerea* and *Alternaria alternata* spore germination. These inducers assayed *in vivo* were also able to significantly reduce disease symptoms caused by *B. cinerea* in tomato leaves by enhancing the biocontrol effect of strain P1. To identify the molecules acting as 'biocontrol inducers', HPLC analysis of the samples is in progress.

A BACTERIAL DISEASE OF SAFFRON CAUSED BY *BURKHOLDERIA GLADIOLI* AND *PSEUDOMONAS* SPP.. M. Fiori, S. Virdis and A. Schiaffino. *Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; E-mail: fiorim@uniss.it.*

Symptoms of a disease of possible bacterial origin were observed in the leaves and corms of some saffron plots in Sardinia since October 2003. The leaves had reddish brown spots, that were either isolated or coalescing and were surrounded by a large chlorotic halo. The corms had yellowish-brown rounded spots, which tended to become black with time. Isolation on nutrient agar from corms and leaves produced numerous cream to yellowish-brown and white-cream bacterial colonies mixed with others characterized by a yellow hue. Based on their frequency, ten colonies were selected, four of which were cream to yellowish-brown, four white-cream, and two yellowish. In pathogenicity tests, only four of ten isolates reproduced the symptoms on saffron leaves and corms, and three could also infect tomato plants. These four virulent isolates were identified with conventional tests and the computerised system BIOLOG. One isolate was identified as *Burkholderia gladioli*, one as *Pseudomonas corrugata* while the remaining two were *P. fluorescens* biotype F. The latter three isolates reacted positively with a serum to *P. corrugata* anti IPVSS-6FP. Fatty acid profiles of two representative isolates confirmed that one was *Burkholderia* sp., and the other *Pseudomonas* sp. (*P. fluorescens/P. marginalis/P. putida*). We suggest that isolates of *B. gladioli* and *Pseudomonas* spp. are responsible for the saffron disease found in Sardinia. *B. gladioli* has only been reported once in the literature. Genomic characterization of the other three pseudomonads is underway.

BIOCONTROL OF *VERTICILLIUM DAHLIAE* ISOLATED FROM OLIVE TREES BY *TRICHODERMA* SPP.. L. Flamini[1], A. Dirotti[2], A. Prodi[3], P. Nipoti[3], M. Sportelli[2] and L. Pizzichini[1]. *[1]ASSAM - Agenzia Servizi Settore Agroalimentare Marche - Servizio Fitosanitario Regionale, Via Alpi 21, 60131 Ancona, Italy. [2]C.D.F. s.r.l., Via Amendola 40, 48022 Lugo, Ravenna, Italy. [3]Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi di Bologna, Italy. Fax: +39. 051.2096722; E-mail: flamini_lucio@assam.marche.it.*

In the year 2004 *Verticillium dahliae*, a fungus causing a severe vascular disease of horticultural and woody plants, was isolated from declining olives in groves of Ascoli Piceno (Marche region, central Italy). During investigations on *V. dahliae* done by ASSAM, preliminary *in vitro* trials were carried out to determine the potential biocontrol activity of three *Trichoderma* isolates against this fungus. Two *Trichoderma* strains were isolated from symptomless olive roots in the same groves where *V. dahliae* was found. Both *Trichoderma* strains were observed under a light microscope and classified as *T. viride* and *T. harzianum* following the taxonomic key of Rifai. Another *Trichoderma* strain was isolated from a compost used for *Agaricus bisporus* cultivation and it was characterized by molecular techniques (PCR, amplicon cloning and sequencing) to verify whether it was the non aggressive biotype (Th1-*T. harzianum* complex). In a dual plate test, all *Trichoderma* strains showed biocontrol activity against *V. dahliae*, by reducing radial growth, as compared with the control. Although they all affected *V. dahliae* growth, their mode of action showed differences in terms of hyphal lyses and mycoparasitism.

NATURAL INFECTION PERIODS OF *DIPLODIA CORTICOLA* IN A DECLINING CORK OAK FOREST. A. Franceschini, B.T. Linaldeddu and F. Marras. *Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; E-mail: afran@uniss.it.*

Diplodia corticola is the causal agent of cankers, vascular necrosis, and dieback on various oak species. This fungus has been frequently associated with oak decline in Mediterranean countries, although many epidemiological and pathogenic aspects

of its relation with the host are still to be clarified. The results are reported of a study carried out in a declining cork oak forest, in order to investigate the dynamics of natural infections by *D. corticola*, the climatic predisposing factors, and the organs that are infected. Groups of 15 seedlings of 2-year-old cork oak were exposed every month for a year to natural inoculum of the pathogen. Two months after exposure, seedlings were examined for the presence of the fungus in the leaves, buds, trunk and roots. Infections occurred only in six months, mainly May, September, and December, with the highest frequency in the trunk. This aspect becomes relevant in the *D. corticola - Quercus suber* pathosystem, since cork oak trees undergo cork extraction, which exposes a large trunk surface to the pathogen. No significant relationships were observed between incidence of infections and meteorological parameters (temperature, relative humidity, and rainfall) recorded in the forest. In *in vitro* tests, the optimum temperature for mycelial growth and conidial germination of *D. corticola* was 25°C. This fungus, therefore, is a mesophilic microorganism, even though capable of infecting the wood also in December, with temperature ranging between 6 and 14°C.

A MULTIPLE APPROACH TOWARDS SOIL-BORNE DISEASE BIOCONTROL. S. Galletti, P.L. Burzi, C. Cerato, S. Marinello and E. Sala. *Consiglio per la Sperimentazione e Ricerca in Agricoltura (C. R. A.) - Istituto Sperimentale per le Colture Industriali, Via Corticella 133, 40129 Bologna, Italy. Fax: +39.051. 6316856; E-mail: c.cerato@isci.it.*

The difficulty encountered in controlling soil-borne diseases, even by chemicals, is probably due to the complicated ecosystem of the soil, where a number of interactions occur. The adoption of correct agronomic practices normally prevents damages, but in the presence of favourable conditions diseases spread rapidly almost without any possibility of control, apart from methods with high environmental impact, like soil fumigations. Milder alternatives are represented by biological methods but the awareness of their moderate effectiveness suggests combining some of them in a multiple integrated approach. In this view, methods aimed at lowering pathogen inoculum in the soil could be envisaged at different levels: (i) directly, by green manure of biocidal crops, like *Brassicaceae*, which exert a natural fumigating effect by releasing fungitoxic compounds; (ii) indirectly, by treating *Brassicaceae* seeds with a selected biological control agent like *Trichoderma*, for facilitating the establishment of the antagonist, so as to enhance soil suppressiveness, and provide a source of inoculum for the subsequent crop; (iii) indirectly, on the crop, by treating seeds with *Trichoderma* strains selected for rhizosphere competence and antagonistic effectiveness towards the pathogens. Data are reported from preliminary experiments carried out in greenhouse and field, towards soil-borne pathogens of sugar beet according to the above mentioned approaches

FIRST REPORT OF *MYCOSPHAERELLA* SP. ASSOCIATED TO GUM SPOTS OF CITRUS LEAVES IN ITALY. S. Grasso, A. Catara and F.M. Grasso. *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. Fax: + 39. 095.7147287; E-mail: cataraan@unict.it.*

Gummy spots of citrus leaves have been reported from different countries. In some cases the disease is associated with fungal infection. In Japan, it was shown that *Mycospaerella horii* is the causal agent of certain spotting conditions, whereas in Florida *M. citri* was shown to induce "greasy spots". During the years 2004 and 2005, which were characterized by unusually abundant spring rains in southern Italy, many citrus groves suffered extensive shedding of mature leaves (of the previous year) that showed typical greasy spots on the lower page. Sweet orange, lemon, grapefruit, mandarin, and mandarin hybrids were heavily affected in some areas of Sicily and Calabria (southern Italy). Defoliation reduced fruit size and spring blooming, and induced many growers to treat with fungicides. Old yellow leaves showing severe symptoms collected from the soil or from the lower canopy and incubated under high humidity, showed groups of perithecia developing from the spots, which contained asci morphologically similar to those of the genus *Mycospaerella*. Corn or potato agar culture of gummy mesophyll tissues yielded slow growing brown-greenish colonies, bearing usually multiseptate conidia (sometime deprived of septa), similar to those of the genus *Cercospora*. The high frequency of the association of this fungus with typical greasy spot symptoms, the early shedding of affected leaves, the cyclical appearance of symptoms during autumn, are consistent with the hypothesis of a mycological aetiology of the disease. Pathogenicity tests are in progress.

SUSCEPTIBILITY OF CITRUS SEEDLINGS TO *FUSARIUM SOLANI* INOCULATION. V. Grimaldi, V. Coco and A. Catara. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. Fax: + 39. 095.7147287; E-mail: virgilio.grimaldi@unict.it.*

The complex species *Fusarium solani* is associated with dry root rot disease of citrange, as well as other citrus rootstocks. In a large number of experiments, the success of inoculation attempts was heavily affected by plant age, inoculum preparation, and inoculation protocol. Maize meal-sand fungus cultures and the inoculation of perlite-grown young citrange seedlings, turned out to be the most effective. Three *F. solani* isolates, i.e. 1A and R2B (recovered from Troyer citrange roots) and 2B (from sour orange) proved to be pathogenic to citrange seedlings. After 36 days, inoculated seedlings showed root rot, sometime browning of the apex and central region of the roots and of the crown, reduced growth of roots and stem up to -21.4%. No wilting or scorching were observed on the shoots. All symptomatic root apexes were infected. Five different citrus species, including sour orange, sweet orange, Troyer citrange, Parson special mandarin and *Poncirus trifoliata*, were sown in pots containing soil added with maize meal-sand or rice inoculum of isolate 1A. Inoculation resulted in detrimental effects consisting of lower number of germinating seeds and reduced growth of stem and roots.

PRELIMINARY RESULTS OF THE EFFECT OF AGRONOMIC PRACTICES ON EPIPHYTIC BACTERIAL POPULATIONS OF OLIVE. N.S. Iacobellis[1], P. Lo Cantore[1], M. Sileo[1], G. Celano[2] and C. Xiloyannis[2]. *[1]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali and [2]Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. Fax: +39.0971.205702; E-mail: iacobellis@unibas.it.*

In the framework of the European project "Olivero", an experimental olive grove (cv Maiatica) apparently unaffected by olive knot disease was divided in two parts. One, called "organic", was managed by cover cropping and compost amendment and the second, called "tillage", was managed by soil tillage and burning of pruning material. Foliar fertilization at the flowering

and olive stone hardening stages was administered to both areas. In each area, three olive plants in the erosion and accumulation zones, as well as in the zone with a highly active erosive process were selected. Periodically, leaves in the median part of one-year-old twigs were collected and, after washing under agitation, aliquots of the liquid were distributed either on KB or on the semi-selective *Pseudomonas savastanoi* pv. *savastanoi* medium ANSS. The majority of the epiphytic bacteria were Gram-positive and most of them in dual agar plate assays inhibited the growth of *P.s.* pv. *savastanoi*. No bacteria with the feature of *P.s.* pv. *savastanoi* were isolated. In general the epiphytic population density varied with the seasons being higher in late spring (May-June) and lower in late winter (February). In late spring the highest population densities were observed in the accumulation zone where the level of available mineral nutrients was higher. In late winter, the lowest population densities were observed in the accumulation zone of the "tillage" area, though no difference in the nutritional state of the trees between the two areas was observed. Apparently other as yet unidentified factors are responsible for the above effect.

INDUCTION OF RESISTANCE IN SWEET CHERRY FRUITS TREATED WITH SALTS AND *AUREOBASIDIUM PULLULANS*. A. Ligorio, F. Nigro, L. Schena, I. Pentimone and A. Ippolito. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: ippolito@agr.uniba.it.*

Enzymatic activity of chitinase, ß-1,3-glucanase, peroxidase and phenylalanine ammonia-lyase (PAL) in sweet cherries cv Ferrovia and cv Moreau treated with $CaCl_2$, Na_2CO_3 and *Aureobasidium pullulans* (De Bary) Arnaud, strain L47, was assessed. Fruits were individually wounded, treated with a solution of the above salts (2% w/v) or with an aqueous suspension of L47 ($2 \cdot 10^8$ cells ml^{-1}) and kept at 24±1°C. At various time intervals (0, 12, 24, 48, 72, 96 h) tissue samples were collected and total proteins extracted. Fruit wounded and treated with sterile distilled water and unwounded fruit served as controls. In tissues from sweet cherries cv Ferrovia calcium chloride caused the highest increase of endochitinase, peroxidase, and PAL activities, reaching maximum levels 24, 72 and 48 h after treatment, respectively. L47 confirmed its ability to increase the production of ß-1,3-glucanase, chitinase, and peroxidase in host tissue; moreover, in cv Moreau the antagonist enhanced PAL activity as compared to the other treatments. Sodium carbonate did not influence clearly the biochemical response of treated fruits. N-acetyl-ß-D-glucosaminidase activity was not influenced by any treatment. Among the constitutive isoforms of chitinase, polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of additional isoforms of endochitinase in tissues of both cultivars treated with $CaCl_2$ and L47 at different time intervals. In conclusion, beyond the well known activity in enhancing the stability and the integrity of plant cell wall and the inhibition of pectinolytic enzymes of pathogens, calcium, as well as the antagonist L47, is also able to enhance the activity of enzymes involved in host resistance.

CHARACTERIZATION OF HYDROLYTIC ENZYMES PRODUCED BY *AUREOBASIDIUM PULLULANS* STRAIN L47. A. Ligorio, L. Schena, I. Pentimone, F. Nigro and A. Ippolito. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: ippolito@agr.uniba.it.*

The yeast-like fungus *Aureobasidium pullulans* (De Bary) Arnaud, strain L47, isolated from the surface of table grape berries, was able to control postharvest decay of several fruits. Its mechanisms of action have been partially investigated. The production of fungal cell wall-degrading enzymes was suggested to play a role in the antagonist-pathogen interaction. The present study showed that strain L47 was capable of producing the hydrolytic enzymes ß-1,3-glucanase and chitinase. In *in vitro* assays, *A. pullulans* showed an increasing production of endo-ß-1,3-glucanase and endo-chitinase, reaching a maximum level after 96 h and 72 h, respectively. Eso- and endo-ß-1,3-glucanase as well as eso- and endo-chitinase displayed optimal activity at 40°C and at pH 4.0-5.0; their activity was strongly inhibited by $HgCl_2$ and enhanced by the presence of Ca^{+2}, Zn^{+2}, and Mg^{+2}. Polyacrylamide gel electrophoresis (SDS-PAGE) showed the presence of an isoform of endochitinase, with a molecular mass of about 70 kDa, and six isoforms of endo-ß-1,3-glucanase, with molecular masses of 200, 120, 50, 45, 34 and 27 kDa, respectively. By testing the effect of total protein extracts obtained from a culture filtrate of L47 on germination and germ tube elongation of conidia of *Botrytis cinerea*, inhibition of 15% and 7%, respectively, at the lowest concentration (0.98 µg ml^{-1}), was found. No germination was observed at the highest concentration tested (9.8 µg ml^{-1}).

SUPPRESSION OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIA IN THE RIZOSPHERE OF OLIVE AND EGGPLANT BY COMPOST FROM OLIVE OIL BY-PRODUCTS. G. Lima, F. De Curtis and V. De Cicco. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Sezione di Patologia Vegetale, Via De Sanctis, 86100 Campobasso, Italy. Fax: +39.0874.404855; E-mail: lima@unimol.it.*

Olive oil by-products composted in a pilot scale olive plant were characterized and assayed for their suppressive activity against different fungal plant pathogens. Some cured composts consistently reduced the *in vitro* growth of *Verticillium dahliae* and other important fungal pathogens. In two-year experiments with olive and eggplant grown in pot with soil artificially contaminated with *V. dahliae* microsclerotia, the incorporation of 15% (w/w) of a cured compost alone or in combination with the biofungicide TV1 (*Trichoderma viridae*), significantly reduced the density of microsclerotia in the soil as well as *V. dahliae* root infection with respect to the untreated controls. The suppressive activity of the composts seems mainly caused by the beneficial residual microbial population selected during the composting process. In fact, the efficacy of the composts decreased or disappeared when it was autoclaved before use. Several bacteria and fungi, isolated and partially characterized from these composts, showed a highly antagonistic *in vitro* activity against *V. dahliae* and other fungal pathogens. The results of our investigations indicate that composted olive by-products are very promising for applications aimed at controlling fungal pathogens of different crops in organic and integrated agriculture systems.

PHYSIOLOGICAL RESPONSES OF CORK OAK AND HOLM OAK TO THE INFECTIONS OF PATHOGENS INVOLVED IN OAK DECLINE. B.T. Linaldeddu[1], C. Sirca[2], D. Spano[2] and A. Franceschini[1]. *[1]Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale and [2]Dipartimento di Economia e Sistemi Arborei, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; E-mail: ben@uniss.it.*

The endophytic fungi *Biscogniauxia mediterranea*, *Diplodia corticola*, *Discula quercina* and *Pleurophoma cava* are involved in the aetiology of oak decline in the Mediterranean area. Several studies have proved their pathogenicity, but little information is available

on the physiological response of the host to their infection. The aim of this study was to investigate the changes in net photosynthesis and stomatal conductance values in 3-year-old cork oak and holm oak seedlings growing in natural conditions and inoculated with these pathogens. Throughout the three-month experimental period, the evolution of symptoms and the physiological variables were recorded every 10 days. In particular, gas exchange measurements were taken on four mature leaves from each plant exposed to sun, using an ADC 2250 infrared gas analyzer. All pathogens induced necrotic lesions around the site of infection; however, the lesions caused by *D. corticola* were significantly wider in both oak species. Significant and gradual reductions in net photosynthesis and stomatal conductance values were recorded on all cork oak seedlings inoculated. In the plants infected by *P. cava,* these physiological changes appeared just 20 days after inoculation. On holm oak physiological variables were significantly modified only by *D. corticola* and *D. quercina* infections. Seedlings inoculated with *D. corticola*, showed a significant decrease in photosynthetic rate (-87%) and stomatal conductance (-80%) after two months. These results, confirm on the one hand the aggressiveness of the pathogens tested and, on the other, show the different physiological responses of the two host species to infection.

EFFECT OF PHOSPHITE ON *PHYTOPHTHORA* SPECIES ASSOCIATED WITH WALNUT DECLINE AND DEATH. M. Maccaroni, A. Coramusi, M. Galli and A. Belisario. *C.R.A. - Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. Fax: +39. 06.82070316; E-mail: a.belisario@ispave.it.*

The efficacy was tested of phosphite in reducing growth and viability of *Phytophthora cinnamomi*, *Phytophthora cryptogea*, and *Phytophthora nicotianae* isolates obtained from declining and dead English walnut trees. Three isolates of *P. cinnamomi* from different sites were used, since *P. cinnamomi* is the most destructive and widespread species. Different phosphite concentrations added to CMA were tested *in vitro* (i.e. from 2 to 16 ppm and from 1,250 to 20,000 ppm) to determine the dose at which growth and/or viability were compromised. *Phytophthora* species showed a different susceptibility to phosphite doses. *P. nicotianae* was the most susceptible with a significant reduction of growth at 2 ppm, and death at 1,250 ppm. *P. cryptogea* was indifferent to all the low doses, but was killed at 1,250 ppm. *P. cinnamomi* isolates showed a different response, as the isolate coming from Venice was the only one able to grow at 1,250 and 2,500 ppm. Viability of *P. cinnamomi* isolates ceased at 20,000 ppm. The effects of phosphite were also studied on artificially inoculated English walnut logs 1 m long. Only *P. cinnamomi* isolates were used. After 14 days from wound inoculation, logs were dipped 1, 2, 3, 5 min at the dose of 2,500 ppm, and 30, 60, 90, 150 sec at the dose of 5,000 ppm. The dose of 5,000 ppm reduced progressively the dimensions of cankers with the increase of dipping time. Reisolations from the margins of lesions gave a significant reduction of the number of colonies with the increase of dipping time.

ANTAGONISTIC INTERACTIONS BETWEEN FUNGAL ENDOPHYTES AND PATHOGENS INVOLVED IN OAK DECLINE. L. Maddau, B.T. Linaldeddu and A. Franceschini. *Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; E-mail: lmaddau@uniss.it.*

The endophytic communities of oak trees include many saprophytic fungi widespread in nature, some species with antagonistic capability and others showing pathogenetic activity. Such species establish interactions, the knowledge of which is of great importance for understanding their ecological role and detecting potential biocontrol agents. The aim of the present work was to investigate the *in vitro* competitive interactions between 22 endophytic fungi isolated from *Quercus* spp. and three of the main pathogens involved in oak decline: *Biscogniauxia mediterranea*, *Diplodia corticola* and *Discula quercina*. Using dual-plate method, eight fungi, *Bionectria solani*, *Dictyochaeta parva, Penicillium* sp., *Sporormiella minima*, *Trichoderma asperellum*, *T. fertile*, *T. harzianum*, and one yeast-like fungus were selected on the basis of their antagonism index evaluated according to Badalyan *et al.* (*Phytopath. Medit.* 41: 220-225, 2002). These fungi showed a strong competitive ability against all three pathogens, through deadlock at distance with a clear zone of inhibition, and/or overgrowth with complete replacement. The hyphae of the pathogens near the inhibition zone showed irregular growth and severe morphological alterations such as extensive vacuolation, chlamydospore formation, and plasmolysis. Further assays demonstrated that the organic extracts from liquid cultures of *D. parva*, *S. minima* and *T. fertile* possessed toxic activity. In particular, they significantly inhibited mycelial growth of all three pathogens and the germination of conidia of *D. corticola*. These results provide useful guidelines for the development of natural biocontrol strategies against the pathogens involved in oak decline.

SCREENING FOR IDENTIFICATION OF *TRICHODERMA* STRAINS AS POSSIBLE INDUCERS OF SYSTEMIC RESISTANCE. S. Marinello, P.L. Burzi, S. Galletti and C. Cerato. *Consiglio per la Sperimentazione e Ricerca in Agricoltura (C. R. A.) - Istituto Sperimentale per le Colture Industriali, Via Corticella 133, 40129 Bologna, Italy. Fax: +39.051.6316856; E-mail: c.cerato@isci.it.*

Trichoderma spp. are fungi that occur in nearly all agricultural soils and in other environments, such as decaying wood. They have received considerable attention as potential biological control agents for a number of pathogens. Their action seems due to different mechanisms like antagonism, antibiosis, mycoparasitism, induction of defence responses, and other adjunctive mechanisms, which could positively affect plant response to diseases, such as growth promotion. Chitinases are a class of pathogenesis related proteins associated with induced resistance responses in plants. Seven *Trichoderma* strains were screened for their different capability of chitinase induction in sugarbeet. The screening was performed under greenhouse conditions with sugarbeet plants at the six-leaf stage. Two leaves per each plant were treated twice at one-week interval with homogenized *Trichoderma* liquid culture. Treated plants with a chemical inducer of resistance (acibenzolar-S-methyl) and untreated plants were used as controls. Two and seven days after treatment, total proteins were extracted. Chitinases were detected by a sensitive method on chitin agar plates. Using acid-swollen chitin (glycol chitin) as substrate followed by staining with calcofluor white, dark haloes were observed around the wells containing protein extracts under UV light. Halo areas were measured by Quantity One software (Biorad Laboratories, Hercules, CA, USA). Only one *Trichoderma* strain induced a chitinase level in plants higher than the others not differing from that produced by the chemical. These results suggest that this fungal strain should be further investigated in view of its possible use as resistance inducer in sugarbeet.

IDENTIFICATION OF THE MOLECULAR FACTORS INVOLVED IN THE INTERACTIONS AMONG THE BIOCONTROL AGENT *TRICHODERMA ATROVIRIDE*, PLANTS AND FUNGAL PATHOGENS BY A PROTEOMIC APPROACH. R. Marra, V. Cotarelli, P. Scisciola, P. Ambrosino, M. Ruocco, F. Scala, S.L. Woo and M. Lorito. *Dipartimento Ar.Bo.Pa.Ve., Sezione di Patologia Vegetale, Università di Napoli, and CNR-IPP, Via Università 100, Portici (NA), 80055 Italy. Fax: +39.081.2539339; E-mail: lorito@.unina.it.*

Biocontrol agents, such as *Trichoderma* spp., may influence the fate of plant-pathogen interactions by inducing defence mechanisms in plant and limiting directly or indirectly the pathogen attack. In this work we performed bioassays to analyse the differences of proteomes of bean plants interacting with the pathogens *Botrytis cinerea* and *Rhizoctonia solani*, and with the antagonist *T. atroviride* strain P1. Proteins extracted from the antagonist, the pathogen, and the host during their interactions between two, or among three components, were separated using 2D-gel electrophoresis. Many differential proteins, potentially involved in the interactions, were identified using the PD-QUEST software and characterised via MALDI-TOF MS and in silico analysis. Results can be summarised as follows: (i) in bean plant proteome different defence- and pathogenesis-related proteins were induced by the antagonist and the two pathogens; (ii) in *Trichoderma* proteome, besides the metabolic and stress-related proteins, an ABC transporter, different cyclophilins and a hydrophobin were differentially expressed as compared to the control; (iii) in *B. cinerea* proteome virulence factors and defence proteins accumulated. Data obtained demonstrate the usefulness of this approach for the identification of molecular determinants involved in the three-component interactions. This study is, to our knowledge, the first report that analyses the complex factors that play a role during the cross-talk between plants, fungal pathogens and antagonistic fungi.

EFFECTS OF RECYCLED SUBSTRATE ON THE INCIDENCE OF *FUSARIUM OXYSPORUM* F.SP. *RADICIS LYCOPERSICI* IN SOILLESS GROWN TOMATO. A. Minuto, G. Minuto and A. Garibaldi. *Centre of Competence for the innovation in the agro-environmental sector (AGROINNOVA) - Università degli Studi di Torino, Via L. Da Vinci 44, 10095 Grugliasco, Torino, Italy. Fax: + 39.0182.55494; E-mail: andrea.minuto@unito.it.*

One of the most damaging soil-borne diseases of tomato is Fusarium crown and root rot caused by *Fusarium oxysporum* Schlect f.sp. *radicis-lycopersici* Jarvis & Shoemaker. In closed soilless systems the disease is reported to cause serious problems. Preliminary studies carried out in the framework of a national research project involving five research units, showed the presence of suppression against *F. radicis lycopersici* in tomato in recycled substrates sampled from several old closed soilless systems. The suppressiveness seems to be related to substrate composition, the previous cropping history, and the resident microflora. By adopting perlite as substrate and drip irrigation as nutrient solution delivery system, inoculated steamed and non-steamed substrates were suppressive of disease development in tomato seedlings (cv Cuor di bue), while never-used steamed and non-steamed inoculated perlite incited an high disease incidence. Similar results were obtained by testing perlite samples obtained from a tomato crop managed with a sub-irrigation system. By adopting rockwool as substrate and drip irrigation as nutrient solution delivery system, non-steamed inoculated recycled substrates showed a consistent suppressiveness against *F. radicis-lycopersici*. On the contrary, steamed inoculated rockwool seemed conductive to Fusarium crown and root rot, particularly when coming from slabs irrigated with nutrient solution with low electric conductivity (2-3 mS/cm).

DETERMINATION OF THE BIOCONTROL POTENTIAL OF *MONOSPORASCUS CANNONBALLUS* IN EMILIA ROMAGNA AND REPORT OF THE ANTAGONISTIC ACTIVITY OF A WILD STRAIN OF *FUSARIUM OXYSPORUM*. A. Mirotti, M. Sportelli and S. Gennari. *C.D.F. s.r.l., Via Amendola 40, 48022 Lugo (RA), Italy. Fax: +39.0545.35320; E-mail: cdf@cdflugo.it.*

Root rot and decline, caused by *Monosporascus cannonballus* Pollack & Uecker, is a yield-limiting disease in many muskmelon and watermelon growing areas. In the course of a four-year investigation (1999-2002) financed by the regional authorities (L.R. 28/98) of the Emilia Romagna region (northern Italy), a preliminary monitoring was conducted to look for *M. cannonballus*, in view of the conduction of subsequent trials aimed at testing in solarized open field the antagonistic activity of biocontrol agents against root rot and decline and at evaluating rootstock responses to sudden wilt, so as to identify a potential resistant rootstock line. Monitoring was done in sixteen farms. *M. cannonballus* was found in the first year of monitoring in five farms of the Bologna, Ferrara, and Reggio Emilia districts on melon (four farms) and watermelon plants (one farm). Tolerance to the disease of commercial melon cultivars and watermelon rootstocks was experimentally assessed. A pumpkin (RS841) exhibited a significant reduction of wilt incidence. These results were confirmed with a similar experiment in the Marche region (central Italy). In a field naturally infested with *M. cannonballus,* the biocontrol efficacy was tested of *Trichoderma harzianum* and *Trichoderma viride* strains, and of a non-pathogenic wild strain of *Fusarium oxysporum* (FO 47). Both *Trichoderma* species confirmed their alleged efficacy as biocontrol agents. FO 47 showed a higher antagonistic activity, which led to a significant reduction of disease severity. This seems to be is the first time that *F. oxysporum* is shown to have a potential for the control of root rot and decline of muskmelon plants.

***CERCOSPORA BETICOLA* SENSITIVITY TO TRIAZOLES AND STROBILURINES. M. Moretti, M. Saracchi and G. Farina.** *Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133, Milano. Fax: +39.02.50316781; E-mail: maddalena.moretti@unimi.it.*

A study was conducted during 2004 in two different localities of northern Italy (Torremenapace, Pavia and Carbonara di Po, Mantova) to assess the sensitivity of *Cercospora beticola* towards fungicide treatments with triazoles and strobilurines. In each locality four sugarbeet plots were defined and exposed to different fungicide treatment protocols: (i) no treatment (control); (ii) three treatments with Spyrale (difenoconazole 100 g l^{-1} and fenpropidin 375 g l^{-1}); (iii) three treatments with Amistar (azoxystrobin 23,2%) and (iv) three treatments with Sphere, a mixture of the triazole cyproconazole, at 80 g l^{-1} and the strobilurine trifloxistrobin, at 187.5 g l^{-1}. Fungal isolates collected from fields treated with Spyrale showed no variation in their sensitivity to this fungicide, both in Torremenapace and Carbonara di Po, while Amistar and Sphere treatments slightly reduced the sensitivity of *C. beticola* towards azoxystrobin. In Carbonara di Po, where cercosporiosis usually gives earlier and heavier symptoms, *C. beticola* sensitivity to both Spyrale and Amistar appeared generally lower than in Torremenapace. Results showed that the mix-

ture of fenpropidin and a triazole can protect sugarbeet from the build-up of resistance against these fungicides. Since repeated use of strobilurines can determine loss of their activity, it is of paramount importance to design suitable anti-resistance strategies in *Cercospora beticola* leaf spot control.

PRELIMINARY RESULTS OF CLONING AN *ARAC* GENE FROM *PSEUDOMONAS AVELLANAE*. **C. Moretti[1], M. Scortichini[2] and R. Buonaurio[1].** *[1]Dipartimento Scienze Agrarie e Ambientali, Università degli Studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy. [2]C.R.A. Istituto Sperimentale per la Frutticoltura, Via di Fioranello 52, I-00040 Ciampino aeroporto, Roma, Italy. Fax: +39.081.8506206; E-mail: mscortichini@yahoo.it.*

In the past decades, severe epidemics of bacterial canker and decline of hazelnut (*Corylus avellana* L.), caused by *Pseudomonas avellanae* (Psallidas) Janse *et al*., have been reported in northern Greece and central Italy. Greek and Italian strains of the bacterium belong to two separate populations, which can be discriminated by monoclonal antibodies raised against the cell wall polysaccharides and by rep-PCR analysis, using ERIC primer sets. To further characterize at the molecular level the two populations of the bacterium, a specific 700 bp fragment of the Greek strains (BPIC 631), obtained with ERIC-PCR, was cloned in the pCR®4-TOPO vector and sequenced. Its deduced aminoacidic sequence, compared with those from GenBank, showed high similarity with proteins of the AraC-XylS family, which are positive transcriptional regulators involved in the control of many important processes related to sugar catabolism, responses to stress, and pathogenesis. In addition, this sequence showed the characteristic helix-turn-helix motif of the AraC-XylS family in the N-terminal region, when analysed by PROSITE, a database of protein families and domains. Experiments are in progress to clone the complete *araC* gene of *P. avellanae* and to verify if it is present in both populations of the bacterium.

***IN VITRO* TESTS ON THE ANTAGONISM OF ENDOPHYTIC FUNGI FROM *QUERCUS* SPP. TOWARDS *BISCOGNIAUXIA MEDITERRANEA*.** **M. Nasini, A. Mazzaglia and N. Anselmi.** *Dipartimento di Protezione delle Piante, Università della Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy. Fax: +39.0761.357473; E-mail: anselmi@unitus.it.*

Oak woods are cyclically interested by a severe decline. In the Mediterranean area a critical role is assigned to the "weakness pathogen" *Biscogniauxia mediterranea* which causes bark necrosis on stressed plants after an unpredictable endophytic phase. To investigate the possibility of its biological control, the *in vitro* antagonistic activity was evaluated of several non-pathogenic fungal endophytes, commonly found in oaks. Three strains each of *Acremonium* sp., *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Gliocladium roseum*, *Trichoderma viride*, and *Verticillium* sp. were considered. Their potential antagonism against *B. mediterranea* was assessed *in vitro* by dual culture tests on PDA and by measuring the growth rate of the pathogen in PDA containing different concentrations of liquid culture filtrates from the most effective strains. Mycelial growth was recorded daily and the data were statistically worked out. In dual culture tests *A. pullulans*, *C. cladosporioides* and *Acremonium* sp. showed very poor if any inhibition; *E. nigrum* and *Verticillium* significantly reduced *B. mediterranea* growth; one strain of *G. roseum* had both a weak antibiotic and a mycoparasitic action; *Trichoderma* was the most effective in parasitizing the opposite mycelium. Culture filtrates tests showed that *E. nigrum* and *Verticillium* (and very scarcely *T. viride* and *G. roseum*) produce and release in the medium unknown inhibitors, which are more effective as their concentration increases. Colonization of plant tissues, which is fundamental for endophyte life strategy, would depend on their reciprocal competitiveness by the use of different "weapons". Their relative abundance can provide hints on the health trend of the plants.

***IN VITRO* AND *IN VIVO* ACTIVITY OF PLANT VOLATILE COMPOUNDS AGAINST *PENICILLIUM EXPANSUM*.** **F. Neri, M. Mari and S. Brigati.** *Criof - Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, Via Gandolfi 19, 40057 Cadriano, Bologna, Italy. Fax: +39.051.765049; E-mail: fiorella.neri@unibo.it.*

The inhibitory activity of nine plant volatile compounds was screened *in vitro* and *in vivo* against *Penicillium expansum* Link, the cause of blue mould of pome fruits. The result of *in vitro* trials on spore germination and mycelial growth showed a consistent fungicidal activity of the compounds *trans*-2-hexenal, carvacrol, *trans*-cinnamaldehyde and citral, while hexanal, **(-) -** carvone, *p*-anisaldehyde, eugenol, and 2-nonanone exhibited a progressively lower inhibition. *Trans*-2-hexenal was the best inhibitor of conidial germination (MIC 24.6 ml l^{-1}; ED_{50} 10.2 ml l^{-1}), while carvacrol was the best inhibitor of mycelial growth (MIC 24.6 ml l^{-1}; ED_{50} 9 ml l^{-1}). The four most active compounds in *in vitro* studies were tested *in vivo* as fumigants against blue mould on 'Conference' pears. Best control was achieved by *trans*-2-hexenal vapour treatments (12.5 ml l^{-1}) when applied over a 24 h period, beginning 24 h after inoculation. By contrast, carvacrol (12.5-200 ml l^{-1}) and *trans*-cinnamaldehyde (50-400 ml l^{-1}) were not effective. Citral (200 ml l^{-1}) gave only a slight control of blue mould.

INDUCTION OF RESISTANCE TO BLACK ROOT ROT OF TOBACCO *IN VITRO* BY CULTURE FILTRATES OF ISOLATES OF *PENICILLIUM* SPP.. **R. Nicoletti and A. Carella.** *C.R.A. - Istituto Sperimentale per il Tabacco, Via P. Vitiello 108, I-84018 Scafati (SA), Italy. Fax: +39.081.8506206; E-mail: rosario.nicoletti@entecra.it.*

Fungal antagonism against soil-borne plant pathogens depends not only on direct interactions, such as mycoparasitism or antibiosis, but can also be mediated by the plant itself. In fact, some fungi are capable of stimulating a defence reaction which turns to some extent in acquired resistance and may sometimes allow the plant to escape disease. One of the mechanisms involved in this phenomenon consists in the release of oligosaccharides from the cell wall following the action of lytic enzymes produced by the antagonist. A preliminary test was set up to evaluate the biological activity against black root rot (causal agent *Thielaviopsis basicola*) of culture filtrates of some *Penicillium* isolates in which xylanase activity had been stimulated by using xylan as the major carbon source in the growth medium. Culture filtrate of each isolate was added in the substrate of tobacco plantlets growing in sterile plastic containers together with chlamydospores of the pathogen. Culture filtrates were never able to inhibit either germination of chlamydospores or hyphal growth of the pathogen in the agar medium, while a substantial reduction of black root rot symptoms was observed. In particular, isolates of *Penicillium crustosum* completely inhibited the disease onset for one month, while a variable degree of protection was afforded by the other species. Culture filtrates of the same isolates

from Czapek-Dox broth, thus lacking xylanase activity, failed to inhibit black root rot development. Further investigations will be carried out to assess whether resistance induction may depend on xylanase activity on tobacco roots.

SPECIFIC PCR DETECTION OF *PHYTOPHTHORA MEGASPERMA* USING THE INTERGENIC SPACER REGION OF RIBOSOMAL DNA. F. Nigro[1], T. Yaseen[1], L. Schena[1], A. Ippolito[1] and D.E.L. Cooke[2]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Via Amendola 165/A, 70126 Bari, Italy. [2]Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK. Fax: +39.080.5442911; E-mail: nigrof@agr.uniba.it.*

Ribosomal RNA genes (rDNA) possess suitable characteristics for the detection of pathogens at the species level. They occur in multiple copies arranged in tandem repeats with each repeat consisting of the 18S Small SubUnit (SSU), the 5.8S, and the 28S Large SubUnit (LSU) genes, separated by internal spacers (ITS). ITS have proven particularly useful for separation of *Phytophthorae* at the species or genus level, although *P. megasperma* still remain one of the most problematic species in the genus. The utility of the region between the LSU and SSU genes, known as the intergenic spacer (IGS), has not been fully exploited for diagnostic purposes in *Phytophthora.* Thus, to develop a specific molecular tool, the complete sequence of the IGS region in *P. megasperma* was determined. Primer pairs were designed for amplification of the region spanning a portion of the LSU, the IGS, and the 5' end of SSU. The tandem repeated unit was 5,259 bp long appearing to be the shortest rDNA cluster described so far among the available sequences. A pair of oligonucleotide primers (IGph6 and IGph5), designed on the *P. megasperma* IGS sequence, were evaluated for specificity and absence of cross reactivity, by using a wide range of *Phytophthora* species and other fungi, commonly occurring in the soil. The resulting amplicon was of the expected size (176 bp), thus allowing the specific identification of all *P. megasperma* isolates, but not of those known as the AC form (isolate SCRP448).

IDENTIFICATION AND CHARACTERIZATION OF THE AGGRESSIVE BIOTYPE OF *TRICHODERMA HARZIANUM* (TH 2) ISOLATED FROM *AGARICUS BISPORUS* COMPOST IN ITALY. P. Nipoti, S. Sandalo, A. Prodi, S. Medina Estaun and A. Pisi. *"Alma Mater Studiorum" Università di Bologna, Dipartimento di Scienze e Tecnologie Agroambientali, Viale Fanin 40, 40127 Bologna, Italy. Fax: +39.051.4640127; E-mail: paola.nipoti@unibo.it.*

Since the year 2002 several mushroom growers of northern Italy reported remarkable losses of *Agaricus bisporus* production due to heavy infections by "green mould". Colonies of phenotypically different *Trichoderma* spp. were isolated from infected compost. The aim of this study was to distinguish the aggressive biotype of *Trichoderma harzianum* (*Trichoderma aggressivum* f. *europeaum* f. nov. - Th 2) from the other two non-aggressive biotypes (*Trichoderma harzianum* complex - Th 1 and *Trichoderma atroviride* - Th 3). Th 2 has not yet been reported from Italy in *A. bisporus* compost. Observations with the light microscope showed: conidiophores in a pyramidal pattern; primary and secondary branches at right angle; 2-3 bottle-shaped phialides in whorls; subglobose conidia with broadly rounded apex and slightly pointed base; intercalary or terminal chlamydospores. Based on these characteristics the isolated colonies were identified as *Trichoderma harzianum* Rifai. Phenotypic observations of these strains were made on two different media, PDA (potato dextrose agar) and CMD (cornmeal dextrose agar), at 30°C in the dark for 7 days. A good identification of the biotypes was obtained after 4 growing days on PDA and after 7 on CMD. Strains were characterized using sequence data from the nuclear ribosomal internal transcribed spacer regions 1 and 2 (ITS1 and ITS2). In the phylogenetic tree (MegAlign 4.00) most of the sequenced *Trichoderma* strains showed high affinity with the Th 2 reference strain (up to about 98% with an extremely low divergence, 0.2 to 0.7%). Only one strain belonged to biotype Th 1 (affinity 98.9%, divergence 0.3%).

RESULTS OF THE FIRST YEAR OF A SURVEY FOR YELLOW RUST IN SICILY. C. Oliveri[1], V. Coco[1], M. Palumbo[2] and M. Tessitori[1]. *[1]DiSTeF, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. [2]C.R.A., Istituto Sperimentale per la Cerealicoltura, Sezione di Catania, Via Varese 43, 95123 Catania, Italy. Fax: + 39. 095.7147287; E-mail: c.oliveri@unict.it.*

Puccinia striiformis is the agent of yellow rust (or stripe rust) of cereal crops (*Poaceae* family) and grasses, wheat, barley, and rye being the main cereal hosts. Although little common, yellow rust has been considered potentially more damaging than brown rust and black rust, mainly in Northern Italy. Thanks to a Mediterranean network for yellow rust study, coordinated by INRA (Thiverval Grignon-Francia), a survey for *Puccinia striiformis* was initiated in spring 2005 in three fields located in eastern Sicily at different altitudes, i.e. Mineo (250 m a.s.l.), Libertinia (180 m a.s.l.), and Enna (650 m a.s.l.). More than 90 different varieties among durum (25) and bread wheat (30) and barley (36) were examined during the heading-flowering stage. A yellow rust focus was recorded in the field located in Libertinia in four cultivars (cvs Mieti, Kalango, Serio, Asperia) and six new lines (A416, CTT7, CTT25, CTT28, CTT65, CTT97) of bread wheat. Yellow pustules of stripe rust occurred in all aerial parts, but were most frequently seen on the leaves. The pustules were often arranged into conspicuous stripes with a linear orientation between vascular bundles. Apparently, climatic conditions were not favourable to disease development in other areas. These results give a preliminary indication of the rate of resistance of wheat genotypes in the same location. As expected, bread wheat was more susceptible. Moreover, infections of *P. recondita* f.sp. *tritici* were observed in all locations on different cultivars of durum and bread wheat.

STUDY OF BIOFILM FORMATION IN *CANDIDA ALBICANS* MAY HELP UNDERSTANDING THE BIOCONTROL CAPABILITY OF A *FLOR* STRAIN OF *SACCHAROMYCES CEREVISIAE* AGAINST THE PHYTOPATHOGENIC FUNGUS *PENICILLIUM EXPANSUM*. G. Ortu[1], M.A. Demontis[1], M. Budroni[2], S. Goyard[2], C. d'Enfert[2] and Q. Migheli[1]. *[1]Dipartimento di Protezione delle Piante and [2]Dipartimento di Scienze Ambientali e Biotecnologie Agroalimentari, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. [3]Unité Postulante Biologie et Pathogénicité Fongiques, Institute Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France. Fax: +39.079.229316; E-mail: qmigheli@uniss.it.*

The film-forming *Saccharomyces cerevisiae* strain M25 shows significant ability to reduce postharvest decay in apples caused by the phytopathogenic fungus and patulin-producer *Penicillium expansum*. The biocontrol effect depends on medium, growth condition, and age at which the yeast is collected and inoculated into artificially infected wounds. Biofilm formation was studied using the pathogenic yeast *Candida albicans* as a model. The formation of biofilm in *C. albicans* is articulated in three main phases: the first is

identified with the adhesion of the cells to a solid medium and consequent formation of microcolonies; the second with the filamentous growth of the cells in hyphal form, and the last with the secretion of an extracellular matrix stabilizing the structure. A comparative microarray analysis between biofilm and planktonic form on *C. albicans* showed a differential expression of the genes *Pga59* and *Pga62*, encoding two GPI-anchored proteins in the cell-wall. These proteins are implicated in the first phases of biofilm formation, i.e. in cell adhesion to a solid medium. The role of *Pga59* and *Pga62* during biofilm formation was estimated through the analysis of several disruption mutants for sensitivity to Congo red, for capability to hyphal differentiation as well as for their ability to form a biofilm. This study continues with the construction of disrupted mutants of *S. cerevisiae* M25 for *ccw12*, a gene homologous to *Pga59*, in order to determine its possible role in biofilm formation and in the antagonistic activity against *P. expansum*.

PHYTOPHTHORA SPECIES ON ORNAMENTAL PLANTS IN ITALY. A. Pane[1], P. Martini[2], A. Cimento[3], S. Rapetti[2], S. Savona[2], F.M. Grasso[1] and S.O. Cacciola[3]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95125 Catania, Italy. [2]Istituto Regionale di Floricoltura, Via Carducci 12, 18038 Sanremo (IM), Italy. [3]Dipartimento S.En.Fi.Mi.Zo., Università degli Studi di Palermo, Viale delle Scienze 2, 90128 Palermo, Italy. Fax: +39.091.7028855; E-mail: cacciola@unipa.it.*

Root and crown rot caused by *Phytophthora* spp. are responsible for serious damage to pot-grown ornamentals. Several *Phytophthora* spp. were recovered in a recent survey of ornamental nurseries in Liguria and Sicily (northern and southern Italy, respectively). *Phytophthora* spp. were obtained by direct isolation on a selective medium and were identified by morphological, biochemical (electrophoretic patterns of total mycelial proteins and isozymes on polyacrylamide gels), and molecular characters [RFLP and sequence analysis if ITS regions of rDNA (Cacciola *et al.*, 2001, *For. Snow Landsc. Res.* 76: 387)]. The following *Phytophthora* species were associated with symptoms of crown and root rot: *Phytophthora cactorum* (Leb. & Cohn) Schröeter in *Viburnum tinus* L.; *Phytophthora citricola* Sawada in *Cytisus scoparius* (L.) Link and *Polygala myrtifolia* L.; *Phytophthora cryptogea* Pethybr. & Lafferty in *Banksia* spp. and *Lantana camara* L.; *Phytophthora drechsleri* Tucker in *C. scoparius*, *Polygala hedraiandra* de Cok & Man in't Veld in *V. tinus*; *Phytophthora nicotianae* Breda de Haan in *Callistemon citrinus* (Curtis) Skeels, *Callistemon viminalis* (Sol. *ex* Gaertn.) G. Don *ex* Loudon, *Catharantus roseus* L., *Choisia ternata* Humb., Bonpl. & Kunth, *Cyclamen persicum* Mill., *Grevillea* spp., *Gardenia jasminoides* J. Ellis, *Limonium sinense* (Girard) Kuntze, *Mandevilla splendens* (Hook.) Woodson, *Metrosideros polymorpha* Gaudich, *Pandorea jasminoides* (Lindl.) K. Schum., *Phormium tenax* J.R. Forst & G. Forst, *Thymus* spp., and *V. tinus*; *Phytophthora palmivora* (Butler) Butler in *C. persicum, M. splendens, P. tenax*, *Pittosporum tenuifolium* Gaertn, and *Pittosporum tobira* (Thunb.) W.T. Aiton; *Phytophthora tropicalis* Aragaki & Uchida in *Cuphea ignea* A. DC. Moreover, *P. citricola* and *P. nicotianae* were found to be responsible for leaf spots and twig blight of *Laurus nobilis* L..

SEQUENCE POLYMORPHISM OF _BCATRO_, A GENE ENCODING AN ABC TRANSPORTER IN _BOTRYOTINIA FUCKELIANA_. C. Pane and F. Scala. *Dipartimento Ar.Bo.Pa.Ve - Università di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. Fax: +39. 081.7755320; E-mail: catello.pane@libero.it.*

Botryotinia fuckeliana (anamorph = *Botrytis cinerea*) is the causal agent of the grey mould disease on many crops. Phytopathogenic fungi may tolerate exposure to cytotoxic compounds through the action of efflux pumps, such as the ABC membrane transporters. In our laboratory, the *BcatrO* gene, encoding an ABC transporter in *B. fuckeliana*, was cloned and its characterization is in progress. We analyzed the sequence polymorphism of a 0.6 kb *BcatrO* fragment that was amplified by PCR, from 30 isolates of *B. fuckeliana* and one isolate of *B. fabae.* The isolates differed for geographic and host origin, and resistance to azole fungicides. Phylogenetic trees were constructed using nucleotide and the corresponding translated amino acidic sequences. In both cases six groups were found. In particular, the comparison of the amino acidic sequences revealed that 21 isolates from various origins showed a 100% identity. Isolates 154 e 193, collected in Croazia, from strawberry flowers and chrisanthemum, respectively, differed in the first 19 aa. In the other groups, one or two aa substitutions were observed. Our results show that no significant correlation exists between the origin of the isolates and their *BcatrO* sequence polymorphism.

CHARACTERIZATION OF _BCATRO_ KNOCK OUT MUTANTS OF _BOTRYOTINIA FUCKELIANA_. C. Pane, R. Esposito and F. Scala. *Dipartimento Ar.Bo.Pa.Ve - Università di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. Fax: +39.081.7755114; E-mail: catello.pane@libero.it.*

BcatrO is a gene encoding an ABC transporter in *Botryotinia fuckeliana* (anamorph = *Botrytis cinerea*), the causal agent of grey mould of many plants. Preliminary studies indicate that *BcatrO* transcript accumulation is induced by hydrogen peroxide, in a dose-dependent manner, but not by inducers (i.e. cycloheximide and fenpiclonil) of the transcription of other ABC genes in *B. fuckeliana*. Analysis of *BcatrO* during a synchronized infection of bean leaves revealed a peak in the transcript accumulation at 6 h after inoculation. This may indicate that the product of *BcatrO* could be involved in fungal pathogenesis. In order to clarify the role of this ABC transporter, *BcatrO* knock-out mutants were made by replacing a part of the coding region with a hygromycin resistance cassette. Drug sensitivity to several chemicals of *BcatrO* mutants was observed. On Czapek medium added with hydrogen peroxide, the growth of *BcatrO* mutants was either completely inhibited or reduced by 50%, compared to that of the wild type, when hydrogen peroxide concentration was 1 mM or 0.1 mM, respectively. Similarly, mutant growth was reduced in solid media added with 0.03 ppm fenpiclonil. By contrast no change in phenotype was observed in mutants grown on Czapek + 1.0 ppm resveratrol. Interestingly, in the presence of ciprodinil (0.08 ppm and 0.04 ppm) and azoxystrobin (25 ppm), mutants grew better than the wild type. Assays to evaluate the pathogenicity of the *BcatrO* mutants are in progress.

OCCURRENCE OF _CUCUMBER MOSAIC VIRUS_ IN _PASSIFLORA EDULIS_ IN ITALY. G. Parrella[1], A. De Stradis[2] and C. Vovlas[3]. *[1]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. [2]Istituto per la Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. [3]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.081.7758122; E-mail: giuseppe.parrella@ipp.cnr.it.*

During spring 2005, symptoms of a disease consisting of striking coalescent yellow spots, pale mosaic and slight deformations of the leaves were observed on the canopy of some plants of *Passiflora edulis* Sims. (family *Passifloraceae*, commonly known as "pur-

ple passion fruit" or "granadilla", growing in a garden of the Naples area (southern Italy). The same plants showed pale coloration of the flowers whereas fruits were apparently normal. In summer, symptoms on the new vegetation became milder. Uranyl acetate stained leaf-dip preparations observed under the electron microscope showed isometric virus particles *ca.* 30 nm in diameter. Symptoms observed in herbaceous hosts plants, after mechanical inoculation with sap from symptomatic *P. edulis* leaves, and transmission tests with *Myzus persicae* indicated that *Cucumber mosaic virus* (CMV) was the likely agent of the disease. Virus identity was confirmed by DAS-ELISA with commercial antisera against CMV, made with leaf sap extracts from *P. edulis* and artificially inoculated symptomatic hosts (*Nicotiana glutinosa*, *Nicotiana benthamiana*). CMV has previously been reported as the agent of passiflora mosaic disease, from several tropical and subtropical countries (i.e. Australia, Africa, New Guinea, New Zealand), where granadilla cultivations are of economic importance. In Italy, *P. edulis* is grown as ornamental plant, mostly for its attractive flowers. This is the first report of CMV from *P. edulis* in Italy.

EVALUATION OF *LYCOPERSICON* GERMPLASM FOR RESISTANCE TO WHITEFLY-TRANSMITTED VIRUSES IN SARDINIA. G. Parrella[1], L. Manca[2] and A. Sirigu[2]. *[1]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. [2]CRAS, Centro Regionale Agrario Sperimentale della Sardegna, Viale Trieste 111, 09123 Cagliari, Italy. Fax: +39.081.7758122; E-mail: giuseppe.parrella@ipp.cnr.it.*

Whitefly-transmitted viruses (WTVs) are a major threat to the quantity and quality of tomato crops grown under protected conditions in Sardinia. WTVs occurring in the island belong to genus *Begomovirus*, i.e. *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and the recently recorded *Tomato yellow leaf curl virus* (TYLCV), and to genus *Crinivirus*, i.e. *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV). The increased incidence in Italy of criniviruses and their vectors, especially in greenhouses, highlights the need for additional efforts towards resistance and management of whitefly-transmitted viruses. Thus, wild species, commercial varieties and tomato lines were evaluated for resistance to WTVs in two greenhouses where a high incidence of TYLCSV and TICV had previously been observed. During the trials, chemical treatments to prevent or control whitefly infestations were completely abolished. Symptoms development and severity were visually monitored every month. Molecular hybridization with specific TYLCV and TICV riboprobes were used to detect both viruses. Whitefly infestations were also monitored by assessing the presence of adults and juveniles of *Bemisia tabaci* and *Trialeurodes vaporariorum*, on leaf samples of all tomato germplasm being tested. Results showed substantial differences between genotypes in disease incidence, virus spread, symptom severity, and crop yield. Accessions of the wild species *Lycopersicon hirsutum* were apparently the best sources of resistance/tolerance to both viruses, while accessions of *L. peruvianum* and tomato lines carrying *Ty-1* gene were effective in the control of TYLCSV isolates presently spreading in greenhouse-grown tomatoes of southern Sardinia.

OUTBREAKS OF *ONION YELLOW DWARF VIRUS* IN ONION CROPS IN CALABRIA. G. Parrella[1], A. De Stradis[2], C. Vovlas[3] and G.E. Agosteo[4]. *[1]Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. [2]Istituto per la Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. [3]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. [4]Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 2, 89061 Gallina, Reggio Calabria, Italy. Fax: +39.081.7758122; E-mail: giuseppe.parrella@ipp.cnr.it.*

In 2003-2004, chlorotic stripes, yellowing and curling of the leaves were observed in several onion (*Allium cepa* L., cv Cipolla rossa di Tropea) cultivations of Calabria (Lamezia Terme and Ricadi). Diseased plants ranged from 20 to 100%, depending on the field inspected. Electron microscope observations of leaf-dip preparation showed the presence of potyvirus-like particles about 780 nm in length. Attempts to infect mechanically *Nicotiana* spp. and *Chenopodium* spp. failed, but symptoms were reproduced only in onion plantlets inoculated when young. From a set of primer pairs directed against three common viruses infecting *Allium* spp., i.e. *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV) and *Garlic common latent virus* (GarCLV), expected amplicons were obtained only with the primer pair specific for OYDV was used. The 280 bp amplicon, corresponding to the C-terminus of the coat protein gene and part of 3' UTR of OYDV, were cloned and sequenced. Highest similarity was obtained with a strain of OYDV from Holland. Further confirmations of OYDV spread in onion cultivations of Calabria were obtained by dot blot hybridization of sap samples with a specific OYDV riboprobe. Moreover, virus particles were decorated only by IgG raised to OYDV coat protein. Since in some fields the disease affected the totality of the crop, infections by OYDV must be considered as a relevant problem for onion cultivation in Calabria, especially in those areas where the production of local varieties is of high economic relevance.

MOLECULAR SURVEY ON *TOMATO YELLOW LEAF CURL DISEASE* AND ITS VECTOR IN ITALY. G. Parrella[1], L. Scassillo[2], A. Crescenzi[3] and A.G. Nappo[1]. *[1]Istituto per la Protezione delle Piante, Via Università 133, CNR, 80055 Portici (NA), Italy. [2]Dipartimento di Entomologia e Zoologia Agraria, Via Università 100, 80055 Portici (NA), Italy. [3]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, Campus Macchia Romana 3A310, 85100 Potenza, Italy. Fax: +39.081.7758122; E-mail: giuseppe.parrella@ipp.cnr.it.*

A molecular survey of viruses responsible for tomato yellow leaf curl disease (TYLCD) and their associated vector *Bemisia tabaci* Genn., was conducted during 2004-2005 in four regions of southern Italy: i.e. Sardinia (two locations), Sicily (one location), Calabria (three locations) and Campania (two locations). A total of 62 tomato samples were checked for virus infection and for the presence of the vector. In a preliminary screening plants were tested for the presence of *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV) by PCR-RFLP of a 680 bp fragment comprising the coat protein gene. Results clearly showed that in all four regions both viruses are widespread and present singly or in mixture in single plants. The identity of the two viruses was confirmed by total or partial sequencing of field isolates. In particular, the majority of TYLCV isolates found in Italy showed high similarity with TYLCV mild strain (TYLCV-Mld), whereas other isolates shared the highest similarity with an Egyptian isolate (TYLCV-EG). TYLCSV isolates showed a high level of identity with TYLCSV or TYLCSV from Sicily (TYLCSV-Sic). Concerning *B. tabaci* populations associated with TYLCD epidemics, the molecular characterization of COI gene (citocrome oxidase I) showed that the Q biotype was the most prevalent. This may be a consequence of the large use of insecticides against which Q biotype populations easily develop resistance, as observed in some countries of the Mediterranean basin.

A ON-LINE KEY TO THE FUNGAL PATHOGENS OF ITALIAN RICE. A.M. Picco and M. Rodolfi. *Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri, Sezione di Micologia, Università degli Studi di Pavia, Via San Epifanio 14, I-27100 Pavia, Italy. Fax: +39.0382.34240; E-mail: apicco@et.unipv.it.*

In Italy, fungi have been reported to cause more diseases to rice crop than any other group of pathogens. Therefore, a correct identification of rice fungal pathogens, together with information on their presence in growing areas, are necessary to prevent epidemics and to improve crop and seed health programmes. An interactive key to fungal pathogens of *Oryza sativa* L. grown in Italy will become soon available on the web. The basic outline of the key is produced by the programme FRIDA (FRiendly IDentificAtion), patented by the University of Trieste (Italy) and initially developed for the identification of Italian lichens. The interactive identification system is based both on morphological characteristics of the pathogen (i.e. mycelium *in vitro*, spore type, sexual and asexual sporophores, additional structures) and on visible symptoms of infected rice (i.e. necrosis, rots, discolorations, blights, abnormal elongation of plants). The system, that can be continuously updated, currently includes 61 fungal taxa, representative of 33 genera and 50 species. Causal agents of seedling, foliar, leaf sheath, culm, root, crown and grain diseases reported in Italy are included. An illustrated glossary will be of help to the user and, once identification is done, nomenclature for teleomorph and anamorph fungal names, descriptions and illustrations of both pathogen and disease will be available.

ISOLATION OF *COLLETOTRICHUM ACUTATUM* FROM STRAWBERRY SOILS. A. Pisi, P. Nipoti and A.R. Babini. *"Alma Mater Studiorum" Università degli Studi di Bologna, Dipartimento di Scienze e Tecnologie Agroambientali, Viale Fanin 40, 40127 Bologna, Italy. Fax: +39.051.4640127; E-mail: paola.nipoti@unibo.it.*

Strawberry cultivation, either for nursery plants or fruit production, is an important source of income in many agricultural areas of Italy, especially in Emilia-Romagna. Monoculture is frequent and increases the potential inoculum of root and crown pathogens in the soil. Since methyl bromide has been banned, new studies were initiated for identifying alternative techniques to prevent the spread of anthracnose caused in strawberry by *Colletotrichum acutatum.* Anthracnose symptoms are characterized by irregular black leaf spots, flower blight, crown and fruit rotting. As yield losses are remarkable, the utilization of soil free from the pathogen is of utmost importance. Soil samples taken from fields before or during strawberry cultivation were examined for monitoring the presence of the pathogen. To detect *C. acutatum* a semi-selective medium (CMA modified) was used. Results indicate that this medium is reliable only when the potential inoculum of the pathogen is relatively high. Investigations are in progress for a better definition of the amount of fungicides or antibiotics to be added to the medium, for increasing its sensitivity.

PRELIMINARY STUDY ON THE EFFECTS OF TWO SAR INDUCERS AND PROHEXADIONE CALCIUM ON THE DEVELOPMENT OF PHYTOPLASMAS IN VINCA. S. Prati, D. Maffi, C. Longoni, S. Chiesa, P.A. Bianco and S. Quaroni. *Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. Fax: +39.02.50316781; E-mail: piero.bianco@unimi.it.*

The use of natural or synthetic inducers of plant-defence responses in crop protection represents a valuable strategy to avoid environmental risks and the development of resistant pathogen strains. We studied the effects of two plant resistance inducers (aluminium tris and acibenzolar-S-methyl) and a growth regulator (prohexadione calcium) using the plant pathogen system *Catharanthus roseum*-chrysanthemum yellows phytoplasma (CY). Aluminium tris (O-ethyl phosphonate) was one of the first chemicals capable of inducing systemic acquired resistance (SAR) towards pathogenic fungi and bacteria. Acibenzolar-S-methyl (BTH) is a functional analogue of salicylic acid and a particularly effective SAR inducer. Prohexadione calcium, a plant growth regulator, was chosen for its efficacy in controlling apple proliferation, a phytoplasma disease of apple. Fifty-day-old *C. roseum* seedlings were sprayed with the chemicals on trial and after four days were grafted with CY-infected scions of the same species. A second treatment was made 21 days after grafting. Plants were visually observed weekly and tissues, collected below the grafting union, were analysed with a light microscope. CY phytoplasma were stained using either DAPI or Diene's stain. For two month after treatment, all chemicals significantly reduced the appearance of symptoms (yellows and virescence). After three months, aluminium tris-treated plants showed only mild symptoms of yellows while no differences were observed between plants treated with the remaining chemicals and the infected non-treated control plants. The efficacy of aluminium tris to impair phytoplasma development was also assessed by microscopic observation of sampled tissues.

EARLY DETECTION AND K84 STRAIN APPLICATION FOR CROWN GALL PREVENTION IN PEACH NURSERIES. A. Raio[1], G. Popolo[2], R. Peluso[2] and A. Zoina[2]. *[1]Istituto per la Protezione delle Piante - CNR, Via Università, 133, 80055 Portici, Italy. [2]ARBOPAVE - Università degli Studi di Napoli "Federico II", Via Università, 100, 80055 Portici, Italy. Fax: +39.081.7755320; E-mail: zoina@unina.it.*

Crown gall, caused by *Agrobacterium* spp., can be considered a main limiting factor for peach cultivation in the nurseries. Trials were carried out on the early detection of crown gall and the evaluation of biocontrol with the antagonist K84. A PCR-based protocol that allows detection of latent infections by tumorigenic agrobacteria in peach plants was developed. Total DNA was extracted by a rapid procedure and the highly conserved sequences of the virulence (*vir*) region of Ti plasmid of *Agrobacterium* spp. were amplified. Agrobacteria were detected in the roots and up to 20 cm of the stems of artificially inoculated symptomless peach plants. The procedure developed in this study may be a useful tool for the certification of plant propagation material and for epidemiological studies on crown gall disease. The efficacy of the antagonist *Agrobacterium rhizogenes* strain K84 in preventing crown gall of peach was monitored for several years in some nurseries of Southern Italy. In 2001, tumors were observed in K84 protected plants grown in a nursery of the Campania region (southern Italy). Tumorigenic and K84 insensitive strains, originated by plasmid exchange between K84 and autochthonous tumorigenic agrobacteria, were found in tumors. These agrobacteria also showed high rhizosphere competence and may represent a real threat for biocontrol implementation. Although strain K1026 (a K84 derivative) could prevent the selection of dangerous transconjugant agrobacteria, its use is not allowed in the UE for it is regarded as a genetically modified microorganism.

PEACH CALICO DISEASE: CHARACTERIZATION OF *PEACH LATENT MOSAIC VIROID* VARIANTS CONTAINING INSERTIONS WITH DIFFERENT PATHOGENIC PROPERTIES. M.E. Rodio[1], S. Delgado[2], R. Flores[2] and F. Di Serio[2]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, and Istituto di Virologia Vegetale del CNR, Sezione di Bari, 70126 Bari, Italy. [2]Instituto de Biología Molecular Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, 40022 Valencia, Spain. Fax: +39.080.5442911; E-mail: diserio@ivv.cnr.it.*

Peach latent mosaic viroid (PLMVd), a chloroplast replicating viroid, induces a wide variety of symptoms in peach, including a severe chlorosis (albinism) denoted peach calico (PC). PLMVd variants causing PC differ from those inciting mosaics or no symptoms at all because they contain an insertion of 12-13 nt wherein the PC molecular determinant maps. This domain has limited sequence variability, folds itself into a hairpin capped by a U-rich loop, and appears to emerge sporadically *de novo* (Malfitano *et al.*, 2003). We have now identified other natural PLMVd variants from two new PC isolates containing insertions similar to, or very different from those previously reported. These variants are unevenly distributed within field plants according to the symptoms they exhibit. Our studies show that: (i) the position, size and folding are structural constrains of the insertions; (ii) natural variants containing an inserted hairpin capped by a GA-rich loop are latent; (iii) the PC phenotype is determined by variants containing insertions with a U-rich hairpin loop, although the adjacent stem appears to have some influence, and (iv) the insertion can be acquired and lost during infection, suggesting that a latent variant can evolve into a pathogenic one and *viceversa*.

PHYTOSANITARY CERTIFICATION OF GARLIC. C. Rubies Autonell[1], C. Ratti[1], C. Lanzoni[1], A. Pisi[1], A.R. Babini[1], P. Tedeschi[2], A. Maietti[2] and V. Brandolini[2]. *[1]DiSTA-Area Patologia Vegetale, Università degli Studi di Bologna, Via Fanin 40, 40127 Bologna, Italy. [2]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Ferrara, Via Fossato di Mortara 17, 44100 Ferrara, Italy. Fax: +39.051.4640127; E-mail: crubies@agrsci.unibo.it.*

Since in Italy garlic is infected by different viruses, among which *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Garlic common latent virus* (GarCLV), and a number of allexiviruses, the production of virus-free plants was initiated years ago. The sanitary status of the appreciated garlic ecotypes grown in Voghiera (Ferrara) has now been evaluated, by checking 100 different plants for the presence of OYDV, LYSV, GarCLV, allexiviruses, and *Shallot latent virus* (SLV). DAS-ELISA and RT-PCR using leaf tissue extracts yielded similar results for all viruses, whereas RT-PCR was more efficient for virus detection from bulbs. Results showed that: (i) cv Serena was infected by GarCLV (4%), OYDV (97%), and LYSV (88%); (ii) cv Termidrome by OYDV (50%) and LYSV (67%), but not by GarCLV; (iii) cv Bianco Piacentino was 100% infected by OYDV and LYSV, and 33% by GarCLV. All bulbs of the Voghiera garlic ecotype were totally infected by the complex OYDV, LYSV, and GarCLV. SLV was not identified in any tested samples but allexiviruses, whose molecular identification is in progress, were found in all of them. Propagation material of Italian and foreign varieties, and of local ecotypes, proved to be infected by one or more viruses, whereas the official certification protocol requires the absence of viruses, OYDV in particular. Thus, because the unhappy sanitary situation of garlic may be due to inadequate diagnostic techniques, it is necessary to standardize and optimize detection protocols, which should be rapid sensitive and reliable.

HYTRA1, A NEW HYDROPHOBIN FROM *TRICHODERMA HARZIANUM*, MAY BE INVOLVED IN THE INTERACTION MECHANISMS OF THIS FUNGUS WITH PLANTS. M. Ruocco, S. Lanzuise, M. Ferri, F. Scala, S.L. Woo and M. Lorito. *Dipartimento Ar.Bo.Pa.Ve., Sezione di Patologia delle Piante, Università di Napoli, CNR-IPP, Via Università 100, Portici (NA) 80055 Italy. Fax: +39.081.2539339; E-mail: lorito@.unina.it.*

Fungal hydrophobins are small hydrophobic proteins that play an important role in the biology of fungal development. Hydrophobins are ubiquitous in filamentous fungi and, in many cases, their genes have been identified by analysing the mRNAs more abundantly transcribed during developmental processes such as sporulation, fruit body formation, or fungal infection of plants and animals. In our studies on *Trichoderma*-plant interactions we found a new hydrophobin from culture filtrates of *T. harzianum* T22. A 10 kDa protein was purified by a chitin column affinity and the first 15 amino acids were sequenced by the Edman reaction. By using degenerate primers designed on the obtained amino acidic sequence, the whole gene was cloned. From data bank screening, this gene proved to encode a new class II hydrophobin (named HYTRA1). HYTRA1 is a hydrophobin with chitin binding activity, possessing the typical 8 cysteine residues and 4 disulfide bridges. Infiltrations of the fractions purified from the culture filtrate of *T. harzianum* containing HYTRA1 gave a strong HR reaction in tobacco and tomato leaves. HYTRA1 was expressed in *Escherichia coli* under the T7 promoter and its production was tested by using IPTG as an inductor. The protein was found in inclusion bodies but not in the cytoplasmatic fraction. The targeted disruption of the *Hytra1* gene is in progress to study the role of this hydrophobin in the biological and antagonistic activity of *T. harzianum*.

OCCURRENCE OF FUNGAL ROOT ROT IN PINE TREES IN TUSCANY AND ITS RELATIONSHIP WITH *TOMICUS DESTRUENS*. G. Sabbatini Peverieri, C. Villari, R. Tiberi and P. Capretti. *Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale e Entomologia Agraria, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. Fax: +39.055/3288273; E-mail: paolo.capretti@unifi.it.*

In the course of the regional monitoring programme META ("Monitoraggio Estensivo dei boschi della Toscana a fini fitosanitari") several surveys were carried out during the last few years in forest areas of Tuscany. Disorders of pine species (*Pinus pinea* and *Pinus pinaster*) were observed mostly along the Tyrrhenian coast and in the San Rossore Regional Park. Attention was primarily paid to the disease caused to roots by the pathogenic fungi *Leptographium sp.* and *Heterobasidion annosum. Leptographium* species (*L. serpens*, *L. lundbergii*, *L. guttulatum* and *L. wingfieldii*) generally associated with *Tomicus destruen*, were found, and especially in already damaged plants, either fallen or struck by lightning. Isolations were positive from adult beetles and from pine chips removed from around their galleries and emergence holes in the pine bark. *Leptographium* spp. were also associated with breeding beetles. Although carpophores of *H. annosum* were quite rare, the fungal presence was confirmed by trapping basidiospores using fresh wood discs in different areas in the park, also during winter with temperatures close to 0°C. Compatibility tests showed that the *H. annosum* isolates from San Rossore park belonged to the P group. In the same area, the contemporary presence of *Leptographium* spp. and *H. annosum* was sometimes found in dying trees.

DOUBLE STRANDED RNAS AND VIRUS LIKE PARTICLES IN *BOTRYOTINIA FUCKELIANA*. A. Santomauro, M.A. De Guido, R.M. De Miccolis Angelini and F. Faretra. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/a, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: faretra@agr.uniba.it.*

Botryotinia fuckeliana (de Bary) Whetz. (*Botrytis cinerea* Pers.) is a fungal pathogen characterised by a broad variability. Several sources of variation are known, such as heterokaryosis, different levels of ploidy, and transposable elements. Additional sources of variation might be represented by extrachromosomal genetic elements, such as double-stranded RNAs (dsRNAs) and virus-like particles (VLPs). Thirty-three *B. fuckeliana* isolates from different host plants and geographical areas were investigated. dsRNA was detected in 13 isolates with a broad variation in number and molecular size (1.4 to 7.5 kbp). Electron microscopy showed the presence of VLPs in 18 isolates. Isometric particles (around 40 nm in diameter) were the most common, but rigid rods (40-200 nm long) and filamentous particles (400-600 nm long) were also observed. Polyclonal antisera to the VLPs carried by the strains WS276 (isometric) and WS217 (rigid rod) were raised in rabbit, and used in investigations on VLP transmission to the progeny and to other fungal isolates. VLPs were found with a very high frequency (99%) in conidia, whereas their transmission to ascospore offspring did not occur during the sexual process. Transmission by hyphal anastomosis was rarely observed between isolates paired in a same Petri dish. 'Cured' isolates were obtained at low frequency by growing colonies in the presence of the protein-biosynthesis inhibitor cycloheximide. Differences in several phenotypic traits (i.e. vegetative vigour, sporulation, sclerotia production, response to temperature, virulence) could not be detected between VLPs-free isolates and the 'infected' parental strains. Thus, mycoviruses seem to be in a cryptic status in *B. fuckeliana*, as it commonly occurs with other filamentous fungi.

FURTHER STUDIES ON OAK DECLINE DISTRIBUTION IN LOMBARDY. M. Saracchi[1], C. Bottigiola[1], M. Lanticina[2], A.M. Vailati[2], V. Parco[3] and F. Caronni[3]. *[1]Istituto di Patologia Vegetale dell'Università degli Studi, Via Celoria 2, 20133 Milano, Italy. [2]Studio Associato EcoLogo, Via Lamarmora, 12, 20013 Magenta MI, Italy. [3]Parco Lombardo della Valle del Ticino, Via Isonzo 1, Pontevecchio di Magenta, 20013 Magenta MI, Italy. Fax: +39.02.50316781; E-mail: marco.saracchi@unimi.it.*

Oak decline was first observed in Italy in the Circeo National Park in 1986 and was later recorded from other Italian regions. Only a few years ago it was reported also from some woods along the Ticino river. This paper reports the distribution of oak decline in four regional parks of Lombardy. The disease incidence was recorded during field inspections carried out in June and October 2004, in 121 different localities. Symptomatic oaks were recognized, as reported in the literature, based on the presence of epicormic shoots on trunk and branches, withered branches and diebacks, crown thinning, microphyllia, leaf yellowing, and mucilaginous bleeding oozing from the bark. The occurrence of dead trees was also recorded during field surveys. Symptomatic oaks were detected in 89 of 120 localities, mainly in the "Parco Lombardo della Valle del Ticino" (43/48), where the worst situations were encountered. In some cases, over 90% of 30- to 60-year-old trees showed typical disease symptoms and more than 30% were dead. The distribution of oak decline did not seem to follow a particular pattern along the 150 km park length. Less frequently, symptomatic trees were detected in the "Parco Adda Sud" (15/23), in the northern region of "Parco delle Groane" (17/27), and the northern and southern parts of the "Parco della Valle del Lambro" (14/23). In the latter the "Parco Reale di Monza" is included, where the health of oak, robinia and other wood species is seriously compromised.

This study was partially supported by the Regione Lombardia.

ACTIVITY OF *BACILLUS CIRCULANS* JORDAN AGAINST POSTHARVEST ROT PATHOGENS. P. Sardi[1], M. Saracchi[1], M. Moretti[1] and P. Principi[2]. *[1]Istituto di Patologia Vegetale and [2]DISTAM, Facoltà di Agraria, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. Fax: +39.02.50316781; E-mail: paola.sardi@unimi.it.*

During investigations carried out to isolate biocontrol agents, a bacterial strain showing activity towards several wild strains of the genera *Alternaria*, *Botrytis*, and *Penicillium*, was isolated from strawberry leaves collected from plants that had never been treated with pesticides. The strain was named 6B9 and identified as *Bacillus circulans* Jordan. Strain 6B9 inhibited the development of fungal colonies, when grown in dual cultures, and preliminary light microscopy observations showed that fungal hyphae had unusual morphology. To verify the actual effect of strain 6B9 on the development of the hyphae of *Alternaria*, *Botrytis* and *Penicillium*, observations were made with a scanning electron microscope (SEM) on colonies of the pathogens, grown on polycarbonate membranes in the presence of actively growing 6B9 or of its culture filtrate. SEM observations showed heavy morphological alterations in *Alternaria* and *Botrytis*, while *Penicillium* suffered lighter damages. The modifications affected mostly hyphal tips that were frequently swollen and malformed. Hyphae subject to the direct activity of *B. circulans* 6B9 appeared flexuous, frequently collapsed and with altered surfaces. In proximity of hyphal tips, an evident increase of branching was often observed. Colonies grown in the presence of 6B9 culture filtrates showed similar alterations that were, however, of lesser importance and appeared more slowly. The cultural methodology employed in this study prevents the antagonist to come in contact with its target, suggesting that fungal growth inhibition caused by *B. circulans* 6B9 is due to the production of active metabolites.

THE GENE ENCODING CERATO-ULMIN, AN *OPHIOSTOMA*-PRODUCED PROTEIN INVOLVED IN THE DUTCH ELM DISEASE, HAS BEEN INTROGRESSED OR HORIZONTALLY TRANSFERRED IN AN UNRELATED SPECIES OF THE GENUS *GEOSMITHIA*. A. Scala, C. Comparini, L. Carresi and S. Tegli. *Dipartimento di Biotecnologia Agraria, Laboratorio di Patologia Vegetale, Università di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino, Italy. Fax: +39.055.4573232; E-mail: aniello.scala@unifi.it.*

Cerato-ulmin (CU) is a class II hydrophobin protein of about 8 kDa, produced by the Ascomycota *Ophiostoma novo-ulmi*, *Ophiostoma ulmi* and *Ophiostoma himal-ulmi* that are responsible for the Dutch elm disease (DED). Many data suggest the key role of CU in DED pathogenesis. *Ophiostoma quercus*, another *Ophiostomatacea* species non pathogenic to elm trees, possesses the CU protein in the cell walls (Scala *et al.* 1997, Mycological Research 101: 829-834), whose gene was recently isolated and characterized. Until now, attempts to find the *cu* gene and/or CU-like protein in other Ophiostomas were unsuccessful. With this study, a strain of *Geosmithia* sp., a genus unrelated to *Ophiostoma*, was shown to possess the *cu* gene, and to excrete a

CU-like protein in the liquid Takai medium. This strain, named IVV7, was isolated near Vibo Valentia (southern Italy) showing typical DED symptoms. IVV7 was shown to belong to the genus *Geosmithia* on the basis of the sequences of the internal transcribed spacers 1 and 2 and the 5.8S ribosomal RNA gene. The IVV7 *cu* gene was isolated using primers based on published *cu* gene sequence from *O. novo-ulmi*. Sequence analysis showed that the *cu* gene of IVV7 is identical to that of *O. novo-ulmi* (score > 1,000; E value = 0.0). The significance of the *cu* gene presence in a fungal species so distant from the Ophiostomas is discussed.

DETECTION AND QUANTIFICATION OF *PHYTOPHTHORA RAMORUM*, *P. KERNOVIAE*, *P. CITRICOLA*, AND *P. QUERCINA* BY REAL-TIME MULTIPLEX PCR. L. Schena[1] and D.E.L. Cooke[2]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Via Amendola 165/A, 70126, Bari, Italy. [2]Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK. Fax +39.080.5442911; E-mail: leonardo.schena@agr.uniba.it.*

New species of *Phytophthora* such as *Phytophthora ramorum*, *Phytophthora kernoviae*, and *Phytophthora quercina* together with *Phytophthora citricola* are of concern for their impact on forest health, natural ecosystem stability, and international trade. A real-time multiplex PCR approach based on Taq-man PCR was developed to simultaneously identify and detect these four *Phytophthora* species. Specific primer and probes labeled with FAM (*P. ramorum*), YY (*P. kernoviae*), Rox (*P. citricola*) and Cy5 (*P. quercina*) were designed on different regions of the ras-related protein gene. A new set of Black Hole Quenchers (BHQ) which dissipate energy as heat rather than fluorescence was utilized. The method proved to be highly specific in tests with target DNA from 45 *Phytophthora* isolates (31 species). For all pathogens, the detection limit was 1 fg μl^{-1} of target DNA. Cycle threshold (Ct) values were linearly correlated (r=0.997) with the concentration of the target DNA, indicating the appropriateness of the method for qualitative and quantitative analyses. The reliability of the entire procedure was assessed using both artificially and naturally infected leaves of a range of plant species. Combining multiplex PCR with a simple but effective procedure for direct DNA extraction from plant material, it was possible to detect and quantify the four different pathogens in approximately 3 h. Therefore, the method proved to be rapid, reliable, sensitive and cost effective, avoiding specific amplification for each pathogen. The present approach has great potential as a tool for detection and diagnosis in a range of applications from pathogen surveys to statutory testing.

MATING TYPES IN *CRYPHONECTRIA PARASITICA* POPULATIONS FROM CALABRIA. D. Spica[1], G. Sammarco[1], R. Zappia[2], S.O. Cacciola[1] and G. Magnano di San Lio[2]. *[1]Dipartimento S.En.Fi.Mi.Zo., Università di Palermo, Viale delle Scienze 2, 90129 Palermo, Italy. [2]Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 4, 89061 Reggio Calabria, Italy. Fax: +39.0965.689049; E-mail: gmagnano@unirc.it.*

The reproductive biology of the chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr., has ecological as well as genetic effects in determining the structure of pathogen populations and the spread of hypovirulence. Sexual recombination of vegetative incompatibility (*vic*) loci is thought to be the main source of *vc*-group diversity, which correlates negatively with the success of biological control based on hypovirulence. Two alleles (*MAT-1* and *MAT-2*) at a single locus control sexual compatibility in *C. parasitica*. A collection of 179 *C. parasitica* isolates from four locations in Calabria (Acri, Cittanova, S. Lorenzo and Valle di Camera) was characterized. Vegetative compatibility was assessed on Powell's medium, using tester-isolates of the European *vic*-groups, and mating types were identified with the method of Liu *et al.* (1996, *Phytopathology* 86: 1344), using *MAT-1* and *MAT-2* tester-isolates. Isolates of EU1, EU2, EU10 and EU12 *vc*-groups were found in all four locations. EU2 was the prevalent *vc*-group at Acri, S. Lorenzo and Valle di Camera while EU12 prevailed at Acri. Despite the low diversity of *vc*-groups, the frequency of isolates with a hypovirulent phenotype (albino colony) was low in all locations, except for Acri. In all four subpopulations, mating types were found in a balanced (close to 1:1) ratio, suggesting that sexual reproduction occurs frequently. It is likely that in *C. parasitica* populations from Calabria the low diversity of *vc*-groups is the result of founder effect rather than of limited sexual recombination. The prevalence of sexual reproduction could hamper the spread of hypovirulence in these populations.

PRELIMINARY STUDY ON THE CONTROL OF *RHIZOCTONIA SOLANI* IN TOBACCO FLOAT SYSTEM USING REDUCED RATE OF IPRODION ASSOCIATED WITH IPRODION-RESISTANT ISOLATES OF *GLIOCLADIUM ROSEUM*. P. Tarantino, R. Caiazzo, A. Carella, F. Porrone and E. Lahoz. *C.R.A. - Agricultural Research Council, Via P. Vitiello 108, I-84018 Scafati, Italy. Fax: +39.081.8506206; E-mail: ernesto.lahoz@entecra.it.*

Rhizoctonia solani Kühn [teleomorph = *Thanatephorus cucumeris* (Frank) Donk] is a widespread soil-borne pathogen of tobacco, inducing losses against which the use of fungicides has become of interest. Biological control may pursue this goal if used in the framework of an integrated pest management strategy. *Gliocladium roseum*, a mycete common in many different environments (tropical, desert, temperate) is a powerful biocontrol agent (BCA), as shown by its efficiency in controlling *Botrytis cinerea* in strawberry, raspberry, tomato and *Rhizoctonia solani* in tobacco. The extraordinary ecological versatility renders *G. roseum* a good candidate for its use in agriculture. Although control of soil borne diseases with chemical applications at lower rates can be achieved, still it does not exclude the risk that pathogen resistance to chemicals could develop. To minimize these problems, the use of low fungicide rates combined with a BCA can be envisaged. The BCA strain used must be resistant to the chemical, so that different mechanisms can act against the target pathogen. In the present work low doses of fungicide were applied jointly with a resistant *G. roseum* strain against *R. solani*. Results showed that the same level of disease control was obtained in plots treated with a high rate of iprodion or protected with a low rate of fungicide together with an iprodion-resistant strain of *G. roseum*. A lower disease control rate was obtained when iprodion alone was used at low dosage.

SECONDARY METABOLITES PRODUCED BY TWO COMMERCIAL STRAINS OF *TRICHODERMA HARZIANUM*. F. Vinale[1], R. Marra[1], F. Scala, M. Lorito[1], E.L. Ghisalberti[2] and K. Sivasithamparam[2]. *[1]Università di Napoli "Federico II", Dipartimento ARBOPAVE - Patologia Vegetale, Via Università 100, 80055 Portici, Italy. [2]University of Western Australia, Faculty of Agriculture, School of Earth and Geographical Sciences, 35 Stirling Highway, Crawley, Perth, WA, Australia. Fax: +39.081.2539339; E-mail: frvinale@unina.it.*

Trichoderma harzianum, strains T22 and T39, are two microorganisms successfully used as biopesticides and biofertilizers. The

strains used as biocontrol agents show different mechanisms of action in their antagonistic interactions with fungal pathogens. These include antibiosis, mycoparasitism or hyperparasitism, competition for nutrients, cell wall-lytic enzyme activity, and induction of systemic resistance to pathogens *in planta*. Secondary metabolites play an important role during biocontrol, although their modes of action towards microorganisms and plants have not been fully elucidated. In this work, for the first time, we isolated and characterised the major secondary metabolites from strains T22 and T39. Six compounds were extracted and characterized from fungal culture filtrates: two new molecules, a T22 azaphilone (3) and a T39 butenolide (5), and four already known secondary metabolites [1-hydroxy-3-methyl-anthraquinone (1), 1,8-dihydroxy-3-methyl-anthraquinone (2), harzianolide (4), and harzianopyridone (6)]. The compounds isolated showed different levels of antibiotic activity against *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani* and *Pythium ultimum*, and may have specific modes of action and roles in the antagonistic activity of *Trichoderma harzianum*. The antifungal activity of the metabolites and their production were also evident during *in vitro* interaction with *R. solani*.

BIOCONTROL *PSEUDOMONAS* STRAINS AGAINST POSTHARVEST PATHOGENS OF APPLE. F. Vinale[1], A. Fiore[2], V. Fogliano[2], F. Scala[1], M. Gallo[2], S. Gigante[1], G. Cirvilleri[3], A. Catara[3] and M. Lorito[1]. *[1]Università di Napoli "Federico II", Dipartimento ARBOPAVE, Sezione di Patologia Vegetale, Via Università 100, 80055 Portici (NA), Italy. [2]Università di Napoli "Federico II", Dipartimento di Scienze degli Alimenti, Via Università Parco Gussone, 80055 Portici, Italy. [3]Dipartimento DISTEF, Sezione di Patologia Vegetale, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. Fax: +39.081.2539339; E-mail: lorito@unina.it.*

Serious economical losses on fruits and vegetables are caused by post-harvest storage rots. Use of biological control agents may prevent post-harvest diseases and may be a useful alternative to pesticide applications. In this work, different *Pseudomonas* strains (*Pseudomonas corrugata* and *Pseudomonas syringae*) were screened for their antagonistic ability to control *in vivo* the fungal pathogens *Penicillium expansum* and *Alternaria alternata* on apple (*Malus domestica* cv Annurca). Both pathogen and antagonist suspensions were directly co-inoculated on wounded cv Annurca apples and lesion diameter was measured. Results showed that the most promising isolates, *P. syringae 1480A* and *46*, reduced post-harvest symptoms when bacterial suspension and pathogen conidia were inoculated at concentrations of $1\text{-}5\cdot10^7$ CFU ml^{-1} and $1\text{-}6\cdot10^6$ conidia ml^{-1}, respectively. These strains also provided an excellent control of rot development by dipping the apples in the bacterial suspension. To identify the molecules involved in the biocontrol activity of these strains, the production of lipodepsipeptides (LDPs) and various enzymatic activities (glucanase and chitinase) were investigated. *P. syringae* strains *1480A* and *46* did not produce lipodepsipeptides, but had a considerable level of glucanase and chitinase activity.

PCR DETECTION OF FUMONISIN-PRODUCING *FUSARIUM* ISOLATES FROM PIEDMONT. I. Visentin, D. Francia, D. Valentino, G. Tamietti and F. Cardinale. *Università degli Studi di Torino, Di.Va.P.R.A., Patologia Vegetale, Via Leonardo da Vinci 44, 10095 Grugliasco (TO) Italy. Fax: +39.011.6708541; E-mail: giacomo.tamietti@unito.it.*

Fumonisins are a family of mycotoxins produced by *Gibberella moniliformis* (anamorph = *Fusarium verticillioides*) that contaminate maize and maize-based products, notably those produced in Northern Italy, causing great concern for human and animal health. New, sensitive and quantitative diagnostic tools for detection of *F. verticillioides* are badly needed, that would allow following fungal contamination of kernels also in a symptomless phase. In this study, we tested three pairs of primers for their ability to discriminate isolates of *F. verticillioides* from Piedmont. The first pair of primers is supposedly species-specific for *F. verticillioides*, whereas the second is reported to discriminate Italian toxigenic from exotic, non-toxigenic strains isolated from bananas (Patiño *et al.*, 2004, *J. Food Prot.* 67: 1278). The third pair of primers was designed on the key fumonisin biosynthetic gene *FUM1* (Bluhm *et al.*, 2004, *J. Food Prot.* 67: 536). This last set of primers had never been tested on non-toxigenic strains isolated from maize. Indeed it is not known if, in these strains, the *FUM1* gene is deleted, mis-regulated and/or mutated. Preliminary results seem to indicate that the flexibility and robustness of published PCR tools might be heavily influenced by the geographical and host origin of the fungal isolates.

INTRASPECIFIC VARIABILITY OF *CYLINDROCLADIUM PAUCIRAMOSUM* IN INTERACTIONS WITH *TRICHODERMA HARZIANUM* AND IN THERMAL SENSITIVITY. A. Vitale[1] and G. Polizzi[1]. *[1]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. Fax: +39.095.7147283; E-mail: gpolizzi@unict.it.*

Cylindrocladium pauciramosum is a new fungal specie described by Schoch *et al.* (1999). Sexual compatibility and female fertility studies showed that it was recently introduced in Italy, where it causes leaf spots, stem cankers and crown and root rots on several ornamental crops. In order to investigate on the intraspecific variability of *C. pauciramosum*, two dual culture assays with *Trichoderma harzianum* T22 and T39 and a field trial to evaluate the role of soil hydrothermal regimes on the pathogen survival were performed. *T. harzianum* T22 was more effective in reducing carnation leaf colonization and microsclerotia production by eight *C. pauciramosum* isolates than T39 when simultaneously paired. However, antagonistic activity was isolate dependent. Microsclerotia production variability of twenty fungal isolates in different pairings combinations with T22 was examined on carnation leaf tissues. *C. pauciramosum* produced the lowest number of microsclerotia in leaves pre-colonized (4 days before) by the T22 antagonist, while the highest microsclerotia forming ability was detected on carnation leaves inoculated only with the pathogen. However microsclerotia forming ability was very variable among tested isolates and in the corresponding pairings and it was not related with the type of growth (slow o normal). In greenhouse conditions survival of three *C. pauciramosum* isolates, referred to microsclerotia viability from infected carnation leaves placed at a depth of 15 or 30 cm, was different and related to the hydrothermal regimes and the two exposure times (12 and 20 days). These data suggest that intraspecific variability of *C. pauciramosum* population could influence disease control measures.

IDENTIFICATION OF THE CAUSAL AGENTS OF AN ALTERATION OF SEEDS OF THE BEAN ECOTYPE "FAGIOLO DI CONTRONE". S. Vitale[1], A. Del Galdo[2] and M. Zaccardelli[2]. *[1]C.R.A. - Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. [2]C.R.A. - Istituto Sperimentale per le Colture Industriali di Battipaglia (SA), Italy. Fax: +39.06.82070370; E-mail: salvatore.vitale@entecra.it.*

The French bean (*Phaseolus vulgaris* L.) ecotype "Fagiolo di Controne" is a valuable crop grown near the Calore river, in the

province of Salerno (Campania, Italy). This bean ecotype is harvested in autumn and, every year, severe symptoms appear on the seeds before harvesting. Symptoms consist of spots of different size on the seed surface, which initially have a light-brown colour, later turning brown-black. Disease incidence is high (20% of the seeds harvested in the 2004), especially in very humid seasons. Economic loss due to this disease is substantial because of the high market value of the commodity. In this work, the causal agents of this alteration were investigated. Seed samples were subdivided in three classes of disease severity (low, medium, and severe symptoms) and isolations were made on PDA plates. After one-week incubation, developed fungal colonies were observed microscopically for identification. From seeds of all three disease severity classes, *Stemphylium botryosum* and *Alternaria* sp. were isolated. The first fungus prevailed in seeds with low and medium disease severity. Both fungi are responsible of "black pod" disease that appear when plants are weakened or are at the end the vegetative cycle. Inoculation of "Fagiolo di Controne" seeds with *S. botryosum* and *Alternaria* sp. isolates reproduced field symptoms.

SELECTION OF *PSEUDOMONAS* SPP. ABLE TO PROMOTE GROWTH OF TOMATO. M. Zaccardelli, F. Campanile and A. Del Galdo. *C.R.A. - Istituto Sperimentale per le Colture Industriali di Battipaglia, SS 18 n. 204, 84091, Battipaglia (SA), Italy. Fax: +39.0828.340169; E-mail: m.zaccardelli@tiscali.it.*

Plant growth promoting rizhobacteria (PGPR) are effective through direct mechanisms, i.e. production of phytohormone-like substances, or by indirect mechanisms, i.e. increasing synthesis of phytohormones in plants. PGPR activity is often associated with control of plant pathogen by induction of systemic resistance or by producing antibiotics and/or syderophores. Some species of *Pseudomonas* have PGPR propriety. In this work the results are illustrated of the selection of *Pseudomonas* spp. able to produce syderophores and to promote growth of tomato plants. From soils cultivated with different crops (wheat, chickpea, broadbean, and pea) or manured with compost, *Pseudomonas* spp. producing fluorescent or non-fluorescent syderophores were isolated on agar medium without Fe or on KB-agar. Sixteen and eleven fluorescent and/or syderophores producing pseudomonads were obtained from soils cultivated with the different crops or manured with compost, respectively. They were characterized by M13-PCR to select genetically different isolates to be assayed for tomato growth promotion. Fourteen genetically different isolates were inoculated five times every six days to tomato plantlets, in a climatic chamber. After one month, the weight of roots, leaves, and stems were measured. Two isolates showed growth promotion on tomato. In particular, an isolate from soil grown with chickpea, gave an increase of 94% of fresh weight of the roots and the other isolate, from soil grown with pea, gave an increase of 72% of dry weight of the leaves. Inoculation trials of these PGPR pseudomonads on tomato cultivated in the open field are in progress.

SUSCEPTIBILITY TO *PSEUDOMONAS SYRINGAE* PV *TOMATO* OF FOUR TOMATO ECOTYPES AND THEIR COMMERCIAL HOMOLOGUES, CULTIVATED IN TWO LOCATIONS OF CAMPANIA. M. Zaccardelli, M. Parisi and I. Giordano. *C.R.A. - Istituto Sperimentale per le Colture Industriali di Battipaglia, SS 18 n. 204, 84091 Battipaglia (SA), Italy. Fax: +39.0828.340169; E-mail: m.zaccardelli@tiscali.it.*

Pseudomonas syringae pv *tomato* (PST) is the causal agent of bacterial speck of tomato. The damages consist of necrotic spots on the leaves and skin of the fruits, with consequent loss of yield and quality. Tomato genotypes with the resistant gene *Pto* are commercially available. Nevertheless, *Pto* is not introduced in tomato ecotypes and, in any case, different susceptibility exists between different tomato genotypes lacking the *Pto* gene. With this work, the susceptibility to PST of four tomato ecotypes from Campania (S. Marzano, Corbarino, Vesuviano, and Sorrento) and of their commercial homologue genotypes (Ranco F1, Faino F1, Principe Borghese and Cuore di Bue, respectively) was evaluated in 2004 in two location (Angri and Battipaglia) in the province of Salerno (southern Italy) under natural infection. Infection data were collected on the fruits at harvesting. In both locations, no spots on the fruits were observed in S. Marzano and Sorrento ecotypes and in their homologues Ranco F1 and Cuore di Bue. By contrast, heavy attacks to the fruits of the Vesuviano ecotype were observed in both location (incidences 6% at Angri and 11.7% at Battipaglia) but spots were observed on few fruits of its homologue Principe Borghese only at Angri. Symptoms of lower intensity were observed on the fruits of the ecotype Corbarino (incidences 3% at Angri and 4.3% at Battipaglia) and on its commercial homologue Faino F1 (incidence of 1.8% at Angri and 3.3% at Battipaglia). The highest susceptibility of the ecotype Vesuviano confirmed what observed in 2002 at Angri.

USE OF MICROSATELLITE-PCR METHODS FOR THE IDENTIFICATION OF TERVERTICILLATE *PENICILLIUM* SPECIES OF THE *AURANTIOGRISEUM* GROUP. M. Zaccardelli[1], F. Campanile[1], E. Lahoz[2], A. Carella[2] and R. Nicoletti[2]. *[1]C.R.A. - Istituto Sperimentale per le Colture Industriali di Battipaglia, SS 18 n° 204, 84091 Battipaglia (SA), Italy. [2]C.R.A. - Istituto Sperimentale per il Tabacco, Scafati (SA), Italy. Fax: +39.0828.340169; E-mail: m.zaccardelli@tiscali.it.*

Identification of terverticillate *Penicillium* spp. (subgenus *Penicillium*) assigned to the aurantiogriseum group is controversial when morphological or biochemical methods are used. Recently it was shown that molecular methods like sequence analysis of β-tubulin genes, afford a reliable identification. However, the development of molecular methods alternative to sequence analysis, thus accessible to many laboratories, would be highly desirable. We have used a PCR assay with primers designed on repeated minisatellite DNA sequence. After a preliminary evaluation of some minisatellite primers, the primes $(GACA)_4$ and $(GTG)_5$ were chosen and PCR was done with about fifty isolates of *Penicillium* spp. assigned to the aurantiogriseum group, as *Penicillium aurantiogriseum*, *Penicillium commune*, *Penicillium crustosum*, *Penicillium echinulatum*, *Penicillium polonicum*, *Penicillium solitum*, *Penicillium verrucosum*, *Penicillium viridicatum.* Isolates of *Pennicillium* spp. not belonging to the aurantiogriseum group or assigned to subgenera *Biverticillium*, *Aspergilloides*, and *Furcatum*, were used as outgroups. Within each aurantiogriseum species, all isolates were indistinguishable from each other or showed only two types of electrophoretic patterns. The electrophoretic patterns between different aurantiogriseum species were typical for each species and distinguishable from the electrophoretic patterns of the isolates of *Pennicillium* spp. not assigned to the aurantiogriseum group or belonging to subgenera *Biverticillium*, *Aspergilloides* and *Furcatum.* These results suggest that PCR with microsatellite primers $(GACA)_4$ and $(GTG)_5$ is a valuable and rapid method for the identification of *Penicillium* spp. of the aurantiogriseum group.

EFFECTS OF DIFFERENT LEVELS OF IRRIGATION AND NITROGEN FERTILIZER ON SOFT ROT OF POTATO. M. Zaccardelli, A. Pentangerlo, B. D'Onofrio, F. Campanile and A. Del Galdo. *C.R.A. - Istituto Sperimentale per le Colture Industriali, SS 18 n. 204, 84091 Battipaglia (SA), Italy. Fax: +39. 0828.340169; E-mail: m.zaccardelli@tiscali.it.*

The effect of three levels of irrigation (reintegration of 50, 100 or 150% of the water field capacity) was evaluated in combination with three level of nitrogen fertilizer (60, 120 and 180 kg ha^{-1}) on the incidence of soft rot of potato. Cultivar Adora was cultivated in 2005 in Campania (southern Italy). Incidence of plants with soft rot symptoms was evaluated in May, sixty and seventy days after planting. No influence of irrigation levels was observed on soft rot incidence whereas nitrogen fertilization has a bearing on disease incidence. In particular, soft rot incidence was highest (23.3% after seventy days) with the lowest dose of nitrogen and lowest (17.9% after seventy days) with the highest nitrogen dose. Seventeen symptomatic plant samples were collected to isolate pectinolytic agents. Isolations were made using semi-selective media and the 51 isolates obtained were tested for pectinolytic activities on plugs of potato tubers. About 60% of the isolates were not pectinolytic whereas about 40% showed pectinolytic activity. Twenty pectinolytic isolates were analysed by PCR using selective primers designed on the gene encoding pectate liase (*pel*) of *Erwinia carotovora.* Only one isolate gave the typical fragment of 434 bp. Identification of the remaining 19 pectinolytic isolates is in progress.