This study concerns the antagonistic activity of *Pichia guillermondii* isolate 5A against *Penicillium digitatum* on mandarin type fruits cv Fairchild. In order to enhance the inhibitory activity of the yeast 5A, we tested the effect of some salts, Na$_2$CO$_3$ and fruits cv Fairchild. In order to enhance the inhibitory activity of *mondii* no growth were often observed. Germinability of cultures, a typical antagonistic drop growth or a respect zone of 80%, in relation to concentrations of the crude extracts (CEs) or rangia was negatively affected by CEs as already shown with CFs.

Secondly, the use of Na$_2$CO$_3$ alone gave a high inhibition at the three dosages used (6-7%), and the same occurred at the other two dosages. The use of Na$_2$CO$_3$, even at a dosage of 1%, values increased tive trials (90.2, 98.2, 92.2% inhibition). When 5A was combined with the salt Na$_2$CO$_3$, even at a dosage of 1%, values increased between 5-7% and the same occurred at the other two dosages. The use of Na$_2$CO$_3$, even at a dosage of 1%, values increased...
preliminary results are encouraging because effective fungicides were found.

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BIODEGRADATION OF FUMONISIN B1. R. Benedetti, G. Firdasi, N. Nazi and R. Locci. Dipartimento di Biologia Applicata al-la Difesa delle Piane, Università degli Studi di Udine, Via delle Scienze 208, 33100 Udine, Italy. Fax: +39.0432.55850, E-mail: Benedetti@wldef.unito.it.

The presence of Fumonisin B1, produced by Fusarium verticilliodes on maize, is a serious risk to the health of consumers, whether human or animal. The toxin is very stable and difficult to remove from contaminated commodities. No means for the biological decontamination of substrates are at present available. In order to isolate strains able to degrade FB1, 3 samplings from 7 different fields of maize were then subjected to 7 sequential enrichment steps each containing 4 different microbial liquid cultures that were subsequently cultivated in an enrichment culture with FB1 for 15 days. The mixed cultures were then subjected to 7 sequential enrichment steps each consisting in 8 days of incubation with 0.5 mg/ml of FB1. Following this enrichment a bacterial consortium was obtained which completely degraded FB1, as demonstrated by TLC. After several enrichment steps a bacterial consortium was obtained which was used to isolate strains able to degrade FB1, 3 samplings from 7 fields in which maize had been cultivated repeatedly for at least 10 years, were carried out. The soil samples were pooled to obtain 7 mixed microbial liquid cultures that were subsequently cultivated in an enrichment culture with FB1 for 15 days. The mixed cultures were then subjected to 7 sequential enrichment steps each consisting in 8 days of incubation with 0.5 mg/ml of FB1. Following this enrichment a bacterial consortium was obtained which completely degraded FB1, as demonstrated by TLC. After several screenings of the consortium, a FB1 degrading bacterial strain was isolated. This strain was identified as Delftia acidovorans by partial sequencing of the 16S rRNA gene and was demonstrated to grow and degrade the FB1 in saline buffer. The HPLC/MS analysis of the degradation products identified four carbon aliphatic backbone structures of different length. Transposon mutagenesis is in progress to identify the gene(s) involved in the degradation.

EVALUATION OF ARABIDOPSIS THALIANA ECOTYPES FOR RESISTANCE TO DAMPING-OFF CAUSED BY PYTHIUM SLYCITICUM AND STEM ROT CAUSED BY SCLEROTINIA SCLEROROTIORUM. C. Boccongelli1, A. Buzì1, G. Chiò1, P. Magro1 and R.A. Bressan2. 1 Dipartimento di Protezione delle Piane, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy, Fax: +39.0761.357473; E-mail: magro@unitus.it. 2The Horticulture and Landscape Architecture Department, Purdue University, West Lafayette, 625 Agriculture Mall Drive, IN 47907-2010.

Arabidopsis thaliana has become widely used as a model organism for the study of molecular and cellular biology of plants, including gene functional analysis. Currently, functional analysis is largely based on laboratory-induced mutants that are selected in forward and reverse genetic studies. An alternative source of genetic variability is represented by that naturally occurring among Arabidopsis ecotypes. The goal of the present study was to screen different Arabidopsis ecotypes for their natural resistance to Pythium and Sclerotinia, in order to verify the possibility to use them for studying the genetic bases of resistance to these two organisms, comprised among the most destructive soilborne pathogens on a wide range of crops. Pythium and Sclerotinia were isolated and identified from naturally infected A. thaliana plants. Twenty-three A. thaliana ecotypes were soil-inoculated with Pythium and Sclerotinia, then transferred in a growth chamber, under a 16 hr day length at 21°C. A factorial design with three replicates in randomized blocks was applied. Percentage of plant survival at 6 and 14 days post inoculation was assessed. Among tested ecotypes, significant differences were found in the degree of resistance to the diverse pathogens, which probably reflect the existence of a differential gene activation in the same plant genotype. In order to identify differences in Arabidopsis resistance gene expression, molecular analysis on these ecotypes will represent the second step of the present study.

VIRIDEPYRONONE, A NEW ANTIFUNGAL α-PYRONE PRODUCED BY TRICHODERMA VIRIDE. A. Cabras1, L. Maddau1, S. Serra1, A. Andolfi2, A. Motta1 and A. Evidente2. 1 Dipartimento di Protezione delle Piane – Sezione di Patologia vegetale, Università degli Studi di Sassari, 07100 Sassari, Italy. 2 Dipartimento di Scienze del Suolo, dell’Ambiente, Università di Napoli Federico II, 80055 Portici, Italy. Istituto di Chimica molecolare del CNR, 80078 Pozzuoli, Italy.

During an investigation for the identification of fungi liable to be used for the biological control of soil-borne plant pathogens, we found a strain of Trichoderma viride showing antagonistic activity in vitro and in vivo towards Sclerotium rolfsii, the causal agent of crown and stem rot of artichoke. The noteworthy antag-onistic activity exhibited by this Trichoderma species may be explained partly by the production of different classes of bioactive metabolites, including antibiotics, inhibitors of fungal growth, and enzymes. Some of us have previously reported the isolation and have elucidated the structure of a new tetracyclic diterpene, named isoharizandione, from this strain of T. viride able to inhibit fungal growth of S. rolfsii. In further studies for characterizing bioactive metabolites produced in liquid cultures of this antagonist, we have isolated another metabolite showing a significant antifungal activity in vitro and in vivo towards S. rolfsii. This metabolite, which based on preliminary spectroscopic investigations appears to be a new α-pyrones, was named viridepyronone. In this paper we report the structural constitution of viridepyronone as well it antagonistic activity against S. rolfsii.

SELECTION AND CHARACTERIZATION OF MUTANTS OF BOTRYTIS CINEREA CROSS RESISTANT TO NOVEL CLASSES OF AGRICULTURAL FUNGICIDES. G. Carrideo, F. Scala and G. Del Sorbo. 1 Dipartimento Ar.Bo.Pa.Ve., Università di Napoli, Via Università 100, 80055 Portici (NA), Italy. Fax: +39.081.7755320; E-mail: giuseppcarrideo@vodafone.it

Multidrug resistance (MDR) is the simultaneous resistance to chemically unrelated toxic compounds. In most cases, MDR depends on enhanced expression of genes encoding membrane efflux permeases, which are known as ATP binding cassette (ABC) transporters. Therefore, the activity of ABC transporters constitutes a factor influencing the efficacy of agricultural fungicides. Large families of genes encoding ABC transporters are present in all organisms, including phytopathogenic fungi. Sequencing of the whole genome of S. cerevisiae revealed the occurrence of 29 genes encoding ABC transporters. A greater number of ABC genes has been estimated for several species of phytopathogenic fungi. In B. cinerea 15 sequences encoding typical ABC domains have been cloned. However, functional analysis has been done for only three of them. The aim of this study was to identify the major ABC determinants of MDR in B. cinerea. Using substrates containing sublethal concentrations of azoxy-strobilin and fenpicolon we selected ten stable mutants of B. cinerea displaying diverse patterns of cross resistance towards a number of different classes of agricultural synthetic fungicides and natural toxic compounds. The involvement of some of the cloned ABC-encoding genes in the patterns of MDR is being determined by studying either the basal level of expression (i.e. normal vegetative growth) of single ABC genes and expression in response to treatment with different toxicants.
Enzymes from mycoparasitic Trichoderma spp. strains clearing polymers of cell walls play a major role in the antagonism of these biocontrol agents against fungal pathogens, whereas an unequivocal evidence for an analogous role of these enzymes in the activity of postharvest biocontrol yeasts is still lacking. Although transfer of genes encoding these enzymes in crop plants has increased resistance to fungal diseases, the expression of such genes from Trichoderma strains in other biocontrol microbes to improve antifungal efficacy, or to gain indirect evidence for the role of lytic enzymes in the activity of biocontrol yeasts, is yet to be explored. Gene ecb42 encoding a 42KD product from T. barcianum was placed under the control of the constitutive promoter O6C from Aspergillus nidulans. The plasmid obtained (pOecb42) was used with pAN7-1, bearing the selective marker gene bbg B (conferring resistance to Hygromycin B) in cotransformation experiments of Saccharomyces cerevisiae 404 and of the efficient postharvest biocontrol yeast Cryptococcus laurentii LS28. Hygromycin B-resistant colonies were obtained only for strain 404 in electroporation-based cotransformation, whereas no transformants were obtained for LS28 following both electroporation and gene-gun experiments. PCR analyses with primers specific for ecb42 have shown that 20 colonies of S. cerevisiae 404 contain this gene. Characterization of transformants is under way in order to: (i) assess their possible biocontrol activity; (ii) obtain fungicidal culture filtrates with high levels of endochitinase activity. Protocols of Agrobacterium tumefaciens-based transformation are being developed for solving the problems encountered with strain LS28.

The Mediterranean basin is considered an area of genetic diversification of Cucumis melo L. and in Southern Italy, Apulia in particular, an ample and diversified local germplasm of Cucumis melo subsp. melo conv. adzhur is available. This cubitcur is a remnant of melon cultivars selected for use as immature fruits. In screenings for resistance towards Sphaerotheca fusca local germplasm of C. melo subsp. melo conv. adzhur, a plant belonging to the BA7-2 ecotype resulted healthy. In the following screenings carried out on BA7-2/S progeny obtained by self-fertilization of healthy plants, three different reactions towards S. fusca were recorded: plants with severe symptoms, healthy plants and plants showing a restricted development of the disease. This last reaction consisted in the appearance on the leaves of small chlorotic areas covered with thin mycelium and with rare sporulation. On the same areas a netlike necrosis was visible. Single plants belonging to the three typologies of disease reaction were self-fertilized and the progenies were tested. All plants of progenies obtained by self-fertilization of healthy plants resulted healthy; the plants derived by self-fertilization of susceptible plants showed disease symptoms. The progenies derived by self-fertilization of plants showing limited symptoms segregated 39% of healthy plants, 28% of susceptible plants and 33% of plants with limited symptoms. This segregation ratio suggests that the resistance found in the BA7-2 ecotype of C. melo subsp. melo conv. adzhur is oligogenic. Further specific investigations on the inheritance of resistance in the BA7-2 ecotype are in progress.
INFLUENCE OF TRANSGENIC ROLABC CITRUS PLANTS ON ROOT-ASSOCIATED BACTERIA. G. Grivelli1, A. Gentile1, M. Gennari2, Z.N. Deng2, A. Rizzitano3, S. Spina4, F. Dominà1, C. Abbate1 and R. La Rosa1.

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Troyer citrus, an important citrus rootstock, was transformed with rolABC genes to modify the growth habitus and possibly the behaviour against pathogens. Twenty-three clones regenerated from independent transformation events and five wild type plants were grown in pots for two years. Morphological and physiological modifications were detected, including dwarfing (up to 80% height reduction) and increase of root system density. Root-associated bacteria and fluorescent Pseudomonads were monitored. Total bacterial population (between log 7 and log 8 CFU/g of roots) was not significantly different between 23 plant clones and five wild types examined. The number of fluorescent Pseudomonads, below the detectable level in four rolABC clones, ranged between log 4.5 and log 8 in all the others rol clones and wild types. No differences in the antagonistic activity against Fusarium solani and Phoma tracheiphila and in the IAA production of 130 fluorescent Pseudomonads were found between transgenic clones and wild types. The composition of the bacterial community, studied by ARDRA fingerprints (Amplified Ribosomal DNA Restriction Analysis), did not show any significant difference between transgenic and wild type clones. Enzymatic analysis, involving PAL (Phenylalanine Ammonia-Lyase) and PO (Peroxidase) assays, and total phenolic compounds determination were done in root samples. Total phenolic compounds, PAL and PO activities were higher in root samples of transgenic clones as compared to wild types. Investigating root-associated bacteria and potential biocontrol agents on transgenic fruit tree species provides an example for performing risk assessment studies.


Grey mould, induced by Botryotinia fuckeliana (de Bary) Whetzel, (teleomorph of Botrytis cinerea Pers.), causes heavy yield losses on numerous crops all over the world. The control of this disease requires an intensive usage of fungicides. The fungus is well known for its great adaptability and easily acquires resistance to chemicals. Fenhexamid is a fungicide highly effective against grey mould with a novel mode of action. Baseline sensitivity for B. fuckeliana was assessed by colony growth tests: EC50 was 0.1-0.3 mg ml-1; MIC was 0.3-3 mg ml-1. Fenhexamid-resistant laboratory mutants were obtained by plating conidia directly on fungicide-amended medium or previous UV irradiation (60 mJ, 99% lethality). Mutation ratios were 0.6-10-6 for spontaneous mutations, and 1.7-10-6 for UV-irradiated conidia. Two resistance levels were detected in colony growth tests: low resistance (1<EC50>10 mg ml-1; CMI>100 mg ml-1), and high resistance (EC50>100 mg ml-1; CMI>100 mg ml-1). Fenhexamid did not inhibit germination of conidia but proved a powerful inhibitor of germ tube elongation even for highly resistant mutants (EC50=1 mg ml-1). Resistant mutants were crossed with fenhexamid-sensitive reference strains of selected mating type and resistance genome (SAS405, SAR10993, SAR10995). Genetic analysis of ascospore progenies indicated that resistant phenotypes were due to mutations in single major genes inherited in Mendelian fashion in meiotic progeny and unlinked with the genes Mbc1 and Daf1, responsible of resistance to benzimidazoles and dicarboximides, respectively. Resistant ascospore progenies of two spontaneous mutants showed a reduced mycelial growth on fungicide-free medium as compared to sensitive ascospores.
in similar proportions on other plant organs. The majority of Botrytis cinerea (70%) and Botrytis cinerea (55%) isolates were resistant to one or more fungicides, while the majority of Botrytis cinerea (70%) and Botrytis cinerea (85%) isolates showed the wild-type sensitive phenotype. Further investigations are in progress on the role of the various types of B. fuckeliana isolates in the epidemiology of grey mould and fungicide resistance.

A MOLECULAR APPROACH FOR CHARACTERISING P60, A PHYTOXIC GLYCOPROTEIN COMPONENT OF THE MAL SECCIN COMPLEX. M. Reverberi, C. Betti, A.A. Fabbri and C. Fanelli. Dipartimento di Biologia Vegetale, Università degli Studi “La Sapienza” di Roma, Largo Cristina di Svezia 24, 00165 Roma, Italy. E-mail: massimo.reverberi@uniroma1.it

The mitospores fungus Phoma tracheiphila causes stem blight disease “mal secco” mainly in citrus species. Diseased plants show typical symptoms of tracheoemoycsis: chlorosis, phyloptosis, wilt and die-back of twigs and branches. Phoma tracheiphila produces several kinds of extracellular compounds during the infection. Among these products we isolated a 93 kDa toxin, probably responsible for chloroplast degradation, and a 60 kDa toxic glycoprotein, denoted P60. This glycoprotein induces foliar chlorosis followed by necrosis and is effective when the leaves are illuminated. It is probable that this toxin alters photosynthesis through a direct interaction with the chlorophyll b (chlorophyll b complex. Starting from previous studies in which 6 peptides of P60 were sequenced, 10 couple of degenerated primers were designed and used for PCR amplification on genomic DNA of 6 strains of Phoma tracheiphila. Amplifications yielded several fragments, identical in all the strains, that allowed us to deduce the probable location of the 6 peptides in the whole P60 protein. Three amplimers of 0.6, 0.8 and 1.1 kb, obtained under high stringency PCR conditions, were chosen among the generated fragments. These were subsequently cloned in pGEMT easy vector system and sequenced. The alignment were carried on using TBLASTX 2.1 and gave intriguing results that can shed some light on the mode of action of P60. The 1.1 kb PCR fragment was used as a probe in a Northern blot analysis showing that the glycoprotein P60 was expressed by a 1.8 kb mRNA.

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RESVERATROL AND BHA AS INHIBITORS OF THE GROWTH AND TOXIN PRODUCTION BY DIFFERENT TOXIGENIC FUNGI ON CEREALS. A. Ricelli1, P. Trionfetti2, Nisini1, A.A. Fabbri2, F. Taddei2, S. Zjalic2 and C. Fanelli2. 1Istituto di Scienze delle Produzioni Alimentari-CNR, Viale L. Einaudi 51, 70125 Bari, Italy. 2Dipartimento di Biologia Vegetale, Università “La Sapienza”, L.go C. di Svezia 24, 00165 Roma, Italy. E-mail: corrado.fanelli@uniroma1.it

Cereals undergo the attack of different kinds of fungi that leads to alterations of seeds which are dangerous for human and animal health. In particular mycotoxin-producing fungi still represent an unsolved challenge. In this work a novel role for butylatedhydroxyanisole (BHA) and resveratrol is studied. Both these compounds are known for their antioxidant and antimicrobial properties. BHA is already used as a preservative in fat-containing food admitted by European Union regulations. Resveratrol on the contrary, even if is extensively studied for its wide theraeutic effects (antiplatelet aggregation, antiinflammatory, apototosis inducer), has not yet been considered as a food grade preservative agent. We have tested BHA at 0.02 and 0.01% w/w and resveratrol at 230 and 23 ppm on maize seeds inoculated with Fusarium verticillioides (fumonisins producer) or on wheat seeds inoculated with ochratoxin (OTA) producer. Aspergillus ochraceus and Penicillium verrucosum. The samples were incubated for 30 days at 25°C and 0.95 a. for the fumonisins producer strain or at 28°C and 0.85 a. for the OTA producers strains. The experiments were performed on sterilised (g-irradiated) or non-sterilised seeds to investigate the importance of the interaction between the inoculated fungi and the mycoflora naturally present on the seeds. Fungal growth (as ergosterol content) and mycotoxins were monitored by HPLC. The treated samples showed an inhibition of fungal growth and of toxins production higher than

TRICHODERMA APPLICATIONS IN GRAPEVINE NURSERY FOR THE CONTROL OF PHAEOMONIELLA CHLAMYDOSPO-RA INFECTION. S. Di Marco, F. Osti and R. Roberti. Ibmct CNR, Sezione di Bologna, Via Gobetti 101, 40129 Bologna, Italy. Fax: +39.051.6399024; E-mail: s.dimarco@ibimet.cnr.it

Esca is characterized by a prolonged interaction between host-plant and pathogens, from the nursery to the established vineyard. The present study was done in order to assess the effect of Trichoderma spp. applications in the nursery both on vine cuttings and Pheaeomonella chlamydospora, the main pathogen involved in initial stages of esca development. Grafted vines were dipped in a Trichoderma suspension either before or after callusing. Pre-calling Trichoderma applications were replicated for 3 years and gave inconclusive results yielding a better callus formation in treated vines in the first year of trial only. Post-calling Trichoderma applications at the base of rootstocks provided significant development of hairy roots and reduction of the discoloured area caused by P. chlamydospora that had been inoculated in the rootstock, far from the site of application. Thus a plant defence reaction could be hypothesized. Molecular studies are in progress to investigate enzymatic activities of proteins extracted from Trichoderma treated roots. Although further investigations are needed, the use of Trichoderma may favour the production of plants more resistant to stress-related diseases, esca in particular. 

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80%, suggesting a possible use of BHA and resveratrol in the prevention of fungal contamination of food-stuffs.

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**TRAMETES VERSICOLOR INHIBITS AFLATOXIN PRODUCTION BY ASPERGILLUS PARASITICUS.** S. Zjalic1, A.A. Fabbri1, S. Reverberi1, A. Ricelli1, A. L’Aurora1 and C. Fanelli1.

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**Trametes versicolor** is a non-toxic basidiomycete with medicinal properties, widely used in China and other far eastern countries. A protein-bound polysaccharide (PSK) and a low-cytotoxic polysaccharide-protein complex (EPS) from this mushroom are used with some success as scavenger of free radicals, T-cell proliferation inducer and antiviral agent in gastric cancer. *Aspergillus parasiticus* can produce on different foods and feeds, aflatoxins, which are dangerous pollutants, mutagenic and cancerogenic. Previous studies showed a close relationship between oxidative stress and aflatoxin production, in fact, antioxidants and scavengers of reactive species can inhibit aflatoxin production induced by peroxidation. In this work, lyophilised culture filtrates and some purified fractions from *T. versicolor* were assayed as inhibitors of aflatoxin production. Different strains of *T. versicolor* were cultured in malt extract broth for 15 days in a rotary shaker at 25°C. Culture filtrates were collected and lyophilised. Subsequently, the filtrates underwent purification, ethanol precipitation, dialysis and trypsin treatment to collect glycoproteins and polysaccharides (EPS, EPSt). Fractions were analysed by the crocin test to assay their antioxidant properties. The results obtained show that EPS fractions were the best inhibitors of aflatoxin production by comparison with lyophilised and trypsinized fractions. The data obtained with crocin the test allow some speculations on aflatoxins inhibition.

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**POLYGALACTURONASE FROM AN ISOLATE OF FUSARIUM MONILIFORME ESCAPES INHIBITION BY PLANT PGIPs.** L. Sella1, S. Roberti1, C. Castiglioni1, R. D’Ovidio2 and F. Favaron1.

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Interference with pathogenesis mechanisms of plant parasites may be a strategy to be exploited for increasing plant resistance to diseases. Most fungal pathogens produce endo-polygalactur- onases (PG) during infection of plants. Demonstrations of a crucial role of PG in fungal pathogenicity are increasing since the successful development of pg gene knock out experiments. Plants have developed glycoprotein inhibitors to counteract fungal PG activity. These molecules, named polygalacturonase-inhibiting proteins (PGIPs), are effective against PG of most fungi. It has been demonstrated that overexpressed PGIP may be effective to reduce disease symptoms by fungi. However, it may happen that a particular plant PGIP may fail to inhibit a specific fungal PG. So far, however, a PGIP able to inhibit a specific fungal PG has always been detected in cultivated or spontaneous plants. In the present work we report the identification of a fungal PG that is able to escape PGIP inhibition. This PG is produced by the isolate PD of *Fusarium moniliforme* and is resistant to inhibition by PGIP from a number of dicot and monocot plants (common bean, soybean, leek, Arabidopsis, wheat and rice PGIPs). Since other *F. moniliforme* isolates produce PG that are inhibited by at least one plant PGIP, the PG of the PD isolate of *F. moniliforme* is a valuable molecule to understand the structural basis of PG-PGIP specificity. Cloning and sequencing of this particular pg gene can contribute to identify aminoacidic substitutions crucial for the recognition by plant PGIPs.

**USE OF CHEMICAL AND BIOLOGICAL PRODUCTS TO CONTROL THE TAKE-ALL DISEASE OF TRITICUM DURUM: F. L. Flaminì1, P. Nipoti2, A. Pisi3, S. Gennari4 and A. Mirotti1.** 1 ASSAM, Servizio Fitosanitario Regionale, Via Alpi 21, 60131 Ancona, Italy. E-mail: flaminì_lucio@assam.marche.it. 2 Dipartimento di Scienze e Tecnologie Agroambientali, Università Te- logoa, Viale Fanin 42, 40127 Bologna, Italy. 3 C.D.F.s.r.l., Via Amendola 40, 48022 Lugo, Italy.

Take-all is a widespread and destructive disease of wheat and other cereals in temperate climates. The pathogens of take-all are soilborne fungi, *Gaeumannomyces graminis* being one of the most important. Infected plants appear in patches, develop poorly and remain stunted, showing brown-black dry root rot that usually extends from the base of the stem up to the lower leaves. The control of soil-borne pathogens is possible by increasing saprophytic and antagonistic mycoflora and/or treating the seeds in order to protect the seedlings in the initial stage of development. Chemical and biological products were tested in a field experiment in the Marche region. At the beginning of the trial twenty soil samples were examined to monitor the mycoflora in the field. Different Theses were compared: seeds treated with chemical and biological products and a suspension of a natural strain of *Tricho- derma viride* added to the soil before sowing. The efficacy of the treatments was evaluated by agronomic parameters: number of growing plants per m², a scale of 0-9 for the assessment of plant vigour at bunching, number of ears per m² and yield. The treatments showed variable effects on measured parameters. Nevertheless, interesting results were obtained in the initial vegetative stage of wheat in *T. viride* and guazatina treatments.

**TOLERANCE OF A PUMPKIN SELECTION TO MONOSPORA CANNONBALLUS ROOT ROT/VINE DECLINE, USED AS ROOTSTOCK OF MUSKMELON (CUCUMIS MELO L.) AND TELLURIC BIOCONTROL WITH TRICHODERMA SPP.** A. Mirotti1, M. Sportelli1, L. Flaminì2, and L. Pizzichini3.

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Monosporascus cannonballus Pollack et Uecker is a soilborne fungus that causes a severe root rot of muskmelon (*Cucumis melo L.*) and watermelon (*Cucumis citrullus* Ser.) in several areas of the world. The first report of *M. cannonballus* in Italy (Gennari et al., 1999, *Informatore Fitopatologico* 49 (1-2): 38-40) was in 1997 on watermelon in the Emilia Romagna region. During a four-year experimentation (1999-2002) in the Marche region for setting up bio compatible techniques to overcome *M. cannonballus* root rot/vine decline of melon, a tolerant pumpkin selection (RS841), currently used as watermelon rootstock, was identified. In 2002, field trials were carried out to test the tolerance to *M. cannonballus* of RS841 used as rootstock of cv. Century, a sensitive melon variety, and the biocontrol efficacy of *Trichoderma* spp. strains isolated in the Marche region from horticultural soil. In this case studies were carried out by UE (grants PLQLRT1999-00996 and PLQLRT1999-00433).

**MONOSPORACUS CANNONBALLUS ROOT ROT/VINE DECLINE, USED AS ROOTSTOCK OF MUSKMELON (CUCUMIS MELO L.) AND TELLURIC BIOCONTROL WITH TRICHODERMA SPP.** A. Mirotti1, M. Sportelli1, L. Flaminì2, and L. Pizzichini3.

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study, conducted in a field naturally infested by *M. cannonballus*, RS841 showed a good level of tolerance to the fungus, with a significant reduction in disease rating with respect to non-grafted plants of cv. Century. Grafted plants produced also better quality fruits. The Marche strains of *Trichoderma* showed antagonistic efficiency, increasing plants vigour and the productivity by comparison with non-grafted cv. Century plants. This is the first report in Italy of tolerance of a pumpkin selection to Monosporascus root rot/vine decline and of the possible use of this selection as a rootstock for melon.

**VARIATION OF PEROXIDASE ACTIVITY IN SUGAR BEET LEAVES INFECTED WITH CERCOSPORA BETICOLA SACC.**

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*Cercospora beticola* Sacc. is a sugar beet necrotrophic pathogen that finds optimal conditions in warm humid climates. Resistance to *C. beticola* is polygenic and rate reducing. In particular, a reduced and slower growth of the pathogen was observed inside the tissues of “resistant” hosts. In sugar beet-*Cercospora* system, peroxidase activity in foliar tissues was positively related to plant resistance. After a preliminary screening for peroxidase activity among different cultivars, a greenhouse trial was conducted with plants belonging to cvs. Brek partially resistant) and Duetto (totally susceptible). Foliar sample collected every 3-4 days after inoculation were analysed for peroxidase activity in superficial and intercellular washing and in foliar homogenates after washing. On the foliar surface no relevant activity was detected. In the homogenate, the “resistant” cultivar always showed values statistically higher than the susceptible one. On the contrary, foliar tissues of both cultivars responded in the same way to the infection, with a statistically significant increase of peroxidase activity at the onset of necrosis, till a constant value of 18,000 U g⁻¹ of leaf. Cultivar response as judged by intercellular washings was similar but earlier than that observed in foliar homogenates. Four days before lesion appearance, a statistically significant marked increment of activity was detected, that reached a peak of 22,000 U g⁻¹ f.w. then decreased, but retained values higher than those observed before inoculation.

**EFFECTIVENESS OF SOME CHEMICAL TREATMENTS AGAINST HETEROBASIDION SPP. IN SPRUCE FORESTS AND THEIR ECOLOGICAL IMPACT.** P. Gonthier, G. Nicolotti and G.P. Cellerino. Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. Fax: +39.011.6708544; E-mail: paolo.gonthier@unito.it

The *Heterobasidion annosum* complex includes some widely distributed root and butt rot agents of coniferous trees. Anosous diseases are known to spread via root grafts or spores that infect fresh stumps. Stumps are thus potential reservoirs for *Heterobasidion* infection, and stump treatment is the only control method that has proved sufficiently efficient to be widely practised. This paper reports on the effectiveness of five chemical treatments against *Heterobasidion* on spruce (*Picea abies* (L.) Karst.) stumps in the Western Alps (Aosta Valley). The effects of such treatments on non-target fungi were also investigated, to assess their selectivity and their potential ecological impact. Results were compared with those of *Phlebiopsis gigantea* (Rosttop). At least 20 healthy spruce stumps were treated in 1996 and 1997 with each of the following products: borax powder, copper oxychloride (1% w/v), and urea at three different concentrations (30%, 20%, 10% w/v). Their effectiveness was assessed two years after treatment. Most treatments reduced significantly (P< 0.01) *Heterobasidion* infection compared with controls (all infected). The number of uninfected stumps was ranged from 4% for the treatment with 10% urea to 74% for copper oxychloride. *P. gigantea* protected about 80% of stumps. The patterns of colonization of stumps by non-target fungi were investigated one and two years after treatment. Multivariate analyses showed that borax had strong and persistent effects on the natural mycoflora. Transient effects were shown by most treatments, including some that were effective against the pathogen, whereas the influence of *P. gigantea* seemed to increase over time.

**ISOLATION AND CHARACTERIZATION OF A SERIN-PROTEASE FROM PHIALOPHORA MALORUM.** R. Gregori, M. Mari, P. Bertolini and G.C. Pratella. CRIOF - Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Via Gandolfi 19, 40057, Cadorino (Bologna), Italy. E-mail: gre gro@tin.it

Fungal proteases are a wide group of enzymes that catalyse the hydrolysis of proteins and are relevant in the breakdown of host cells. Little is known about their role in the host-pathogen interaction. These enzymes seem to play a role in cell wall degradation and probably stimulate other enzymes involved in the hydrolysis of cell walls. The aim of this study was to determine the protease activity of *Phialophora* sp., the agent of root rot of pear fruits. Enzyme activity was tested in Petri dishes with solid medium and in flasks with liquid medium containing gelatine (2%) and in artificially inoculated fruit. The solid medium, inoculated with pathogen was incubated at 25°C for at least 14 days and the halo surrounding the colony was measured. In liquid medium and in inoculated fruit, protease activity was determined by spectrophotometer at 280 nm after 21 days at 20°C and after 2 months at 0°C. A relationship was found between halo diameter in solid medium and the amount of protease in liquid medium and in rotted fruits. The amount of protease produced by the various *Phialophora* strains ranged from 7.14 mg ml⁻¹ (APO4) to 10 mg ml⁻¹ (CRE2). In order to identify the various proteases produced by *Phialophora* the following inhibitors were tested on solid medium: EDTA, leupeptin, pepsinatin and PMSF. Enzyme activity was inhibited only by PMSF, a serin-protease inhibitor. A protease with a molecular weight of 42 kDa was identified by SDS-PAGE.

**BIOMOLECULAR STUDIES ON COLD INJURY IN ITALIAN SQUASH FRUITS.** S. Gualanduzzi², E. Baraldi¹ and A. De Santis². Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, Via Fainin 46, 40126 Bologna, Italy. E-mail: gualanduzzi@yahoo.it. 2Laboratorio di Fisiologia Vegetale, Università Politecnica delle Marche, Via Brecce Bianche, 60100 Ancona, Italy. E-mail: desantis@agrsci.unibo.it

After several days of cold storage, Italian squash, the same as the majority of fruits and vegetables, undergo physiological changes, causing cellular damages. In this study, the respiratory pathways of isolated mitochondria and some enzymatic activities of fruit tissues that are involved in defence against ROS (Reactive Oxygen Substances) were evaluated during cold storage. In summer 2002, Italian squash fruits (*Cucurbita pepo* L. var. Giamb) were harvested and stored at 0°C or at 10°C. During refrigeration, H₂O₂, lipid peroxidation amount (as TBARS = Thiorbarbituric Acid Reactive Substances) and catalase and peroxidase activity were determined. Results were correlated with mitochondrial respiratory activity determined as COX, AOX and PUMP activities. Storage at low temperature (0°C or 10°C) influenced the activity of antioxidant enzymes, i.e. fruits conserved at 0°C
suffered a major cold injury than those conserved at 10°C. Decreasing levels of \( \text{H}_2\text{O}_2 \) were observed in pulp tissues from the fifth day at 10°C storage. This correlated with a decrease in respiration. Increasing concentration of TBARS was observed from the seventh day of storage. This correlated with no increase in catalase activity and a scarce increase of peroxidase activity. Cold injury caused by oxidative stress was relatively high, for at 10°C an insufficient increase of the antioxidant defence system occurred. Cold injury appeared more severe when fruits were stored at 0°C.

**STUDY OF PYRENOPORA GRAMINEA PATHOGENICITY GENES BY HOMOLOGY-BASED REVERSE GENETICS. A. Haegi, A. Infantino and A. Porta-Puglia. Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00136 Roma, Italy. Fax: +39.06.86802296, E-mail: epid@resist@ispave.it**

Pyrenophora graminea, a hemibiotrophic fungus, is the causal agent of leaf stripe of barley. The biomolecular aspects of the interaction are presently under study with different approaches. To study the late phases of the infection process, especially the switch from biotrophy to necrotrophy, a homology-based reverse genetic approach has been chosen. It is based on the isolation of the homologous of a candidate gene from the organism of interest and on the evaluation of its involvement in pathogenicity. In *Colletotrichum lindenmuthianum* it has been shown that a transcriptional activator belonging to the fungal zinc cluster (Zn [III], Cys] family (Gal4-like protein) is involved in the switch between biotrophic and necrotrophic phases of the fungal infection (Dufresne et al., 2000, *The Plant Cell* 12: 1579-1589). Similarly, we hypothesized that a Gal4-like protein could be involved also in *P. graminea* infection process. To check the presence of a homologous gene in the genomic DNA of *P. graminea* and possibly to isolate this gene, degenerate primers were designed on conserved regions of Gal4-like proteins of several fungi. Twenty-one primers were designed on the binding domain of the protein which includes the zinc cluster (5’ primers) and on the middle homology region (3’ primers). Several combinations of these primers were used for PCR analysis and different amplified bands were identified. Two significant bands have been cloned and sequenced. One of them could represent a part of a Gal4-like protein and will be used to isolate the gene.

**MYB4 REGULATION OF STRESS RESPONSES: NO CHANCE FOR PATHOGEN AND ENVIRONMENT CHALLENGES. M. Iriti¹, C. Vannini², M. Bracale², F. Locatelli², M. Mattana³, G. Farina¹, F. Faoro¹ and I. Corgaggio¹. ¹Istituto di Patologia Vegetale, Università di Milano, Italy. ²DBSE, Università dell’Insubria, Varese, Italy. ³CNR: IBBA e IVV, Sezione di Milano, Via Celoria 2, I-20133 Milano, Italy. Fax: +39.02.30316781, E-mail: coraggio@ibba.cnrs.it**

Osmyb4 encodes a rice transcripion factor, belonging to the Myb family, induced by cold. It has been previously demonstrated that its constitutive expression in transgenic *Arabidopsis thaliana* plants results in improved cold and freezing tolerance. Furthermore, cDNA microarray analysis indicated a comprehensive report of target genes up-regulated by OsMyb4, involved in both defense and abiotic responses. To verify if the overexpression of these genes makes the Osmyb4 transgenic *A. thaliana* plants more tolerant to both biotic and abiotic stresses, we have either inoculated them with different pathogens (*Botrytis cinerea, Pseudomonas syringae pv. tomato, Tobacco necrosis virus-TNV*) or irradiated with UV light (\( \lambda = 254 \times 40 \) min), or fumigated with ozone (300 nL⁻¹ x 3 h). OsMyb4 transformed plants appeared to be resistant to all the tested pathogens as they did not develop any symptoms. In contrast, wild type (wt) plants showed severe symptoms, typical of the inoculated pathogen within 3-8 days from infection (necrotic lesions in the case of TNV, large necrotic areas around infiltration site for *P. syringae* and light brown lesions covered with fungal mycelium for *B. cinerea*). UV treatment or ozone fumigation did not induce any cell death in the transformed plants, in spite of the high levels of irradiation or fumigation, while numerous dead cells were already present in the leaf mesophyll of wt plants 24 h after treatments. These data demonstrate that OsMyb4 overexpression in *A. thaliana* leads to pathogen resistance and abiotic stress tolerance, opening a new interesting perspective in plant protection from both pathogens and environmental injuries.

**VARIATION OF PEROXIDASES, b1,3-GLUCANASES, CHITINASE AND b1,4-GLUCOSIDASES IN TOBACCO PLANTS INDUCED BY AN ISOLATE OF GLOCIADIUM ROSEUM (GNL). E. Laboz, R. Castillo, F. Porrone and A. Carella. Istituto Sperimentale per il Tabacco, Via P. Vitello 108, I-84018 Seaft, Italy. Fax: +39.081.8506206, E-mail: e.laboz@uniplan.it**

Induced systemic resistances (ISR) is defined as the enhanced resistance exhibited by a properly stimulated plant, following challenge inoculation with a pathogen. Hypersensitive reaction was first related to ISR, then it was observed that challenge inoculation of distant tissues resulted in a smaller and a fewer lesions (systemic acquired resistance, SAR) than in non induced plants. This response is related to a several biochemical changes in the plants. In greenhouse experiments, tobacco seedling roots were inoculated with an isolate of *Gloecidiadum roseum* (GNL) well-known as an antagonist of many plant pathogens. Two weeks later, vegetative growth was recorded before the plants were dissectioned in leaves, stalk and root. Lyophilised leaves were extracted with appropriate buffer and extracts were analysed for b1,3-glucanases, b1,4-g1coclases, N-acetyl-b-D-glucosaminidase and b-D-N-N’-diacyctyl-chitobiode activities. The methods used were: quantification of reducing sugar using laminarine as substrate for glucanase, and spectrophotometric evaluation of the release of p-nitrophenol from p-nitrophenyl-b-D-glucopyranoside, p-nitrophenyl-N-acetyl-b-D-glucosaminide and p-nitrophenyl-b-D-N-N’-diacyctylchitobiose for the last three enzymatic activities, respectively. Peroxidase isoyme patterns were determined by polyacrilamide gel electrophoresis (PAGE). Leaves of plants inoculated with GNL, in comparison with the uninoculated control showed: (i) an increased activity of b1,3-glucanases, b1,4-g1coclases and N-acetyl-b-D-glucosaminidase; (ii) a new band and an increased intensity of other two bands in peroxidase isoyme pattern; (iii) no increase in the level of chitobioside activity. This is the first demonstration of such a behaviour by a *Gloecidiadum roseum*.
Inorganic and organic salts, organic acids and natural gums, mainly consisting of natural compounds used as additives in the food industry, were assayed in vitro and in vivo in order to improve the activity of Cryptococcus laurentii (Cl) and Aureobasidium pullulans (Ap), two antagonist yeasts of postharvest pathogens. In vitro tests, based on their combined effects on the two yeasts and on the postharvest fungal pathogens Botrytis cinerea and Penicillium expansum, the compounds were ranked as follows: (i) products increasing or not affecting the growth of one or both the antagonists but inhibiting the pathogens; (ii) products inhibiting one or both antagonists but increasing pathogen growth; (iii) products inhibiting both antagonists and pathogens; (iv) products either increasing or not the growth of both antagonists and pathogens. Generally, the radial growth of B. cinerea in comparison with that of P. expansum was more consistently inhibited by the same substance. In in vitro tests, the selected substances, alone or combined with the yeasts, were applied on wounded apples inoculated with P. expansum. Different compounds significantly increased the antagonistic activity of the yeasts and showed additive or synergistic effects. The results of this investigation demonstrated that some substances can potentially be used as additives to improve the efficacy of yeast-based biofungicides.

AGROBACTERIUM-MEDIANED TRANSFORMATION OF DIAPORTHE HELIANTHI. B.A. Maimone Mancarello1,2, M. Finetti Sialer3, M.G. Li Destri Nicosia3, G. Del Sorbo1, A. Zoina1, G. Vannacci and G. Del Sorbo1. 1Dipartimento Ar.Bo.Pa.Ve., Università di Napoli, Portici (NA), Italy. 2Dipartimento S.En.Fi.Mi.Zo., Università di Palermo, Italy. 3Dipartimento S.En.Fi.Mi.Zo., Università del Molise, Italy. E-mail: bartolo.m@libero.it

Diporthe helianthi is the causal agent of sunflower stem canker. Molecular analyses showed that isolates originating from France and former-Yugoslavia, where the disease causes severe yield losses, form a monophyletic group which differ from isolates originating from other countries, including Italy, where the disease occurs to a limited extent. RAPD analysis allowed detection of a polymorphism between French and Italian isolates. Sequence analysis of a 580 bp polymorphic band from a highly virulent French isolate revealed high homology with fungal genes encoding polyketide synthases in several species of filamentous fungi (i.e. mckA and mckB of P. citrinum, kwbB of A. terreus, fun3 of G. moniliformis, pks1 of C. heterostrophus, pks1 of G. fujikuroi). For this reason, the fragment was designated Dhpks1 (= D. helianthi gopyketic synthase 1). Ultrastructural studies showed that production of phytotoxins occurs during pathogenesis of D. helianthi. A polyketide phytotoxin, named phomozin, is produced during Diporthe pathogenesis in sunflower plants. The sequence of Dhpks1 was extended to 2309 bp and an Agrobacterium-mediated transformation system for D. helianthi was set up. The possibility that virulence of D. helianthi could depend on the ability to produce a polyketide toxin is being studied in mutants selectively disrupted in Dhpks1.

AGRONOMICAL TECHNIQUES TO INDUCE SOIL SUPPRESSIVENESS TOWARD SOIL-BORNE PATHOGENS. PRELIMINARY RESULTS AFTER A THREE-YEAR FIELD TRIAL. L.M. Manici, F. Caputo and P. Bonora. Research Institute for Industrial Crops (ISCI), Via di Corticella 133, 40128 Bologna, Italy. Fax: +39.051.374857; E-mail: lmanici@isci.it

Root rot is an economically important root disease occurring in vegetable crops, fruit trees, and field crops throughout the world. It is caused by several saprophytically living pathogens among which Rhizoctonia and Pythium are the most frequent. An open field trial has been carried out since 1999 to evaluate the impact of several microbial parameters of soil suppressiveness towards the root rot complex. Five soil management treatments were applied to a field without root rot problems that had undergone crop rotation in the past, to induce differences in the microbial populations and to study their potential role in soil suppressiveness towards root rot.
pathogens. The trial, performed at the ISCI experimental farm, in the East Po Valley (Budrio, Bologna), was organized in a randomised block design with three replicates. The five treatments were: (i) monoculture of 3 cover crops: cereal, crucifer and leguminous species; (ii) a 3-year rotation of winter cover crops; (iii) continuous weed-free fallow. In early May, cover crops were cut and the above ground part of each crop was removed, then tillage to a depth of 20-25 cm was done several times until the next autumn. After three years fallow, the microflora was lower than in all cultivated plots, despite an unvaried level of organic matter. *Rhizoctonia solani* AG 2-2 isolated from sugar beet, artificially inoculated in pots containing soil coming from each treatment, gave the highest disease rate on sugar beet seedling in soil from fallow plots. These results are taken as an indication that a 3-year fallow is the treatment with the lowest suppressiveness towards *Rhizoctonia*.

**COMPOT AS VECTOR OF A TRICHODERMA STRAIN: PRELIMINARY RESULTS.** M. Montanari, M. Ventura and G. Innocenti. Dipartimento di Protezione e Valorizzazione Agroalimentare, Sez. Patologia Vegetale, Alma Mater Studiorum, Università degli Studi di Bologna, Viale Fanini 46, 40127 Bologna, Italy. Fax: +39. 051. 2096365, E-mail: innocenti@agrsci.unibo.it

Composting is a biological process in which organic biodegradable wastes are converted into a hygienic, humus-rich product (compost) used in agriculture as amendment to improve soil structure and to promote plant growth. Compost has recently been shown to have also the potential to provide biological control against plant diseases. However this suppressive effect, which is related to indigenous microbial consortia, is not stable, depending mostly on the origin and quality of the compost. The enrichment of compost with selected strains of microbial antagonists could improve the stability and reproducibility of biological control effect. The establishment of a *Trichoderma atroviride* strain in different organic products was studied. Spent mushroom (*Agaricus bisporus*) compost taken immediately after steaming at the end of the production process or three months later, mixed compost, derived from household organic and green wastes, taken one month after heat peaking were enriched with a spore suspension of the antagonist fungus. The survival, saprotrophic activity, and root colonisation ability of the *T. atroviride* strain over a period of 140 days were evaluated. The results of this study indicated that old compost seems to be more suitable than any of the other tested products for the establishment of the antagonistic fungus. In fact, this product was able to stably sustain the population level of *T. atroviride* obtained by enrichment with conidial suspension at the beginning of the experiment over the experimental period. Moreover, this product promoted the highest levels of saprotrophic activity and root colonisation ability of the fungus added.

**EFFECTS OF TETRACONAZOLE ON SENSITIVE AND RESISTANT STRAINS OF CERCOSPORA BETICOLA SACCO.** M. Moretti1, M. Saracchi1, G. Farina1 and F. Gozzo2,1Istituto di Patologia Vegetale, Università degli Studi di Milano. 2DISMA, Università degli Studi di Milano, Via Celoria, 2, 20133 Milano, Italy. Fax: +39.02.50316781; E-mail: gandolfina.farina@unimi.it

This study reports the effects of tetraconazole, one of the most effective IBS fungicides used to control *Cercospora* leaf spot of sugar beet, on growth, micromorphology of fungal structures, and sterol composition of two strains of *C. beticola*: a wild type field isolate (M3), and an UV mutant strain highly resistant to tetraconazole (3a). The relationships between morphological alterations observed by light microscopy and sterol biosynthesis inhibition induced by tetraconazole treatment were evaluated on broth cultures. The effects of fungicide presence on hyphal development were also investigated by scanning electron microscopy on agar cultures. Morphological alterations in broth cultures, consisting in irregular swelling and excessive septation and branching of hyphae, were profusely present at 0.1 ppm tetraconazole in M3, while in the resistant strain 3a, at the same dose, alterations were rare. To have the same level of morphological alterations as in M3, a dose of 25 ppm tetraconazole was needed. Sterol composition was qualitatively the same in untreated M3 and 3a. The sensitive strain accumulated higher amounts of 14-methylsterols under the effect of tetraconazole treatment. Growth inhibition and morphological alterations were correlated, more than with a depletion of functional sterols, with the accumulation of abnormal sterols, which induced morphological alterations that appear to anticipate growth inhibition. This study showed that, in the fungal strain under study, tetraconazole resistance is probably not due to a target site mutation. Preliminary tests suggest that an energy-dependent efflux of the fungicide may be involved.

**COMPETITION FOR SPACE AND NUTRIENTS PLAYS A ROLE IN THE BIOCONTROL ACTIVITY OF CANDIDA GUILLERMONDI AND SACCAROMYCES CEREVISIAE AGAINST PENICILLIUM EXPANSUM ON APPLE.** G. Ortu, B. Scherm, A. Muzzu, M. Budroni, G. Arras and Q. Miglieli. Dipartimento di Protezione delle Pianta, Università di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; E-mail: migieli@uniss.it

Penicillium expansum causes severe rots of apple fruits during storage and shelf life. Aiming at the development of new antagonistic yeast active in controlling postharvest pathogens of fruits, several yeast isolates were obtained from fig (*Ficus carica*) and prickly pear (*Opuntia ficus-indica*) grown in untreated orchards in Northern Sardinia (Italy). Two strains of the yeast *Candida guillermondii* were selected for their remarkable antagonistic properties against *P. expansum* in apple. A film-forming strain of *Saccharomyces cerevisiae* isolated from wine was also included in the experiments. In trials carried out in cv Golden Delicious and Fuji, the yeasts applied alone or in the presence of various additives reduced apple rot up to 100%. Killed yeast cells and culture filtrates had no biocontrol activity. Addition of different sugars to apple wounds had no detrimental effect of the biocontrol potential of the tested yeasts. Conversely, several nitrates significantly inhibited the antagonistic capability of both *C. guillermondii* and *S. cerevisiae*, thus suggesting that competition for nitrogen may play a role in the biocontrol properties of the tested antagonists.

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**ULTRASTRUCTURAL ANALYSIS OF VITIS VINIFERA LEAF TISSUES SHOWING ATYPICAL SYMPTOMS OF DOWNY MILDEW.** R. Musetti1, L. Stringher1, S. Borselli1, A. Vecchione2, F. De Luca2, L. Zulini2 and I. Pertot2,1Dipartimento di Biologia Applicata alla Difesa delle Piante, Università di Udine, Via delle Scienze 208, 33100 Udine, Italy. 2Istituto Agrario San Michele all’Adige, Via Machi 1, San Michele all’Adige (TN), Italy. Fax: +39.0432.558501; E-mail: Rita.Musetti@pdelfe.unipd.it

In a biodynamic farm of Tuscany a progressive regression of downy mildew outbreaks year after year and a decrease of the virulence, vigour and fertility of the causal agent (*Plasmopara viticola*) were observed. In particular, anomalous lesions caused by the fungus in leaf tissues of sensitive cultivars of *Vitis vinifera* were observed. These atypical symptoms were often associated with typical “oil spots” and occurred in favourable environmental conditions for a normal development of the disease. To understand
the cause of this phenomenon, an ultrastructural study was carried out on grapevine leaf tissues to detect possible alterations of P. viticola mycelium and the possible presence of endophytes. Moreover, isolations were made on PDA medium from grapevine leaves by strip, washing the surface with sterile water, and sampling small leaf sections. Ultrastructural analysis by transmission electron microscopy (TEM) showed that P. viticola hyphae were present in grape leaves showing typical “oil spot” symptoms: they were localized in the substomatal zone and in the intercellular spaces of spongy parenchyma and appeared generally vacuolated. Well-structured haustoria were also present. On the contrary, samples from atypical leaf symptoms had empty intercellular hyphae and necrotic haustoria, while the surrounding asymptomatic leaf tissues contained no hyphae and showed heavy cellular alterations (phenolic accumulation and chloroplast damage, dark material in the xylem, and fibrils differing from P-protein in the phloem). A number of bacteria, filamentous fungi, and yeasts were isolated from the leaf surface, while from inner leaf tissues fungi belonging to the genera Aspergillus, Fusarium, Alternaria, Stemphylium, and Phoma were recovered. All these isolates were tested as possible antagonists of P. viticola, and showed different efficacy against the pathogen.

SEARCHING PENICILLIUM ISOLATES ANTAGONISTIC TO RHIZOCTONIA SOLANI IN SUPPRESSIVE SOILS IN SALENTO. R. Nicotelli, F. Raimo and A. Carella. Istituto Sperimentale per il Tabacco, Via Vittello 66, 84018 Scatarti (SA), Italy. Fax: +39.081.8506206; E-mail: ronietc@libero.it

Rhizoctonia solani is the cause of damping-off and sore of tobacco skin. These diseases have been observed in all the main tobacco-growing areas in Italy except Salento, where their occurrence seems to be restricted to seedbeds. Rather than agronomic factors, suppressiveness of local soils toward R. solani may be related to the presence of fungal antagonists. Several fungal species, i.e. Verticillium biguttatum, Gliocladium roseum, Leucanicium psalliota, Trichoderma aureoviride and T. barzianum, have already been recovered from soil samples collected in the area that showed a remarkable inhibitory capacity in vitro and/or in vivo. So far antagonism of Penicillium species against R. solani has been little investigated. However, since in a previous study some isolates proved to be effective antagonists of the pathogen, a more thorough study on the presence and activity of Penicillium spp. in soils cropped to tobacco was started. Isolations were carried out on a semi-selective substrate (G25N). About 40 isolates were collected and classified according to updated taxonomic keys. The most represented species belonged to the section Dicharactrium in the Furcatum subgenus (e.g. P. janzewskii, P. walkeri). Their antagonistic behaviour against R. solani was preliminarily assessed in vitro, both in dual cultures and by addition of 20% (v/v) concentrated culture filtrates in the growth medium of the pathogen. About 50% of the isolates tested showed some inhibitory capacity. Their secondary metabolite production will be further investigated.

EXPRESSING OF BCA-TO, A NOVEL GENE ENCODING AN ABC TRANSPORTER OF BOTRYTIS CINEREA, IS INDUCED BY OXIDATIVE STRESS. C. Pane, P. Ambrosino, M. Pasqua-riello, F. Scala and G. Del Sorbo. Dipartimento Ar.Bo.Pa.Ve., Università di Napoli, 80055 Portici (NA), Italy. Fax: +39.081.7755320; E-mail: castello.pane@libero.it

ABC transporters are widespread ATP-driven efflux pumps which secrete endogenous and exogenous compounds. In Botrytis cinerea, as in other fungi, these permeases play an essential role during pathogenesis in protection against plant defense com-

pounds and fungicides. Using degenerate primers designed on highly conserved sequences we cloned BcatrO (GenBank Acc. No. AF259075), a novel gene encoding an ABC transporter in B. cinerea. Among the closest homologues of the BcatrO-encoded protein there are products of genes involved in fungal pathogenesis, such as Mgmtr2 of Mucor pusillus, fumigatus, ABC1 of Magnaporthe oryzae and Ghb1 of Gibberella fujikuroi. BcatrO expres-

sion was studied by RT-PCR on RNA isolated from mycelium of the wild-type strain SANS6 of B. cinerea grown in vitro and in samples collected from infected bean leaves. Transcript accumulation of BcatrO is induced by hydrogen peroxide treatment in a dose-dependent manner but not by known inducers (i.e. cycloheximide and fenpropimorph) of transcription of other ABC genes of B. cinerea. Analysis of BcatrO during synchronized infection of bean leaves revealed a peak of transcript accumulation 6 h after inoculation, when the growth of fungal biomass, as determined by expression of the actin gene, occurs to a limited extent. These evidences indi-

cate that the product of BcatrO could be involved in protection of B. cinerea from reactive oxygen species (= ROS) which are generated during the first stages of the fungus-plant interaction.

A SCANNING ELECTRON MICOSCOPY STUDY OF ROOT CA-POLYGALACTURONATE FIBRILS IN THE PRESENCE OF ALUMINUM. A. Pisi, T. Mimmo, C. Marzadori, G. Filippini and C.E. Gessa. Dipartimento di Scienze e Tecnologie Agroambientali, Laboratorio di Microscopia Elettronica ed Area chimica agraria, Alma Mater Studiorum, Università di Bologna, Viale Fainini 50, 40127 Bologna, Italy. Fax: +39.051.2091439; E-mail: apisi@agrsci.unibo.it

Electron microscopy studies have demonstrated the presence of mucilaginous fibrils at the soil-root interface. This mucigel greatly increases the area of contact between root surface and soil mineral particles, influencing the transport of ions through
shows the effect of Al\textsuperscript{3+} on the morphological characteristics of the root mucigel and the primary cell wall. Individual fibrils within aggregates are curved, branched and intimately associated with each other to form complex, three-dimensional webs. This complex system, which results from fibrillar arrangements of the polymer, defines the free space volume that mainly depends on the type of the reticulating ion. Aluminium toxicity is one of the major stress factors in acid soil. The mucigel is able to bind a large amount of aluminium contributing significantly to the total amount of the metal in the roots. Almost no informations are available about the effect of the binding of aluminium onto the fibrillar structure of the Ca-PG present at the soil-root interface. This study, using scanning electron microscopy techniques, shows the effect of Al\textsuperscript{3+} on the morphological characteristics of the Ca-PG network, used as a model of the soil-root interface.

CHANGES OF FREE AMINO ACID LEVEL IN SHOOT CULTURES OF CATHARANTHUS ROSEUS INFECTED BY PHYTOPLASMAS. E. Raffone\textsuperscript{1}, F. Nae\textsuperscript{2}, G. Mastrolantonio\textsuperscript{1}, P. Carillo\textsuperscript{1}, A. Fuggi\textsuperscript{1}, S. Paltrinieri\textsuperscript{2} and A. Bertaccini\textsuperscript{1,2}.\textsuperscript{1}Dipartimento di Scienze della Vita, Seconda Università di Napoli, Via Via- valdi 43, 81100 Caserta, Italy. \textsuperscript{2}DiSTAV - Patologia Vegetale, Università di Bologna, Via F. Re, 40126 Bologna, Italy.

Free amino acids have a fundamental role in plant metabolism, being intermediates of many metabolic pathways involving the synthesis of all nitrogen-containing compounds; therefore changes in their concentrations should occur when plants activate defence mechanisms against pathogens. Pattern of free amino acids in phytoplasma infected micropropagated shoots was studied to look for specific changes that could represent early markers of pathogen presence and indicate metabolic alterations that could help in understanding infection mechanisms. Tissues shoot culture of Catharanthus roseus L. (periwinkle) were grown in vitro on Murashige and Skoog’s (MS) nutrient medium supplemented with BAP 0.1 mg l\textsuperscript{-1} under controlled conditions. Shoots were either infected by phytoplasma strains belonging to 16SrXII-A (stolbur group), 16SrV-A group (elm yellows) and 16SrI-B group (aster yellows) or healthy. Phytoplasma identification was performed by PCR/RFLP analyses of 16S ribosomal genes. Free amino acid contents were determined by an HPLC system. Multivariate analysis was done through the MINITAB software package. After 30 days of growth, disease specific symptoms were present in infected shoots. Among the amino acids that showed differences between phytoplasma-infected and healthy shoots the main changes occurred in arginine, valine, tyrosine, alanine and lysine concentration that was around 70% lower in the infected materials.

The work was supported by “Seconda Università di Napoli”, “Ministero dell’Università e della Ricerca Scientifica e Tecnologi- ca”, and CNR of Italy.

ENDOPHYTIC AND ENZYMOPATHOGENIC ROLE OF TWO STRAINS OF BEAUVERIA BASSIANA (BALS.) VUILL.: PRELIMINARY APPROACHES. M. Rodolfi, R. Groppalli and A.M. Picco. Dipartimento di Ecologia del Territorio e degli Ambienti Territoriali, Università degli Studi di Pavia, Via S. Epifanio 14, 27100 Pavia, Italy. Fax: +39 0382 34240; E-mail: apicco@et.unipv.it

Endophytic mycobiota have been reported from a variety of worldwide hosts but, with few exceptions, their role within plant tissue is poorly understood. During a three year investigation on the endophytic colonisation in eighteen ryegrass (Lolium perenne L.) ecotypes of Northern Italy, two strains of Beauveria bassiana (Bals.) Vuill. were constantly detected. They were repetitively isolated from ryegrass collected from two pastures in the provinces of Bergamo (strain BG01) and Bolzano (strain BZ01). The isolation from both seeds and ovaries (frequency ranging from 2 to 32%) suggests an endophytic behaviour of the fungus. Beauveria bassiana isolates able to grow systemically in the plant may represent one of the most interesting candidates for use as microbial control agents. Therefore, we are evaluating the virulence of the strains towards insects. Nymphae of Gryllus domesticus L. were reared in a net cage and fed with bran, bread and a daily concentration of 10⁸ conidia/ml distilled water. After 7 days, both strains caused very low mortality (1%). Differences in fungal isolates activity were detected after 1 month, with 100% and 50% mortality caused by BZ01 and BG01, respectively. All the nymphae treated with BG01 died after 59 days. Beauveria bassiana was reisolated from all died nymphae. Further investigations concerning insect pests belonging to the same order (Orthoptera) are in progress.

ENGINEERING AND CHARACTERISATION OF SINGLE CHAIN ANTIBODY FRAGMENTS (SCFV) SPECIFIC TO GRAPEVINE VIRUSES. G. Nolke\textsuperscript{1}, M. Orecchia\textsuperscript{1}, P. Saldarel\textsuperscript{2}, M. Dell’Oro\textsuperscript{2}, A. Minafra\textsuperscript{2}, G. Martelli\textsuperscript{2}, R. Fisher\textsuperscript{3} and S. Schillberg\textsuperscript{3}.\textsuperscript{1}RWTH Aachen, Institute for Biology VII, Wor- ringerweg 1, 52074 Aachen, Germany. \textsuperscript{2}Instituto di Patologia delle Piante e Microbiologia Applicata, Università degli Studi di Bari, and Istituto di Virologia Vegetale del CNR, sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. \textsuperscript{3}Fraunhofer-Institute for Molecular Biology & Applied Ecology (IME), 52074 Aachen, Germany. E-mail: cschvps04@area.ba.cnr.it

Antibody-based resistance is a useful tool for producing crop plants expressing resistance to viruses. Theoretically, inhibitory antibodies can be generated that bind to any pathogen or protein involved in pathogenesis. Generation of pathogen-resistant plants requires the production of recombinant antibodies (rAbs), their cloning, efficient expression, stabilization, and targeting to an appropriate cell compartments. Cytoplasmic expression of rAbs, a prerequisite for inhibiting viral pathogens, has been difficult and only single chain fragment variable (scFv) can be reliably expressed. The coat protein of Grapevine leafroll-associated virus 3 (GLRaV-3) was chosen as a target for generation of specific rAbs that could interfere with viral infectivity. Two scFVs (scFvLR3-41 and scFvLR3-43) were cloned from an existing hybridoma line producing a monoclonal antibody specific to the coat protein of GLRaV-3. ELISA tests demonstrated that scFvLR3-41 and scFvLR3-43 specifically reacted to GLRaV3-CP. In order to target the antibody fragments to the plant cell cytosol, where most processes involved in viral infection take place, the scFv cDNA was cloned into a pSS plant expression vector, and transient transformation of tobacco leaves was done to verify its accumulation level and stability. While the cytosolic expression of scFvLR3-41 was not detected, scFvLR3-43 was stable and accumulated to high levels in the plant cytosol, retaining its antigen binding specificity and activity in such a reducing environment. This scFv may be a good candidate for further grapevine resistance studies. Transformation of grapevine is in progress and transgenic plants will be analysed for recombinant protein accumulation and resistance to GLRaV-3.

STUDIES ON THE ROLE IN PATHOGENESIS OF CERATOPLATANIN, A SMALL PROTEIN FROM CERATOCYSTIS FIMBRIATA ESP. PLATANI: A. Bennici, S. Boddi, R. Calamassi, G. Cappugi, L. Carresi, C. Capparini, G. Del Sorbo, B. Mori, B. Pantera, L. Pazzagli, A. Scala, F. Scala, S. Schiff, A. Sereni and S. Tagli. Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università degli Studi di Firenze, Via della Lastruccia 10,
Cerato-platanin (CP) is a moderately hydrophobic protein 120 amino acids in size from the Ascomycete Ceratocystis fimbriata (Ell. and Halst.) Davidson f. sp. platanii Walter. C. fimbriata f. sp. platanii (Cf) is the causative agent of the canker stain of the plane trees, a severe disease showing high incidence and severity in the European populations of Platanes acerifolia (Air.) Willd. According to various databases, CP is the reference protein of a family including proteins produced by different Ascomycota: the snod-pro1 protein from Phaeosphaeria nodorum, the allergen Asp f 15 precursor (Asp f 13) from Aspergillus fumigatus and the heat-stable 19 kDa antigen (CS-Ag) from Coccidioides immittis. The members of the CP protein family are characterized by a high degree of sequence homology, but not by clear functional similarities. However, all these proteins are secreted or potentially secretable, and in some cases seem to be involved in biological recognition phenomena. In the present paper we focused attention on some characteristics of CP suggesting that CP could be one of the first Cfp proteins involved in the plane canker stain pathogenesis, after the physical fungus-plant contact has occurred. CP has been shown by immunoﬂuorescence and immuno-gold labelling assays to be localized onto the mycelial cell walls of Cfp: moreover, CP is early and abundantly released in culture. CP is able to interact with the plane leaves by eliciting phytoalexin synthesis, extended cell plasmolysis and crushing, and abundant starch accumulation in the chloroplasts.

THE ROLE OF ABC TRANSPORTERS OF BOTRYTIS CINEREA IN PROTECTION AGAINST THE BIOCONTROL AGENT TRICHODERMA VIRENS.

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Some of the most studied and promising strategies to reduce the agricultural fungitoxic applications foresee the use of biocontrol agents of the genus Trichoderma against plant pathogens. Trichoderma virens produces antibiotics and lytic enzymes that synergize in inhibiting the development, both in vitro and in planta, of fungal pathogens like Botrytis cinerea. One of the key mechanisms by which target organisms defend themselves from antibiotics is the ATP-dependent efflux, mediated by membrane permeases known as ABC (=ATP Binding Cassette) transporters. In B. cinerea 15 sequences encoding typical ABC domains have been cloned. We studied the role of BcatrA and BcatrB, two ABC transporter-encoding genes in the interaction of B. cinerea with T. virens and with gliotoxin, one of the most fungitoxic compounds produced by T. virens. Expression analysis by RT-PCR revealed that either BcatrA and BcatrB transcription is induced by treatment with T. virens cultures filtrates or with gliotoxin. DBcatrB mutants displayed a higher sensitivity in vitro towards the antagonistic activity of the T. virens than the parental wild-type strain or a DBcatrA mutant. In planta virulence tests on petals of Gerbera jamesonii previously treated with T. virens germinals, showed a significant reduction of the lesion size in DBcatrB mutants in comparison with those caused by the parental wild-type strain. These results indicate that ABC transporters provide protection to B. cinerea against biocontrol agents.

PHYTOTOXIN PRODUCTION BY SEVERAL ISOLATES OF PHAEACREMONIUM AND PHAEOMONIELLA SPP. AND THEIR ANTAGONISM VS. FOMITIPORIA MEDITERRANEA.

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Isolates of Phaeacreamonium aleophilum (Pdale), P. angustius (Pang), P. inflatiipes (Pinfl), P. parasiticum (Ppa), P. rubrigerens (Prub), P. stictola (Pstic), Phaeomoniella chlamydospora (Pch) and Fomitiporia mediterranea (Fme) were used for toxin production and antagonistic action. Culture filtrates of all strains, assayed on detached leaves of ‘Italia’ grapevine, caused chlorosis and necrosis. Several phytotoxic metabolites were extracted from culture filtrates of each fungal strain. Two of these metabolites were the naphtalenedione pentaketides scytalone and isoscerolone. Isoselectrione was produced by Pal, Pch, Pin, Pan, Pru whereas scytalone was produced by Pal, Pch, Pin and Pan. In dual cultures, Fme and all strains of Phaeacreamonium and Phaeomoniella spp. grew antagonistically. The antagonism vs. Fme was clearly shown on agarised plates with Fme colonies grown together with colonies of the other fungi. The margin of the Fme colonies then turned brown, became thicker, and aerial hyphae formed a ridge-like barrier between the two. This experiment showed that the antagonistic effect vs. Fme was probably due to the production by Phaeacreamonium and Phaeomoniella spp. of substances that freely spread through the medium and reached the Fme colony. Pal and Pch strains were used for the concentration and desalting of culture filtrates through anisotropic membranes. Retentate sample of Pch, containing substances with molecular weight (mwt) up to 10 kDa was toxic to Fme. Retentate sample of Pal, containing substances with mwt between 3 and 10 kDa completely inhibited Fme since the start-up, whereas retentate up to 3 kDa inhibited Fme after a longer latency.

THE CMYLCV PROMOTER, AN EFFECTIVE ALTERNATIVE TO THE CAMV 35S PROMOTER.

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 Appropriately regulated gene expression requires a suitable promoter. Only a few promoters functional in plants drive high levels of constitutive expression of transgenes. The genomic promoter of Cauliflower mosaic virus (CaMV 35S) is one of the most efficient, and thus most used. However, transgene silencing has been associated with the presence of identical or homologous 35S sequences in transgenic plants, thus in many cases this promoter is not a safe choice. We have characterized a novel plant viral promoter conferring stable, strong and constitutive gene expression in plants. We isolated the genomic promoter of Cestrum yellow leaf curling virus (CmYLCV) a double-stranded DNA plant virus belonging to the Caulimoviridae family and tested its activity in several types of plant protoplasts and in transgenic Arabidopsis thaliana, Nicotiana tabacum, Lycopersicon esculentum, Zea mays and Oryza sativa. The CmYLCV promoter is highly active in callus, meristems, and vegetative and reproductive tissues in both monocotyledonous and dicotyledonous plants. The level of expression is comparable to, or higher than, that of the CaMV 35S, the ‘super-promoter’ or maize ubiquitin 1 promoters. Low sequence homology was found between the CmYLCV and the CaMV 35S promoter or any other of the characterized caulimovirus promoters. This, together with the restricted host range determined for CmYLCV, minimizes the risk of transgene silencing and makes the CmYLCV promoter a better alternative to, or a safe partner for, the CaMV 35S promoter, suitable for regulating transgene expression in a wide variety of plant species.
ELICITATION OF COUMARINS IN LEMON FRUITS AFTER YEAST INOCULATION. L. Strano1, A. Campisano2, A. Renda1, S. Di Silvestro1 and G. Ruberto1. 1Consorzio Catania Ricerche, Via S. Maria del Rosario 9, 95131 Catania. 2DISTEF - Facoltà di Agraria, Università degli Studi di Catania, Via S. Sofia 100, 95100 Catania. 3CNR-Istituto di Chimica Biomolecolare - Sez. di Catania, Via del Santuario 110, 95028 Valverde (CT), Italy.

Four yeasts (Pichia guilliermondii NRRL Y 18314, P. anomala J121, Debaryomyces hansenii DBVPG 4025 and Saccharomyces cerevisiae P1.6) assayed in different citrus species have shown different antagonistic activity against Penicillium digitatum, apparently due to a multiple mode of action. Since D. hansenii DBVPG 4025 has a very high extracellular b-glucosidase activity among the assayed yeasts (1789.15 nmol pNP l^-1 min^-1) the ability of this yeast to produce scoparone (6,7-dimethoxycoumarin) and scopoletin (7-hydroxy, 6-methoxycoumarin) was investigated. Quantitative assessment of scoparone and scopoletin in the rind of lemon fruits was done 72 h post inoculation by single ion monitoring (SIM) in fast gaschromatography – mass spectrometry (GC-MS) analyses. Tissues inoculated with different yeast species showed different accumulation of scoparone (highest with D. hansenii, while P. anomala was the least effective). Production of scoparone was not related to antagonistic activity (P. guilliermondii > P. anomala > D. hansenii > S. cerevisiae). Co-inoculation of yeast and P. digitatum reduced elicitation. Water inoculated tissues showed only a slight increase in scoparone concentration. Scopoletin was not found in any case.

**ISOLATION, CHARACTERISATION AND USE OF BI- AND POLYNUCLEATE RHIZOCTONIA STRAINS AS BIOCONTROL AGENTS. L. Ferraris, D. Valentino, F. Cardinale and G. Tamietti. Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali – Patologia vegetale, Via L. da Vinci 44, 10195 Grugliasco (TO), Italy. Fax: +39.011.6708341; E-mail: giacomo.tamietti@uni.to.it**

Twelve Rhizoctonia strains were isolated from Chenopodium album, Artemisia vulgaris, Silene cucubalus, and characterised for the number of nuclei, cardinal temperatures, pathogenicity, enzymatic activities, and, preliminarily, for their anastomosis group. Seven strains were binucleate and showed reduced pathogenicity and radial growth (35% less) in comparison with the polyonucleate isolates. One polyonucleate isolate (R3) showed a phenotype similar to that of the binucleate strains. Three and four electrophoretic patterns relative to the mycelial hydrosoluble proteins were obtained from the binucleate and the polyonucleate isolates, respectively; all, however, differed from that of the control R. solani strain from French bean. Patterns of mycelial hydrosoluble proteins correlated well with enzymatic activities and cardinal temperatures, and only partially with pathogenicity and vegetative compatibility. Two isolates, one binucleate (R2) and one polyonucleate (R3) showed a restricted host range and low virulence and were tested as biocontrol agents against R. solani on radish. In presence of a disease incidence of 89-99% on the control, R2 and R3 reduced symptoms by 12-77% and 38-76% respectively, both in heat-treated and non-treated natural soil. The best activity was observed with antagonist/pathogen inoculum ratio ranging from 40 to 50. R2 and R3 were avirulent on radish. ITS1, ribotyping of the potential biocontrol agents as compared to virulent Rhizoctonia strains is under way, to the purpose of taxonomic comparison and of diagnosis/quantification of both groups in future biocontrol assays.

**ENZYMATIC ACTIVITIES IN CULTURE FILTRATES OF SOME FUNGAL ANTAGONISTS OF RHIZOCTONIA SOLANI. A. Trincone, R. Nicoletti, A. Giordano and A. Carella. Istituto di**

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Fungal antagonists exert their biocontrol potential against plant pathogens by several biochemical mechanisms. A critical role is played by glycosyl hydrolases involved in the degradation of the carbohydrate polymers underlying the cell wall. Therefore, a thorough evaluation of the antagonistic potential of fungal strains should also concern the assessment of the pertinent enzymatic activities. There is an increasing interest in finding new biological sources of enzymes suitable for biotechnological applications, and fungal strains kept in mycological collections deserve a thorough screening to this regard. Production of chitinases, >1,3-glucanases, xylanases, mannosidas and fucosidas by 22 isolates belonging to 13 different species of fungal antagonists of R. solani was evaluated in culture filtrates prepared by using either chitin, curdlan, xylan or directly the R. solani mycelium as the fundamental carbon source in the growth medium. Enzymatic activities in the culture filtrates were assessed by release of nitrophenol from different chromogenic substrates and TLC-detection of hydrolysis products from curdlan and xylan. Preliminary results show that the ability to degrade the different polysaccharides is quite variable from isolate to isolate, and that the availability of the specific polysaccharide in the culture medium generally stimulates the pertinent lytic activity. The use of R. solani mycelium as fundamental carbon source in the growth medium appears to be useful for a preliminary appreciation of the general pattern of enzymatic activities necessary for biological action.

**ISOLATION AND SEQUENCING OF AN ENDOPOLY- GALACTURONASE GENE IN DIAPORTHE HELIANTHI. M. Vergara1, C. Regis1, D. Rekab2, G. Firrao2 and G. Vannacci1. 1Dipartimento di Coltivazione e Difesa delle Specie Legnose “G. Scarumuzzi”, Università degli Studi di Pisa, Via del Borgortto 80, 56124 Pisa, Italy. Fax: +39.050.343564; E-mail: rvergara@agr.unipi.it. 2Dipartimento di Biologia Applicata alla Difesa delle Piantate, Università degli Studi di Udine, Via delle Scienze 208, 33100 Udine, Italy. Fax: +39.0432.538903; E-mail: firrao@pdiet.univud.it**

Diaporthe belianthi, the causal agent of stem canker, is an important pathogen of sunflower in Europe. Its pathogenetic mechanisms are still poorly understood, although the production of phytotoxic compounds has been demonstrated. Degradation of cell walls by fungal specific enzymes, primarily pectinases, has been shown to be involved in plant pathogenesis. By now many fungal endopolygalacturonase (endoPG) sequences have been isolated from phytopathogenic microorganisms. In D. belianthi there is no information about endoPG enzymes produced or about their coding regions. In order to analyse the endoPG genomic region in a French D. belianthi isolate (8/96), heterologous primers, designed on fungal conserved endoPG genes, were used. The endoPG genomic region was isolated by screening four differently digested D. belianthi genomic libraries, constructed according to the Genome Walker system (Clontech). Several amplicons obtained by walking both in 3' and in 3' direction were cloned and sequenced: the partial sequences obtained were overlapped and specific external primers were designed on the D. belianthi region. By amplification and cloning of the complete genomic region, the full gene sequence was determined. Sequence analysis by means of BLAST programs confirmed its endoPG nature. Further studies about the relatedness of D. belianthi sequence to the known fungal endoPG genes and about functional and structural domains in its deduced protein are in progress.

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Many pathogens can cause severe damage to potato both in the field and during storage. Dry rot, caused by F. sambucinum, F. solani var. coeruleum and F.avenaceum, and soft rot caused by Erwinia carotovora subsp. carotovora and Erwinia carotovora subsp. atroseptica, are a serious disease of stored potatoes. Dry and soft rot can be controlled chemically but increasing social pressure against the use of chemicals makes it desirable to reduce the need for chemical fungicides by growing resistant varieties of potatoes.

In this work, 16 breeding clones from the National Project “Potato Breeding” and two commercial variety (Spunta and Arima), were screened for resistance to F. sambucinum 1228mc ISPaVe and Erwinia carotovora subsp. carotovora 009 ArBoPaVe. These isolates resisted highly virulent in in vitro artificial inoculation tests. Potato tubers utilised in these tests were grown in the winter-spring cycle in Southern Italy (Angri). Genotype CS 90–164–13 and E 2121 proved resistant both to F. sambucinum and E. carotovora subsp. carotovora. Furthermore, the genotypes ISCI 88/95 39 and MN 1512 R11 showed a good agronomical performance when grown in extra-seasonal cycle in three experimental fields of the Project (Siracusa, Angri, Bari), and a moderate resistance to F. sambucinum.

Susceptibility to DC 3000 and did not have effector genes.

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Plants elaborate a number of compounds useful for pharma-ceutical and phytotherapeutic uses. Saponins are glycosides occurring in more than one hundred plant families. Species belonging to family Asteraceae, produce high level of triterpenoid saponins which are isopr enoidal natural products with remarkable biological activities (antitumor, antifungal, antibacterial and antiviral effects). Preliminary biological tests with leaf extracts from Aster sedifolius against Tribolium viride and Botrytis cinerea showed inhibition of fungal mycelium growth. In this work, a chemical fraction constituted of three purified triterpenoid saponins with oleane-type skeleton, isolated from leaves of Aster sedifolius, was evaluated for its property to inhibit in vitro growth of nine and five phytopathogenic fungi and bacteria, respectively. The growth of Rhizoctonia solani, Sclerotinia sp., Fusarium solani and, especially, Sclerotium rolfsii, was significantly inhibited the same as the growth of Xanthomonas campestris pv. campestris and Xanthomonas axonopodis pv. vesicatoria. No effect was observed on Alternaria sp., Fusarium oxysporum, Pyrenochaeta lycopersici and on Pseudomonas syringae pv. tomato, Erwinia carotovora subsp. atroseptica, Erwinia carotovora subsp. carotovora. Further analyses using separately the three purified triterpenoid saponins are in progress to ascertain which of them is potentially useful in plant disease control.

Biological control is an alternative control method of plant diseases. Many studies supported the use of antagonistic fungi and/or bacteria to control soil-borne plant pathogens. In this work the characterization was done of antagonistic bacteria, mainly isolated from potato tubers and tomato rhizosphere. Over 200 bacterial isolates, collected from cultivated fields in the Campania region, were evaluated for their ability to inhibit in vitro the growth of Rhizoctonia solani and Fusarium solani. Twenty-two bacterial isolates from potato tubers, six from rhizosphere and three from air, were chosen for their antagonistic activity and isolated for DNA polymorphism. Ten most active and genetically different isolates, were chosen for in vivo tests on potato shoots. Isolate 3 R II bac gave the best control against Rhizoctonia solani (65.9% disease control), increased plant height and the number of tubers (24.6 cm and 1.7 tubers/plant) compared with the untreated control (22.2 cm and 1.0 tubers/plant) and Rhizoctonia inoculated plants (14.8 cm and 0.26 tubers/plant). Isolate 7 IV bac gave the best control of Fusarium sambucinum (51% disease control) and increased plant height (22.3 cm) compared with Fusarium-inoculated plants (17.7 cm). All data were analysed by ANOVA and proved statistically different (P< 0.05). The two bacterial isolates capable to control these two diseases were Gram positive and produced endospores (Bacillus spp.); moreover, isolate 3 R II bac produced volatile substances that inhibit the growth of Rhizoctonia solani. Experimental field trials of biological control on potato are in progress using these selected Bacillus spp. isolates.

Experimental plantings were carried out on Flavescence dorée (FD) infected vines during winter 2001 with the aim of acquiring more information about the possibility of obtaining sanitation or
symptom remission. FD-infected grapevines of different cultivars (Barbera, Cabernet Sauvignon, Chardonnay, and Lambrusco) were pruned so as to completely eliminate symptomatic canes in January 2001. Insecticide treatments were regularly made in the vineyard to prevent possible re-infections of the grapevines under examination. Later, in 2001 and 2002, grapevines were checked for the presence of symptoms twice a year. In the same time, PCR-RFLP analyses were conducted on leaf samples collected from each examined vine. Barbera and Cabernet Sauvignon showed several cases of recovery (absence of symptoms and of phytoplasmas) while Chardonnay and Lambrusco exhibited several cases of symptomatic and infected plants both in 2001 and in 2002. The results of this work suggest the possibility to reduce the incidence of FD disease in some grape varieties using a careful winter pruning associated with an efficient control of the insect vector.

OXIDATIVE STRESS IN CHILLING INJURY DURING COLD STORAGE OF AUBERGINE. P. Zubini and E. Baraldi, Dipartimento di Protezione e Valorizzazione Agrumi, Università degli Studi di Bologna, Via Fannini 46, 40127 Bologna, Italy. E-mail: ebaraldd@agrsci.unibo.it

The involvement of oxidative stress in chilling injury during cold storage of aubergine (Solanum melongena) was studied. Chilling temperatures increase the development of reactive oxygen species (ROS) in plants. Sub-tropical species like aubergine are particularly sensitive to ROS when they are exposed to temperatures below 10°C. Plants have an enzymatic and a non-enzymatic antioxidant system to prevent damage from ROS and to scavenge these dangerous compounds. In order to study the role of the enzymatic antioxidant system during post-harvest cold storage of aubergine, we used real time PCR to monitor changes in the transcript level of genes encoding ROS scavenging enzymes. Changes in Mn-superoxide-dismutase, catalase, glutathione reductase and ascorbate peroxidase gene expression were analysed during a period of storage of 10 days at 0°C or at 10°C. Expression of catalase and ascorbate peroxidase was slightly stimulated during storage at 10°C, and inhibited at 0°C. Similarly, glutathione reductase gene expression was stimulated at 10°C. Conversely, Mn-superoxide dismutase transcript level decreased at both temperatures. This suggests that oxidative stress can damage aubergine during cold storage. Contrary to cold storage at 0°C, the expression of ROS scavenging enzymes is positively regulated at 10°C, indicating the occurrence of oxidative stress and a tissue reaction.

FUNGI ASSOCIATED WITH ROOT ROT OF A LENTIL LANDRACE FROM SICILY. L. Riccioni, G. Di Giambattista, M. Valvassori, R. Malta and A. Porta-Puglia, Istituto Sperimentale per la Patologia Vegetale, Via C.G.Bertero 22, 00156 Roma, Italy. E-mail: luca.riccioni@ispave.it

Lentil is an old traditional crop at Villalba (Caltanissetta province, Sicily) and the landrace grown there is very appreciated for its agronomical and nutritional characteristics. During the last spring, yellowing and decay symptoms, associated to root rot, were observed in different lentil fields. Samples of diseased plants were collected and analysed for identifying the causal agent(s). Moist chambers and isolations on PDA from diseased crown and root tissues were carried out. Species of Fusarium were most frequently isolated; among them F. oxysporum and F. culmorum had the major incidence; F. solani, F. equisetii and F. graminearum, as well as other soil-borne fungi like Sclerotinia sclerotiorum and Cylindrocarpon sp., were less frequently recovered. Artificial inoculations of some Fusarium isolates were carried out in the greenhouse on lentil, tomato, bean and wheat to investigate their role in root rot development. Two inoculation methods were used: (i) sterilised seeds were sown in pasteurised soil infested with the fungus grown on autoclaved millet grains; (ii) 15-day-old seedlings were inoculated by pouring a homogenate of the fungus grown on PDA in the soil near the roots. Preliminary results showed a high degree of virulence of an isolate of F. culmorum and an isolate of F. oxysporum that probably belongs to the “forma specialis” lentis as it induced vascular discolorations in inoculated lentil plants.

YELLOW RUST AND SEPTORIA/STAGONOSPORA BLOTCHES IN WHEAT: DISEASE DEVELOPMENT AND HOST RESISTANCE. M. Pasquini, L. Gazzà and A. Iori. Istituto Sperimentale per la Cerealicoltura – Sez. Genetica Applicata, Via Cassia 176, 00191 Roma, Italy. Fax: +39.06.36306022; E-mail: pasquini@maclink.it

Wheat disease monitoring was carried out in different Italian cereal growing areas, from 1999 to 2002, in the framework of the Cereal Interregional Sperimentation Program (SIC) of the Italian Ministry of Agriculture and Forestry. Among the diseases surveyed, yellow rust and Septoria/Stagonospora blotches appeared to be of interest. Yellow rust, which usually is not a problem in Italy, caused severe epidemics, especially in 2000 and 2001. An increase of Stagonospora nodorum blotch was observed, while Septoria tritici blotch was present with a lower frequency. The observations carried out in the field for evaluating the behaviour of durum and bread wheats cultivars grown in Italy showed the absence of genotypes completely resistant to Septoria/Stagonospora diseases and the susceptibility to yellow rust of many bread wheat cultivars. By contrast, durum wheat appeared to be more resistant. Preliminary studies were carried out in the greenhouse to evaluate the virulence of Italian populations of Puccinia striiformis tritici. Different pathotypes were identified showing a wide virulence to many known seedling carrying resistance genes. Many bread and durum wheat cultivars proved to be susceptible to these pathotypes. Durum wheats artificially inoculated at the seedling stage with Stagonospora nodorum isolates were moderately or completely susceptible. Studies are in progress to investigate the behaviour of the same cultivars after artificial inoculation in adult stage.

BIOLOGICAL CONTROL OF MAJOR POSTHARVEST PATHOGENS ON CV. ANNURCA APPLES WITH TRICHODERMA SPP. AND SEPEDONIUM CHYRYSOPERMUM. F. Vinale, S. Woo, K. Abadi, M. Ruocco, D. Scognamiglio, F. Scala, A. Zoina and M. Lorito, Dipartimento ARBOPAVE – Sez. Patologia Vegetale, Università di Napoli, and CNR IPP Sez. di Portici, Via Università 100, 80055 Portici (NA), Italy. E-mail: lorito@unina.it, frvsole@unina.it

Postharvest storage of fruits and vegetables experiences serious economic losses worldwide, even when fungicide treatments are applied. Development of resistance to chemicals by major postharvest pathogens, and concerns for public safety have supported the expanding interest in biological control methods of fruit decay, for example, by using microbial antagonists. The objective of this study was to investigate the biocontrol potential on cv. Annuanca apples of Trichoderma spp. and Sepedonium chrysoperum against Penicillium expansum, Botrytis cinerea and Alternaria alternata, three of the major postharvest pathogens of this commodity typical to the Campania Region. Trichoderma (strains P1 and T22) and C. chrysoperum (strain 704) showed a strong biocontrol effect during in vitro tests performed against P. expansum, B. cinerea and A. alternata. They inhibited both spore germination and germ-tube elongation of the pathogens by the simple application of
crude culture filtrates from the antagonist. In vivo bioassays, using antagonist propagules (P1, T22, 704) inhibited root development for at least 10 days. In order to understand the role of specific metabolites in this plant-antagonist interaction, culture filtrates were analysed biochemically and mutants with known up- and down-regulated genes possibly involved in post-harvest biocontrol were tested in comparison with wild types.

REMEDIATION OF POLLUTION BY USING BIOLOGICAL SYSTEMS BASED ON BENEFICIAL PLANT-MICROORGANISMS INTERACTIONS. F. Vinale, K. Abadi, M. Ruocco, R. Marra, F. Scala, A. Zoina, S. Woo and M. Lorito. Dipartimento ARBOPAVE – Sez. Patologia Vegetale, Università di Napoli, and CNR IPP - Sez. di Portici, Via Università 100, 80055 Portici (NA), Italy. E-mail: lorito@unina.it, frvinale@unina.it

Physical and chemical techniques are the most commonly used methods for remediation of pollutants. The high cost of these methods, however, have stimulated the interest in alternative biologically-based systems, in particular bioremediation and phytoremediation. Bioremediation consists of adding microorganisms to a polluted system, in the presence of nutrients, for degrading toxic compounds. Phytoremediation uses plants to degrade or remove toxicants from soil lacking a defined or managed root-microbial population. The objective of this study was to develop a method for remediating polluted waters or soils by using a novel technique of combined bio- and phytoremediation. Plants (i.e. cotton, beans or corn, as well as ferns and other ornamental plants) were grown on a polluted site (i.e., heavy metals, arsenic, cyanide and metallo-cyanides, nitrates, etc.) in association with one or more rhizosphere competent microorganisms (eventually acting synergistically) which are non-toxic or pathogenic and nutritionally sustained by plant root exudates (i.e. fungi, Trichoderma spp.; and bacteria, Bacillus, Pseudomonas, and Burkholderia). The plant-organism system was introduced to the polluted site, permitting an efficient and rapid uptake of the toxic elements before removal of the plant. In the case of plants associated with Trichoderma spp., we obtained evidence of a strong increased uptake of nutrients or toxicants by the plants; an efficient degradation of cyanide and accumulation and degradation of metallo-cyanides; strong reduction of toxicity caused by polycyclic aromatic hydrocarbons and pheno-lic compounds present in water and soil.

PLANT PROTEINASE INHIBITORS TO CONTROL PHYTOPATHOGENIC FUNGI. D. Turra, R. Hermosa, L. Zara, G. Cecere, F. Vinale, R. Marra, K. Abadi and M. Lorito. Dipartimento ARBOPAVE - Sez. Patologia Vegetale, Università di Napoli, and CNR IPP - Sez. di Portici, Via Università 100, 80055 Portici (NA), Italy. E-mail: lorito@unina.it

Pathogenesis of the plant, by biotic or abiotic factors, elicits the synthesis of plant PR proteins such as proteinase inhibitors (PIs). Plant PIs are able to affect chitin synthesis in fungi, reduce insect growth and microbial protease activity in vitro. Much is known about the effect of PIs on insects, but it is not clear if PI gene overexpression can increase plant resistance against pathogenic fungi such as Botrytis cinerea, Alternaria alternata and Fusarium oxysporum. Significant proteinase activity was found only in cultures of fungal pathogens grown on media containing plant material. Commercial PI preparations were used in in vitro and leaf bioassays to select the PI family (serine proteinase inhibitor) most active in controlling fungi and the production of their proteinases. These PIs were purified from potato shoots by affinity chromatography and partially sequenced, thus obtaining amino acid sequences of at least two different PIs. A new gene, PKI1 (666 bp), isolated by PCR corresponded to the coding region of a serine proteinase inhibitor. BlastP analysis of this sequence showed a 98% homology with a putative Kunitz-type proteinase inhibitor from Solanum tuberosum. Southern analysis performed at high stringency, using potato DNA and a PKI1 probe, indicated that this gene occurs as a single copy in S. tuberosum cv. Desiree. Northern and RT-PCR analysis indicated an absence of introns and a basal expression of the PKI1 gene in leaf tissues. The coding sequence was subcloned into an overexpression vector to fully exploit the biological activity of the inhibitor.

ABC TRANSPORTER GENES OF TRICHODERMA SPP., AND THEIR INVOLVEMENT IN BIOCONTROL. S. Lanzuise, M. Ruocco, L. Catapano, V. Scala, R. Ciliento, P. Ambrosino, S. Woo, F. Scala, G. Cecere, G. Del Sorbo and M. Lorito. Dipartimento ARBOPAVE - Sez. Patologia Vegetale, Università di Napoli, and CNR IPP - Sez. di Portici, Via Università 100, 80055 Portici (NA), Italy.

The fungi of the genus Trichoderma are widely studied for their antagonistic ability. The molecular mechanisms that regulate the fungus-fungus and plant-fungus interactions involved in biocontrol have been investigated, but the role of ABC transporters has not yet been assessed. These transporters may function in the recognition of the plant or pathogen by Trichoderma spp., as well as in the ability of this fungus to withstand the effect of toxins either produced by soil microflora or human activity (i.e. fungi-cides, heavy metals, etc.). Four different sequences of ABC transporters (tABC1, tABC2, tABC3, tABC4) were isolated from the biocontrol agent Trichoderma atroviride P1. PCR-based characterization showed the involvement of these genes in the interaction between Trichoderma and its fungal hosts. For example, tABC2 was overexpressed when Trichoderma was grown in the presence of Botrytis cinerea, Rhizoctonia solani or Pythium spp. or with the culture filtrate of the plant pathogens. Genes tABC1 and tABC3 were involved in the resistance to procloraz and benlate, whereas the expression of tABC2 was down-regulated by proclo-raz and slightly induced by benlate and dicylorn.

PROTEOMIC STUDIES OF ANTAGONIST TRICHODERMA STRAINS. P. Ambrosino, V. Scala, M. Ruocco, R. Marra, S. Woo, F. Scala and M. Lorito. Dip. ARBOPAVE – Sez. Patologia Vegetale, Laboratorio di Lotta Biologica, Università di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: lorito@unina.it

Trichoderma-based formulations represent an effective alternative to chemical control for their ability to function as biopesticides and biofertilizers. However, poor knowledge about the molecular mechanisms involved in the three-way plant-pathogen-biocontrol fungus interaction limits the exploitation of this beneficial microorganism. Numerous laboratories are actively involved in the identification of genes and gene products with biotechnological value from Trichoderma antagonistic strains. Proteome analysis, a powerful experimental tool, has greatly enhanced our ability to conduct holistic and genome-based functional studies. Technical developments in 2-D gel electrophoresis permit the production of reproducible maps of the entire fungal proteome, thus permitting a rapid identification of many proteins related to a specific function in the biological process. We performed proteome studies to isolate differential proteins from the mycoparasite Trichoderma harzianum strains T22 and A6, grown under inducing and repress-ive conditions both in vitro and in vivo during the interaction with tomato and several phytopathogenic fungi (Botrytis cinerea, Rhizoctonia solani and Pythium spp.). 2-DE maps of extracellular and intracellular proteins were generated at three different pl ranges (3-10, 4-7, 6-11) and analysed to identify proteins differen-
tially expressed and potentially involved in antibiotic and enzyme biosynthesis. Proteins of interest were recovered from gels and subjected to MALDI-ToF mass spectrometry. The data obtained were matched with those available in databases, with the aim of identifying genes useful for agricultural and industrial applications.


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In gene for gene interactions, plant defence responses are activated by specific recognition of a pathogen avirulence gene (Avr) if the matching resistance gene is present. Fungus-plant interaction between Cladosporium fulvum and tomato has been studied at the molecular level in great detail. The C. fulvum avirulence genes Avr4 and Avr9 encode race-specific elicitors that induce a hypersensitive response in tomato plants carrying the resistance gene Cf-4 and Cf-9. The mycoparasite Trichoderma atroviride strain P1 has been studied extensively for its biocontrol ability against numerous plant pathogens, and produces compounds that are detected by the plant and systemically increase disease resistance. In addition, Trichoderma promoters inducible in the presence of various pathogenic fungi or active during the interaction with the plant are available for use in the expression of foreign genes. Avr4 from C. fulvum was transferred to T. atroviride by using various regulatory regions, including the GPD promoter of Aspergillus nidulans. Several transformants were obtained and analysed by PCR, Southern and Northern analysis; these tested in vivo by competition assays and in field tests on tomato lines C4+ and C4-. In order to verify if the Trichoderma genome has Avr-like genes, protein extracts from the Trichoderma-plant interaction were analysed by 2D-electrophoresis. Differential proteins highly homologous to a C. fulvum Avr product were found. The relative nucleotide sequences were obtained and characterized.

**PROTEOMIC ANALYSIS OF PLANT-PATHOGEN-ANTAGONIST INTERACTIONS.** R. Marra, V. Scala, M. Ruocco, S. Woo, P. Ambrosino, F. Scala and M. Lorito. Dipartimento ARBOPAVE – Sez. Patologia Vegetale, Università di Napoli, Via Università 100, 80055 Portici (NA), Italy. E-mail: lorio@unina.it

Molecular factors involved in the interaction between plants, pathogens and biocontrol fungi are very complex and still poorly understood. To date, few studies have looked at the mechanisms involved in the establishment pathogenic processes and/or plant resistance in the presence of the beneficial activity of microbial biocontrol agents. The activity of antagonistic strains of Trichoderma spp. and Gliocladium spp. in preventing plant diseases, and at the same time, improving plant growth has been clearly demonstrated. The biocontrol ability of some Trichoderma strains depends on the production of a set of antifungal hydrolytic enzymes such as chitinases and glucanases. This study deals with the interactions between plants and microorganisms (beneficial and pathogenic), which includes a functional analysis of the three-way plant-pathogen-biocontrol fungus interaction. Proteins produced during confrontations between different crop plants (potato, tobacco, bean and tomato), pathogens (Botrytis cinerea, Rhizoctonia solani) and antagonistic fungi (Trichoderma spp.) were separated by 2-D gels and characterized. Gel analysis indicated that many differential proteins are produced and many variations in gene expression occur, depending upon the experimental conditions used. Some of the differential proteins were isolated, sequenced and characterized by mass spectrometry, with the aim of cloning the respective encoding genes. Attention was also given to the isolation of fungal promoters up-regulated during the concurrent interaction with the plant and the pathogenic host, in order to exploit those involved in the activation of plant defence response and/or biocontrol.


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Some strains of Trichoderma atroviride are commercially applied as biological control agents since they are parasites to a number of plant pathogenic fungi. The mycoparasitic interaction is host-specific, therefore, signals from the host fungus are recognised by T. atroviride which elicit the production of anti-fungal compounds associated with morphological changes typical of mycoparasitic processes (i.e. appressorium formation). For some plant pathogenic fungi, signal transduction cascades following activation by inducers have already been characterised. In mycoparasitic fungi, several morphological changes are highly similar to those associated with pathogenicity. The formation of appressoria-like structures, but also the secretion of hydrolytic enzymes appears to be a general mechanism of virulence in plant pathogens as well as in mycoparasites. To study host specificity in detail during biocontrol, the receptor and several key components of the cAMP and MAP kinase signaling pathways involved in T. atroviride virulence were identified. Genes encoding components of the cAMP pathway, catalytic and regulatory subunits of protein kinase A and two G proteins were isolated from T. atroviride P1. Furthermore, two more MAP kinase-encoding genes (tmk1 and tmk2) showing high similarity to MAPKs involved in pathogenicity of M. grisea, and one gene (tgbl1) encoding a Gb-subunit of heterotrimeric G proteins were isolated. To test the functionality and involvement of these factors in mycoparasitism, targeted gene disruption of tmk1, tgbl1 and tpkar1 (encoding the regulatory subunit of cAMP-dependent protein kinase) was performed.

**GENOMIC STUDIES OF TRICHODERMA SPP. STRAINS FOR APPLICATIONS IN INDUSTRY AND AGRICULTURE.** R. Giletto, P. Ambrosino, V. Scala, M. Ruocco, R. Marra, S. Woo, F. Scala and M. Lorito. Dipartimento ARBOPAVE – Sez. Patologia Vegetale, Università di Napoli, and CNR IPP – Portici Section, Via Università 100, 80055 Portici (NA), Italy. E-mail: lorio@unina.it

Biotechnology derived from the Trichoderma genome has applications in a wide range of agro-industrial, environmental and medical activities, which may lead to innovations in the management of pests by reducing chemically-based applications. This work, funded by a new European Union project entitled "Functional genomics and proteomics of Trichoderma antagonist strain for industry and agriculture” (TRICHOEST), is aimed at researching the full exploitation of the genes and gene products from this fungus for both agricultural and industrial processes. In the work performed so far, we have selected two strains of Trichoderma harzianum (T22 and A6) to obtain extensive EST libraries of genes expressed in conditions related to naturally occurring Trichoderma-
pathogen-plant interactions. These include simulated mycoparasitism/antagonism situations, as well as biocontrol in vivo in semi-field/greenhouse conditions. Novel methods have been established to obtain clean mRNA sets from cultures grown in conditions that closely resemble the concurrent natural interaction of Trichoderma spp. with plants and pathogenic host. Total RNAs were pooled for each antagonist strain, thus obtaining libraries containing a great variety of transcripts of interest. The mRNA isolation was carried out by using Gen-Elite Direct mRNA Mini prep Kit, and the resulting cDNAs were cloned in 1 tripEX2 phage vector and packed with the Gigapack Gold system. The two large cDNA libraries obtained was macro-arrayed and probed with a variety of sequences.

TARGETED DISRUPTION OF A NEW ENDOCHITINASE-ENCODING GENE IN TRICHODERMA ATROVIRESCENS. R. Ciliento, S. Woo, P. Ambrosino, V. Scala, M. Ruocco, R. Marra, F. Scala and M. Lorito. Dipartimento ARBOPAVE – Sez. Patologia Vegetale, Università di Napoli, and CNR IPP – Portici Section, Via Università 100, 80055 Portici (NA), Italy. E-mail: lorio@unina.it

The applications in agriculture of Trichoderma spp. include not only direct inhibition of phytopathogenic fungi but also the induction of SAR and IR, improved plant vigour and increased tolerance to abiotic stress. In addition, strains of Trichoderma spp. are largely studied and applied in industrial production of enzymes, synthesis of antibiotics and recycling of organic residues. This has stimulated intense research activity to understand the molecular basis of the biocontrol mechanisms of these antagonists. A novel 36 kDa endochitinase, CHIT-36, with strong antifungal activity was isolated and characterized from Trichoderma harzianum strain TM. Partial amino acid sequences obtained from the purified protein were used to clone the fungal cDNA, based on the polymerase chain reaction performed with degenerate primers. Southern analysis, by using the full gene sequence as a probe, demonstrated the presence of CHIT-36 in Trichoderma atrovirens strain P1 as a single copy. A 900 bp portion of the gene was used for targeted gene disruption experiments. Hygromycin resistant progeny was selected and analysed at molecular level to verify the lack of gene expression for this particular enzyme activity. Progeny was also tested in in vivo biocontrol assays to determine the role of this gene during Trichoderma-fungal pathogen biocontrol interactions.

TRICHODERMA STRAINS DELETERIUS FOR COMMERCIAL MUSHROOM PRODUCTION. S. Woo, K. Abadi, M. Ruocco, R. Ciliento, S. Gigante, P. Ambrosino, V. Scala, M. Sentatore, F. Vinale, F. Scala and M. Lorito. Dipartimento ARBOPAVE – Sez. Patologia Vegetale, Lab. di Lotta Biologica, Università di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: woo@unina.it

The filamentous fungus Trichoderma, found in natural and agricultural ecosystems, is an ubiquitous saprophyte and strong secondary colonizer; it grows rapidly and is a great producer of spores and antibiotics. Trichoderma spp. have been studied mainly as biocontrol agents against a variety of fungal plant pathogens. However, recently they have also been identified as significant pathogens of commercial mushrooms. Trichoderma green mold infestations of Agaricus bisporus (champignon) emerged in Northern Ireland in 1985, and have since caused epidemics in mushroom production throughout Europe. When the disease was reported in North America, the economic losses amounted to $US10 million. Normally, mushroom compost hosts both aggressive and non-pathogenic Trichoderma species, which cannot be morphologically differentiated. Four fungal biotypes were isolated from composts, only T. aggressivum f. aggressivum (Th4), were found to cause mushroom losses. About three years ago, in northern Italy, green mold became a limiting factor in the production of Pleurotus ostreatus, and one year ago it was reported as a problem in the south of Italy. This study was initiated to isolate and identify Trichoderma spp. from the different phases of Pleurotus production; to determine the stage when the infection occurs; to morphologically, physiologically and genetically characterize the isolates; to compare the problems and possible solutions in Agaricus and Pleurotus production; to test the effect of different biotic and abiotic factors on the growth and development of the fungi, and finally to elaborate effective control methods.
hibitor of ATPase-mediated active transport, was added to the medium in order to test its effect upon Zn uptake. No substantial modification of the growth curve of *D. belanzethi* was observed in the presence of sodium vanadate, thus suggesting that the uptake of heavy metals is not mediated by ATPase. Further studies giving more insight into the mechanisms regulating both the uptake and the accumulation of heavy metals in *D. belanzethi* might allow a more efficient use of this fungus in bioremediation strategies.

**SANITARY STATUS EVALUATION OF OLIVE CULTIVARS IN CALABRIA AND SICILY.** G. Albanese, F. Faggioli, L. Ferretti, R. Sciarroni, R. La Rosa and M. Barba. Dipartimento di Agrochimica e Agrobiologia, Università degli Studi Mediterraneo di Reggio Calabria, Piazza S. Francesco 2, I-89061 Reggio Calabria, Italy. Fax: +39.965.689049; E-mail: galbanese@unirc.it

Recent investigations carried out in Italy by dsRNAs analysis showed a very high percentage of virus-infected olive trees. To assess the sanitary status of olive cultivars grown in Calabria and Sicily and to identify the viruses prevailing in these regions, molecular analyses were done. To this effect, shoots were collected from trees of 34 different olive cultivars, from the germplasm collections of the University of Catania and the Istituto Sperimentale per l’Olivicoltura di Cosenza. None of plants showed visible symptoms. Samples of cv. Carolea were also collected in Calabria from commercial orchards where clear, even if sporadic, leafy symptoms. Samples of cv. Carolea were also collected in Calabria from commercial orchards where clear, even if sporadic, leafy symptoms. Samples of cv. Carolea were also collected in Calabria from commercial orchards where clear, even if sporadic, leafy symptoms. Samples of cv. Carolea were also collected in Calabria from commercial orchards where clear, even if sporadic, leafy symptoms.

**INFLUENCE OF MEDIA AND TEMPERATURE ON MICROCONIDIAL FORMATION IN *FUSARIUM* SPECIES SECTION LISEOLA.** L.W. Burgess, M.P. Aleandri, T. Petrovic, H.T. Phan, H.N. Tran and B.A. Summerell. 1School of Land, Water & Crop Sciences, McMillan Bldg, A05, The University of Sydney, NSW 2006, Australia. 2Dipartimento Protezione delle Plante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. 3Royal Botanic Gardens, Sydney, Mrs. Macquaries Road, Sydney, NSW 2000, Australia.

Isolates of *Fusarium subglutinans*, *F. anthophilum*, *F. gloeosporioides*, and *F. verticillioides* were grown in two different culture media (CLA-agar, carnation leaves and SNA, i.e. nutrient agar with a sodium nitrate base). *Fusarium subglutinans* microconidial species considered were better expressed in CLA medium in order to test its effect upon Zn uptake. No substantial modification of the growth curve of *D. belanzethi* was observed in the presence of sodium vanadate, thus suggesting that the uptake of heavy metals is not mediated by ATPase. Further studies giving more insight into the mechanisms regulating both the uptake and the accumulation of heavy metals in *D. belanzethi* might allow a more efficient use of this fungus in bioremediation strategies.

**STUDIES ON VARIABILITY OF THE COAT PROTEIN (CP) GENE OF *CITRUS PSOROSIS VIRUS* (CPV) IN EGYPT.** H. Famby, M. Malfitano, K. Djelouah, A. D’Onghia and D. Allioto. 1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli, 80053 Portici, Napoli. 2Istituto Agroimmo Mediterraneo, 70010 Valenzano, Bari, Italy.

Variability of the coat protein (CP) gene of *Citrus psorosis virus* (CPV) of CPV isolates from Campania was assessed serologically and by sequence analyses of two genomic regions located in 3' (region C) and 5' (region V) halves of the gene. To investigate the genetic variation of CPV from other geographic areas, eight field isolates, collected from different orchards of the Delta region in Egypt, were also analysed. Within the Egyptian sources, the same serological profile was found which was similar to serogroup A profile, found in some Italian sources. Sequence analyses of the eight Egyptian field isolates showed lower genetic diversity values of the CP gene (0.003 for both genomic regions C and V) than those of different isolates from Campania (0.012 and 0.017 for genomic regions C and V, respectively). Phylogenetic analysis of the V and C regions of the CP showed that the Egyptian isolates grouped together with Italian isolates, even if they formed a different cluster. Both Egyptian and Italian sources were clearly separated from the CPV-4 isolate from Florida.

**GENETIC VARIABILITY OF ITALIAN ISOLATES OF *PYRENOCHAETA LYCOPERSICIS*** M. Aragona, A. Infantino, A. Brunetti, E. Lahoz, A. Oliva and A. Porta-Puglia. Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. Fax: +39.06.86802296, E-mail: epid&resist@ispave.it

Corky root is a disease of tomato caused by *Pyrenochaeta lycopersici*. The disease is present in many tomato growing areas in Italy and all over the world, causing up to 40-70% yield losses. *P. lycopersici* has also been isolated from roots of other species, including pepper, melon and lettuce. Forty-three Italian isolates of *P. lycopersici* were collected from tomato and melon plants from fields all over the country and from greenhouse isolates of different origins. In this study we were effective in distinguishing *P. lycopersici* from other closely related species, such as *Pyrenochaeta terestris*, and from the weak pathogenic species *Rhizopus nigricans* which is often present on tomato roots. ITS data provided useful information for the design of specific primers for the rapid identification and detection of *P. lycopersici*. The interaction between *P. lycopersici* and tomato is under investigation at the transcriptional level during the very early contact between roots and fungus in order to study genes differentially expressed during infection.

**DEVELOPMENT OF A DOT-BLOT HYBRIDISATION ASSAY FOR DETECTION OF *CITRUS TRISTEZA VIRUS*.** L. Barbarossa, O. Putere and V. Savino. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: csvvlb07@area.ba.cnr.it

*Citrus tristeza virus* (CTV), an aphid-borne RNA virus, is the causal agent of a major disease of citrus. The virus has been disseminated to most of citrus-growing areas through infected plant material. Subsequent spread by aphids has led to major epidemics. Since effective CTV detection is critical for tristeza management, sensitive, specific and reliable diagnostic methods are required. The development of a sensitive, specific and reliable diagnostic tool is essential for effective CTV management, sensitive, specific and reliable diagnostic methods are needed.
needed. Routine detection of CTV is still mainly performed serologically. Testing of field material by ELISA is used to identify individual trees as CTV-positive or CTV-negative. Estimation of disease incidence is essential for tristeza management decision-making. However, ELISA is not reliable throughout the year for its effectiveness depends on antigen titre and its results are not always clear-cut. As an alternative to ELISA, a nonradioactive molecular hybridisation assay for CTV detection was developed. A DIG-labeled minus-sense riboprobe, complementary to the coat protein gene sequence, successfully hybridised total RNA extracts spotted on nylon membranes, thus allowing detection of CTV in citrus plants grown in a screenhouse or in the field. By contrast ELISA failed to detect the virus in 22% and 28% of the same samples, respectively. The high sensitivity and reliability of molecular hybridisation make it a good alternative to serological methods for CTV detection to be used in surveys and CTV eradication programmes.

GENETIC DIVERSITY OF PSEUDOMONAS SAVASTANOII PV. SAVASTANOII STRAINS IN OLIVE ORCHARDS. A. Campisano, P. Bella and V. Catara. Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Italy. 2Parco Scientifico e Tecnologico della Sicilia S.c.p.a., Catania, Italy.

REP and BOX PCR of Pseudomonas savastanoi pv. savastanoi isolated from olive knots in Sicilian orchards and type strains allowed the strains to be separated into three group according to core profiles defined by the electrophoretic pattern. Polymorphisms delineated by minor bands in REP-PCR profiles were not utilised. Numerical analysis (Dice-UPGMA) of REP PCR polymorphism by GELCOMPAR® software revealed isolates distance to be 65 to 100%. Clusters with a similarity of 77% were detected, each comprising 6 to 22 isolates. Strains from the same orchard, as well as those from different orchards, could cluster either within the same or in different groups. The analysis suggests that different bacterial populations coexist in some of the orchards. The strains within each cluster were only those having REP and REP III or only REP II profiles, with the exception of the cluster including strains from international collections that clustered separately, and the type strain (CFBP1670) clustering the cluster including strains from international collections that REP and REP III or only REP II profiles, with the exception of the cluster including strains from international collections that clustered separately as well as strains of races 2, 3A, 4A and 6, thus confirming previous results obtained by CHEF, REP and multiplex PCR. Local isolates clustered together and separately from other clusters. AFLP molecular typing allowed discrimination of strains belonging to different races, as well as discrimination of strains belonging to the same race.

FOOT ROT OF WHEAT IN ITALY. L. Corazza and A. Santori. Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. Fax: +39 0682070370; E-mail: l.corazza@ispave.it

An increasing incidence of foot rot was observed both on durum and bread wheat in Italy. Foot rot is considered one of the most damaging disease of winter cereals, able to attack also wheat, barley, oats, spelt and triticale, although with different severity. This is a complex disease, since several fungi, mainly Fusarium spp., may be involved at same time; the fungal composition is variable under different agroclimatic conditions. Within the National Project S.I.C., financed by MiP AF, in the last four years (1999-2002), surveys were carried out in order to monitor the phytosanitary conditions of both durum and bread wheat and the incidence of the causal agents of foot rot in Italy. The data obtained confirmed the main role in the etiology of foot rot of Fusarium culmorum, particularly in Southern Italy, and of Fusarium graminearum in Northern and Central Italy. F. culmorum can prevail in the first stages of development of wheat and, later
on, a higher incidence of *F. graminarius* can occur in the same field. *Microdochium nivale*, *Bipolaris sorokiniana*, *Gaumannomyces graminis* and *Rhizoctonia cerealis*, although less frequently, were found in several localities.

**GENETIC VARIATION OF CITRUS TRISTEZA VIRUS DISCOVERED IN CITRUS ORCHARDS IN ITALY.** S. Davino1, L. Rubiò2, V. Savino1 and M. Davino1. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95100 Catania, Italy. E-mail: davinowalter@libero.it. 2Instituto Valencia de Investigaciones Agrarias (IVIA), ctra. Moncada-Da-Naguera km. 4, 46113 Moncada-Valencia, Spain. 3Dipartimento di Protezione delle Piante e Microbiologia Applicata, Via Amendola 165/A, 70126 Bari, Italy.

**Citrus tristeza virus** (CTV) is the causal agent of the most important virus disease of citrus. Numerous CTV isolates differing in biological and molecular characteristics have been reported worldwide. In 2002 CTV was detected in Italy in citrus crops from three separate areas: (i) Naveline in Massafra, province of Taranto, (ii) Tarocco in Belpasso, province of Catania and (iii) Forune in Cassibile, province of Siracuse. CTV isolates from Massafra and Cassibile were mild, whereas isolates from Belpasso induced severe symptoms. The genetic variation of 50, 300, and 100 isolates from Massafra, Belpasso and Cassibile, respectively was examined using RT-PCR, single strand conformation polymorphism (SSCP) and nucleotide sequence analysis of CTV gene p20. All isolates from each area showed the same SSCP pattern suggesting a homogeneous population probably originating from a single focus. Massafra and Cassibile isolates had a nucleotide identity higher than 99% with the Spanish mild isolate T385 and Belpasso isolates showed nucleotide identity of 98% with two seedling yellows-inducing isolates: SY568 from California and NuAa from Japan. These results suggest three recent and independent introductions of CTV from different origins in Italy.

**SPREAD OF ARMILLARIA MELLEA GRAPEVINE ROOT ROT IN PIANA ROTALIANA, TRENTINO REGION, ITALY.** F. De Luca1, M. Sannicolo1, D. Rigling2 and I. Pertot1. 1Istituto Agrario di S. Michele A/A, Via E. Mach 1, 38010 San Michele a/A (TN), Italy. E-mail: federica.deluca@ismaa.it. 2WSL Birmendorf Eidg. Forschungsanstalt Zürcherstrasse, 111CH-8903 Birmendorf, Switzerland.

In Trentino Region (North East Italy), *Armillaria mellea* root rot is a severe and increasing problem in grapevine. In the vineyards, the disease appears in patches of different size. Infected areas and the incidence of the disease in a valley called “Piana Rotaliana” were evaluated. Somatic incompatibility test was used to characterize 53 diploid isolates of *A. mellea*, collected in 40 representative different locations (patches) of the valley. This test, useful for the identification of individual mycelium (genet), and the Geographic Information System (GIS) approach were used to create a genets map of the area. Two replicates of every possible isolate combination were paired on Petri dishes and incubated at 25°C for 3–4 weeks. The formation of barrage lines between isolates of differing genotypes was recorded. The analysis showed the presence in the region of at least 23 different genets of *A. mellea*. Each genotype included either one or many isolates. Three main groups (which include respectively 13, 8 and 6 isolates) were found in more than a single location in the valley. Since infection of grapevine by *A. mellea* spores is considered as irrelevant, two hypotheses can put forward: (i) the area was infected long time ago by *A. mellea* starting from several locations (forest and fruit trees, bushes, etc.) with relevant fungus movement in the soil; (ii) *A. mellea* rhizomorphs and mycelium were carried by soil transported by farmer during crop activities or by river floodings.

**RESULTS OF A THREE-YEAR SURVEY OF PNRSV, APMV AND ACSV IN THE MAIN STONE FRUITS GROWING AREAS OF BASILICATA REGION.** A. Faniigliulo1, S. Comes1,

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**PRESENCE OF RUBUS STUNT IN BLACKBERRY IN NORTHEASTERN ITALY.** P. Ermaca1, L. Carraro, M. Martini, F. Ferrini and N. Loi. Dipartimento di Biofisica applicata alla Difesa delle Piante, Università di Udine, Via delle Scienze 208, 33100 Udine, Italy. Fax: +39.043 2558501.

The presence and distribution of a rubus stunt phytoplasma disease was monitored in an orchard of *Rubus fruticosus* cv. Lochness near Treviso (Northeastern Italy). This orchard was established in spring 2000, using plants imported from France. During the first year of cultivation no typical phytoplasma symptoms were observed. In 2001, 5 of the 650 plants showed clear symptoms of phytoplasma infection: yellowing of the leaves, bushy stunt, proliferation, virescence and phyllody. In spring 2002, 130 plants (20%) showed symptoms. DAPI staining confirmed the presence of phytoplasmas in all the samples randomly collected from symptomatic plants. Further on, 5 plants were analysed by nested-PCR and RFLP. After DNA extraction following an enrichment procedure, PCR/RFLP analyses were performed using P1/P7 primer pair followed by R16F2n/R2 and R16(V)F1/R1 in nested PCR. All samples were positive for the presence of phytoplasmas. R16F2n/R2 PCR-products were digested with *Tus*I, *Tsp*509I, *Bfa*I enzymes. The RFLP patterns permitted to identify the phytoplasmas as belonging to the Elm yellows (EY) group (16S V), rubus stunt subgroup 16S V-E. During 2002 a control program was implemented based on eradication of symptomatic plants and insecticide treatments according to organic procedures. In 2003 only four symptomatic plants were identified.

**ONE-STEP RT-PCR FOR A RAPID DETECTION OF VIRUSES IN OLIVE TREES.** F. Faggioni, R. Sciarraoni, L. Ferretti, V. Luria, G. Albanese, G. Passuini and M. Barba. Istituto Sperimentale per la Patologia Vegetale, Mi.P.A.F., Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39 6.86802296; E-mail: virologia@ispave.it

A rapid and accurate detection of viruses is essential in the aim of regulating marketing of olive propagative material and improving the sanitary and qualitative requirements for the safe movement of germplasm around the world. Molecular techniques have improved the diagnosis of viruses in olive plants as compared with the biological and/or serological methods used in the past. In this frame, we developed a one-step RT-PCR protocol for a rapid and sensitive detection of the most important and spread olive viruses: ArMV, SLRSV, CLRV, ORLSV, CMV, OLV-1, OLV-2, and OLYaV. In particular, for SLRSV, ArMV, ORLSV, CMV and OLV-1 new specific primers were designed, whereas for OLV-2, OLYaV and CLRV oligonucleotides published in previous papers were used. Viral RNA was extracted from 0.1 g of bark tissue, collected in the spring from virus-affected trees, by a commercial “RNeasy Plant Mini Kit” (Qiagen). The protocol was very rapid for the analysis of 48 samples requires only 7 h from RNA extraction to the visualisation of results. The results were the same as those obtained by nested RT-PCR protocols. The developed one-step RT-PCR protocol provides rapid, reliable and sensitive diagnosis and its employment in mass-scale analysis for olive certification programs is suggested.
During the last decade stone fruit crops have undergone a strong expansion in Metapontum area (Basilicata region), covering up to 7500 ha. The widespread presence of different virus diseases affecting these crops in different Italian regions and the more and more high request of plant propagation material represent a serious risk for agriculture in the Basilicata region. A survey was carried out for monitoring the sanitary status of stone fruits (in particular peach, plum and apricot) with reference to PNSRV, ApMV, ACLSV. It was carried out in spring of 1999, 2000 and 2001 in the main stone fruits growing areas of Metapontum, using aerial photos and cadastral maps. Selected fields were homogenous for age and stone fruits species and cultivars. A total of 1,679 ha of surface, corresponding to about 22.4% of selected areas, was used for sampling. Leaf samples were collected from symptomless and symptomatic plants (8 plants/ha) with a diagonal scheme of sampling, and were tested for the presence of PNSRV, ACLSV and ApMV by ELISA, using commercial kits (Sanofi-Pasteur). Incidence of infections during the three-year survey was as follow: 4.1±0.2% for ACLSV, 10.0±2.7% for PNSRV and 6.5±2.0% for ApMV. The highest percentage of infections was found in the oldest orchards (about 10 years of age), where some plants had mixed infections by two or three of the tested viruses.

SPHAEROPSIS SAPINEA PATHOGEN OF PINUS RADIATA IN COMMERCIAL PLANTATIONS IN SARDINIA. A. Franceschini, B.T. Linaldeddu, P. Marongiu and M.A. Mannoni, Dipartimento di Protezione delle Pianete – Sezione di Patologia vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; E-mail: afman@uniss.it

In recent years in Sardinia there has been an increase of decay of Pinus radiata plants near commercial maturity in various artificial stands planted to meet the growing demands of the paper industry. Affected plants are easily recognised because the tree top is first chlorotic, then withers to an extent varying from plant to plant. These symptoms then appear in the distal parts of the branches scattered in the crown, and eventually affect the whole plant. Dead needles remain attached to the branches for a long time, and 2-3 cm long elliptic cankers often form on the branch-es. Sphaeropsis sapinea (Fr.:Fr.) Diko et Sutton, was repeatedly isolated from woody tissues and needles of symptomatic branches. This pathogen is widespread in both hemispheres, causing significant economic damage to pines and other conifers. In Italy it has been reported on various Pinus spp., but never before on P. radiata. Consequently, the pathogenicity of Sardinian S. sapinea isolates on 1-to-3-year-old P. radiata seedlings was tested. The isolates from both woody tissue and needles were highly aggressive for 3-5 weeks after inoculation the tips of the crowns of all the infected plants bent over, and the branches then dried out acropetally. Because there are four known morphotypes of this fungus, morphological and molecular studies were made to characterise the Sardinian isolates. The results showed that all the isolates belonged to type A, as defined by Palmer et al. (1987), and Smith and Stanoz (1999).

MOLECULAR DIAGNOSIS OF PSEUDOMONAS SYRINGAE PV. TOMATO. V. Fanelli1, C. Cariddi1, M. Finetti-Sialer2 and D. Gallitelli3. 1Dipartimento di Protezione delle Pianete e Microbiologia Applicata, Università degli Studi, Via Amendola 163/A, 70126 Bari, Italy. Fax: +39.080.544291; E-mail: corrado.cariddi@agr.uniba.it. 2Dipartimento di Agrochimica e Agrobiologia, Università degli Studi Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales, 89061 Gallina (RC), Italy.

Pseudomonas syringae pv. tomato, the causal agent of tomato bacterial speck, is an harmful pathogen for which early diagnosis in plant propagation material is mandatory (EEC directives 93/61 and 93/62). By analysis of the polymorphic amplicons generated with the primer OPA-19 from the DNA of ten isolates of P. syringae pv. tomato collected from different geographical areas, a 250 bp fragment was identified and labelled with DIG-probe synthesis kit (Roche). In Southern blot analysis this fragment hybridised specifically to a fragment with similar size of theRAPD pattern generated by the type strain 1106 NCPPB of P. syringae pv. tomato and used as control and by the other well-characterised strains ISFP 111, PD 170 and IPV BO 1611, IPV BO 2548 of P. syringae pv. tomato kindly supplied by Dr. Marco Scortichini and Prof. Carlo Bazzi, respectively. No hybridisation signal was obtained with DNA extracted from pathogens actinidiae, populans, persicae, lacrymans, japonica, panic, pisi, aptata morprunorum, syringae and from P. savastanoi pv. savastanoi. The 250 bp fragment was ligated to the pGEM-T plasmid (Promega) and cloned in E. coli. The nucleotide sequence was determined and deposited in EMBL database. When compared with other available sequences, this fragment was found 98% homologous to a region flanking a heat shock protein gene (section 16-21 of the complete genome) of the strain DC 3000 of P. syringae pv. tomato (Acc. No AE016871). The use of this fragment as P. syringae pv. tomato diagnostic tool is proposed for large scale testing.

MOLECULAR CHARACTERIZATION OF MYCOCENTROSPORA CLADOSPORIOIDES (SACC.) P. COSTA EX DEIGHTON, THE CAUSAL AGENT OF OLIVE CERCOSPORIOSES. P. Gallone, L. Schena, A. Ippolito and F. Nigro. Dipartimento di Protezione delle Pianete e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 163/A, I-70126 Bari, Italy. Fax: +39.080.5442911; E-mail: nigrof@agr.uniba.it

A collection of 200 isolates of Mycocentrospora cladosporioides from different olive tissues (leaves and drupes) and regions in the Mediterranean basin was tested to determine the extent of intraspecific genetic variability by random amplified polymorphic DNA-polymerase chain reaction (RAPD–PCR). Eight 10-bp primers (OpD20, OpH13, OpH20, Ap1, Ap5, Ap17, H3, H4), preliminarily screened among seventy, were used. The isolate 159.48 kindly supplied by Mr. Marco Scortichini and Mr. Marco Scortichini and from CentralBureau von Schimmel Cultures, formerly known as Cercospora cladosporioides (Sacc.), served as a reference strain. Results indicated that isolates from leaves have a higher intraspecific genetic variability than isolates from fruits. Specific RAPD markers related to the geographical origin and host’s matrix (leaf/fruit) were obtained and the phenogram generated by UPGMA analysis grouped the isolates under six significantly different clusters. The Internal Transcribed Spacer (ITS) region, including the rDNA 5.8S from 12 representative isolates of M. cladosporioides and several other species in the genera Cercospora and Mycocentrospora, was amplified and sequenced. ITS sequences from M. cladosporioides isolates showed 98% homology with Cercospora kalmiae Ell. & Everh and 95% with other species belonging to the genus Cercospora. ITS sequences were aligned and screened for differences among several fungal species. Primers specific for M. cladosporioides were designed and tested on DNA from Mycocentrospora spp., Cercospora spp., and many other fungal species. Two primers pairs (MC1-MC6 and MC2-MC6) were specific for M. cladosporioides amplifying a unique DNA fragment of the expected size. The availability of species-specific primers will facilitate new studies on the biology of M. cladosporioides.
DIVERSIFICATION OF MORPHOTYPES OF PSEUDOMONAS CORRUGATA IN HETEROGENEOUS ENVIRONMENT. S. Greco1, A. Ferrari1, P. Bella1, V. Catara2 and S. Guglielmino1. 1Parco Scientifico e Tecnologico della Sicilia S.c.p.a., Catania, Italy. 2Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università degli Studi di Catania, Italy. Fax: +39.095.7147278; E-mail: scatara@unic.it. Dipartimento di Scienze Microbiologiche, Genetiche e Molecolari, Università degli Studi di Messina, Italy.

Bacteria produce morphological variants and phenotypic conversion often has a pleiotropic effect on different bacterial activities such as virulence, antagonism, and enzyme production. The occurrence of colonies with morphology different from that of inoculated strains was observed during the re-isolation of Pseudomonas corrugata strains in the course of studies on soil survival and plant infection. This study investigated the effect of ecological opportunity on the occurrence of morphological variants by replicating ancestral morphotypes of P. corrugata and the related species P. mediterranea propagated in spatially heterogeneous environments provided by static LB-broth cultures. The upper and bottom phases of the cultures were destructively sampled every 24 h for 15 days and monitored for changes in colony morphology, growth, protein content and oxidative metabolism. The last three parameters were lower in the upper phase than in the bottom phase for both strains. All the parameters of P. corrugata cultures investigated peaked near the 8th day and P. mediterranea on the 9th day. At this time interval, several morphologically different colonies were observed on nutrient dextrose agar and the colonies could be differentiated on the basis of their entire or curled margins, rough or smooth surfaces, raised, flat or umbonate elevation. Fitness of representative morphotypes was evaluated. Morphological diversification of some of the variants had a pleiotropic effect on the capacity to determine HR on tobacco, virulence on tomato, antagonistic activity profile and a number of enzymatic activity.

EPIDEMIOLOGY OF POWDERY MILDEW OF GRAPEVINE IN SOUTHERN ITALY. H. Hajjeh, M. Miazzi 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

Recently, two genetically distinct biotypes were found in European and Italian isolates of Uncinula necator (Schw.) Burr., the causal agent of powdery mildew of grapevine. It has been hypothesized that the two biotypes correspond to the two overwintering modes of the pathogen, mycelium and conidia in buds or cleistothecia, and cause, respectively, early symptoms in young shoots and leaves (“flag shoots”) or late infections in bunches. Occurrence and frequency of “flag shoots” and cleistothecia were monitored in 29 vineyards located in South Italy in 2000-2003. “Flag shoots” were recorded with a low frequency (0,3% of shoots) in 33% of vineyards, 40% of “flag shoots” were originated from the two proximal buds of the canes. In different years, 30% of “flag shoots” appeared on a same vine, and 16% on a same branch. Cleistothecia were found in 46% of vineyards. Viability and ripening of cleistothecia on the leaves was followed in 16 vineyards. In spring, the numbers of cleistothecia was up to 1,056 cleistothecia/g of leaves with 10% of viable ascospores. Biotype-specific RAPD (Random Amplified Polymorphic DNA) markers were sequenced and SCAR (Sequence Characterised Amplified Region) primers designed. PCR reactions with the primer pair UnE-UnF distinguished “flag shoot” and “ascospore” isolates. The assay was used on 235 isolates sampled in 6 vineyards from April to September in 2000-2002. The “flag shoot” biotype was prevalent until June; later the “ascospore” biotype became prevalent in fungal populations. Further investigations are in progress on the significance and role of the two biotypes in the epidemiology of the disease.

FOUR-YEAR MONITORING OF THE MYCOFLORA ON WHEAT GRAINS IN ITALY. N. Pucci, A. Infantino, G. Conca, G. Di Giambattista and A. Porta-Puglia. Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertore 22, 00156 Roma, Italy. E-mail: epiderresis@istpave.it

During the period 1999-2002 the occurrence of fungal species on wheat kernels has been studied to evaluate their role in direct damages to seed quality and indirect damages caused by the presence of mycotoxin. For each year, two bread wheat (‘Centauro’, ‘Eridano’) and three durum wheat cultivars (‘Appulo’, ‘Simeto’, ‘Zenit’) were studied. They were grown in experimental fields located in the main cultivation areas of the species in Italy. For each variety and site, two replicates of 100 g each were analyzed by the freeze blotter method. During the evaluation period, the incidence of mycotoxa appeared greater in grains grown in northern Italy compared to those produced in the central and southern areas. Average infection rates were 19.9% in the north, 12.9% in the centre and 2.1% in the south. The species most frequently isolated were Microdochium nivale, Fusarium graminearum and Fusarium poae, these latter two known to be toxicogenic. Other Fusarium species, among which F. semitectum, F.avenaceum, F. moniliforme, F. culmorum and F. equiseti, had a minor incidence, variable with the year and the geographic location. However, the average incidence of Fusarium spp. in bread wheat was lower than in durum wheat. Among the cultivars tested, the highest infection level (23.4%) was detected on ‘Simeto’ in 2000.

IDENTIFICATION OF NEW TRICHOVIRUSES AND FOVEAVIRUSES IN PLUM AND APRICOT. D. Liberti1, A. Marais2, L. Svanella-Dumas3, P. Gentil1, A. Ragozzino1 and T. Candresse2. 1Dipartimento AR.BP.AVE. Università di Napoli “Federico II”, università 100, 80035 Portici (NA), Italy. 2Equipe de Virologie UMR GDPP, IBVM, INRA, BP 81, 33 883 Villenave d’Ornon cedex, France. 3Cfl, Centre de Lanzade BP21, 24130 La Force, France. Fax: +33.05.57122384; E-mail: dilbert@unr.bordeaux.fr

Stone fruit-affecting viruses have been studied in the Campagna region. Besides the diseases caused by known viruses (PPV, ApMV, PNRSV, PDV, ACLSV), the identification of etiological agents of diseases not yet related to any known virus is an obvious priority. This applies to apricot-ring-pox symptoms found in 1985 in apricot in an orchard of Pontecagnano (Salerno), and for severe stem-growth symptoms found in several plums collected from different orchards of the Vesuvian area. In an attempt to characterize severe isolates of ACLSV, a degenerate-primers nested-PCR technique (PD0), previously developed for the polyan lent detection of viruses belonging to the Fovea-, Tricho- and Capillovirus genera, was used. Surprisingly, this technique revealed the presence, in all plum and apricot plant accessions, of a new virus related to the Trichovirus genus, which was systematically found in mixed infection with ACLSV. To evaluate the possible presence of other viral agents, double-stranded RNAs (dsRNAs) purified from infected GF305 indicators were used as templates in polyan lent PCR (DOP-PCR). In this way cDNAs corresponding to an additional viral agent from the apricot-ring-pox sources, were amplified and sequenced. The agent appeared to represent a foveavirus closely related to Cherry green ring mottle virus (CGRMV). This is the first report of natural infection of CGRMV in apricot.
IMPROVED MOLECULAR DETECTION OF CITRUS PSOROSIS VIRUS AND CITRUS VARIEGATION VIRUS BY HYBRIDIZATION ON TISSUE-PRINTING. G. Loconsole, M.T. Fatone, M.A. Castellano and A. Minafra. Dipartimento di Protezione delle Pianta e Microbiologia Applicata, Università degli Studi, and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy.
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Citrus psorosis virus (CPV) and Citrus variegation virus (CVV) are the agents of major diseases of citrus. Since their absence is required for the production of citrus propagating material of both “certified” and “C.A.C.” categories, the availability of sensitive, reproducible, specific, and cheap diagnostic tools for their detection is desirable to facilitate sanitary controls. Detection of CPV and CVV was improved by developing a protocol of molecular hybridization on tissue prints, with two digoxigenin-labelled riboprobes complementary respectively to a sequenced region of the coat protein gene of local isolates of CPV (IAM UBA ps101) and CVV (UBA-CVV301). Different tissues and organs of several citrus plants were compared: CPV-infected leaves, flowers and flavedo, fresh or stored at 4°C for a week; CVV-infected young and old leaves, petioles, shoots and flowers, fresh or stored at 4°C for a month. The results showed that detection of CPV was reliable when the hybridization was done on prints from ovary, whereas CVV was satisfactorily detected in all analyzed plant parts, except for old leaves.

OCCURRENCE OF CITRUS VARIEGATION VIRUS IN APULIA. M.T. Fatone, G. Loconsole and M.A. Castellano. Dipartimento di Protezione delle Pianta e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: mt.fatone@agr.uniba.it

Infectious variegation of citrus is a disease reported from all major citrus growing regions of Italy including Apulia, but little information about its incidence in this latter region is available. Field diagnosis based on symptoms observation is often difficult, because of latent infections under certain environmental conditions (high temperature) and graft combinations. The present work reports the first results of an investigation, carried out in spring 2002, for assessing the incidence of Citrus infectious variegation virus (CVV), the agent of the disease, along the Ionian coast of Apulia (province of Taranto). During field surveys, carried out in several commercial orchards and varietal collections, 936 leaf samples were collected from plants showing symptoms recalling those elicited by CVV (crinkled and/or variegated leaves) or by other viruses, or showing non apparent signs of infection. Diagnostic tests were performed by dot-blot hybridization of total nucleic acids extracted from leaf tissues, using a digoxigenin-labelled riboprobe corresponding to a part of the CVV capsid protein sequence. The results showed that 19 out of 653 (about 3%) samples from commercial orchards were infected by CVV, whereas no virus was detected in samples collected from varietal collections. Twelve CVV-infected plants showed crinkled leaves, two of which had also leaf variegation. The seven remaining CVV-positive trees were either asymptomatic or showed symptoms of other virus or virus-like diseases, i.e. crustacitos, concave gum, leaf flecking and/or ringspot, bark scaling, oak leaf pattern, tree decline, bud union disorders.

OCCURRENCE OF ENDOPHYTIC FUNGI IN THE CROWN OF OAK TREES IN SOUTHERN ITALY. F. Mannerucci, R. Ubaldo, G. Campanile, S.L. Giove, A. Gatto, G. Sicoli and N. Luisi. Dipartimento di Biologia e Patologia vegetale, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy.
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Endophytic fungi are commonly detected in the crown of plants of genus Quercus, especially in declining trees, and their pathogenicity is often a point of discussion among scientists. Preliminary results on the variability and incidence of endophytes collected in 20 oak woods of four regions in southern Italy are reported. In each wood, samples were collected in spring and autumn 2002, from four healthy and/or declining Q. pubescens Willd. and Q. cerris L. adult trees. Isolations were obtained in vitro in agar media from 30 buds, bark, and wood of 15 one-year-old and 15 three-year-old twigs per species and oak wood. The colonisation frequency of each endophyte was based on the formula CF = (N/Nt) x 100 (Nt = number of isolations yielding the same endophyte and N = number of all isolations done). The data from the spring survey showed that Discala quercina (West.) v. Arx (CF=17.3%), Biscogniauxia mediterranea (De Not.) O. Kuntze (CF=12.0%) and Aureobasidium pullulans (De Bary) Arz. (CF=5.5%) were the most frequent fungi regardless the Quercus species. Isolations from samples collected in autumn proved that D. quercina was still the most frequent (CF=13.9%), followed by Triboderma sp. (CF = 12.6%), Botryosphaeria stevensoii Shoemaker (CF = 6.4%) and A. pullulans (CF=5.5%), while B. mediterranea was considered as “occasional” (CF<5.0%). Other “occasional” endophytes belonged to the genera Alternaria, Cytospora, Phoma and Phomopsis. In general, D. quercina and A. pullulans occurred mainly in both buds and one-year-old twigs, B. mediterranea was mostly isolated from three-year-old twigs, while 18% of samples did not originate any colonies. This percentage rose to 41% if only wood samples were considered. Since some of the recorded endophytes are well-known weak pathogens, this investigation points out the additional role that endophytes from buds and bark tissues can play in oak decline, especially when trees are weakened by environmental stress factors.

POLYMERASE CHAIN REACTION IDENTIFICATION OF PHELLINE TORULOSUS. G. Campanile1, L. Schena2 and N. Luisi1. 1Dipartimento di Biologia e Patologia vegetale, Università degli Studi di Bari, Via G. Amendola, 165/A, 70126 Bari, Italy.
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Phellinus torulosus (Pers.) Bourd. et Galz. is the causal agent of the white alveolar wood decay found on several species of trees including a large number of forest trees. Immediate detection of the fungus is essential to identify the diseased trees before it spreads to healthy plants. However, current detection methods based on isolation from infected tissues on semi-selective media are laborious, time consuming (20-30 days), and require skilled expertise to identify the pathogen after isolation. These constraints have encouraged the search for rapid and reliable alternative approaches. In the present study a molecular method based on polymerase chain reaction (PCR) has been developed. The internal transcribed spacer (ITS1 and ITS2) regions were amplified from 10 different P. torulosus morphotypes using the universal primers ITS1 to ITS4. Nucleotide sequences were identical in all isolates. ITS1 and ITS2 DNA sequences of P. torulosus were analysed using of BLAST (basic local alignment search tool) to exclude the presence of identical sequences in other microorganisms among available DNA databases (GenBank). Similar sequences from different fungi were aligned using the “Clustalw” software (EMBL, European Bioinformatics Institute) and screened for differences to design specific primers around sequences unique to P. torulosus. In particular, two primer pairs amplifying unique DNA fragments of 150 bp (Pt1 and Pt2) and 450 bp (Pt3 and Pt4) confirmed to be specific for P. torulosus
when tested against a large number of other fungal isolates from different hosts and geographic areas. The short DNA fragment amplified with the primer Pt1 and Pt2 is suitable for developing a real-time detection system.

STUDIES ON THE EPIDEMIOLOGY OF STEMMHYLLUM VESICARUM ON PEAR. E. Maccarferi, M. Collina and A. Brunelli. Dipartimento di Protezione e Valorizzazione Agricoltura e Foreste, Università degli Studi di Bologna, Viale G. Fasin, 40127 Bologna, Italy. Fax: +39.051.4640127; E-mail: mccollina@agrsci.unibo.it

Stemphyllium vesicarium (Wallr.) Simm (teleomorph Pleospora allii (Rabenhorst) Cesati & de Notaris), the causal agent of brown spot of pear, occurs in different European Countries (Italy, Spain, France, The Netherlands, Belgium). Since 1970s, many pear orchards in the Po Valley (the main pear growing area in Italy) have been affected by this fungal disease that may cause heavy losses (sometimes close to 100%), due to its necrotrophic action and to the high sensitivity of pear fruits during the whole season. Many studies on disease control have been carried out, while most of epidemiological aspects need to be clarified as, for instance, the role of the teleomorph Pleospora allii. Pseudeothyecia of P. allii (occurring on affected pear leaves on the ground) produce normally ascospores but, on the basis of aerobiological monitoring and studies carried out in field and laboratory, these do not seem to have a primary role on the spread of the disease on pear trees in spring. In fact ascospores are rarely caught in pear orchards and mainly in periods when pear is not susceptible to the disease (autumn). This suggests that ascospores release and germination are not necessarily linked to pear tree. Furthermore, captures of conidia in places different from pear orchard (e.g. apple orchard), indicate that this pathogen can sporulate far from pear. Moreover, conidia rarely occur on affected pear fruits and leaves in the field, although in laboratory assays the fungus completes its cycle on pear tissues. Hence, survival of S. vesicarium might not be linked with pear trees. These results are consistent with the saprophytic nature of Stemphyllium vesicarium.

COLLETOTRICHUM GLOEOSPORIOIDES CAUSAL AGENT OF OLIVE ROT IN SICILY. G. Scarito, A. Pane, F. Raudino, S. Frisullo and S.O. Cacciola. Dipartimento di Scienze Ettomologiche, Fitopatologiche, Microbiologiche Agrarie e Zootecniche, Sezione di Patologia Vegetale, Università degli Studi di Palermo, Viale delle Scienze 2, I-90128, Palermo, Italy. Fax:+39.091.423238; E-mail: cacciola@unipa.it

In late autumn 2001, a rot of ripe olives was noticed in many olive-growing areas of Sicily. Symptoms resembled those of anthracnose caused by an olive-specific Colletotrichum sp. endemic in some humid areas in Calabria, Apulia and Sardinia (southern Italy). COLLETOTRICHUM isolates were obtained consistently from rotten olives and were compared with a wide collection of reference isolates of Colletotrichum species. Polymerase chain reaction (PCR) with species-specific primers and random amplified poly-morphic DNA (RAPD) analysis were used to characterize the Colletotrichum isolates. Primers for taxon-specific amplification included the conserved primer ITS 4 coupled with specific primers for C. acutatum (CGInt 1 and CGInt 2) and C. gloeosporioides (CGInt). For RAPD-PCR, 16 decamer primers were used (Operon Technologies, Alameda, CA, USA). All olive isolates from Sicily produced amplification products only with the C. gloeosporioides-specific primer CGInt and showed RAPD banding patterns very similar to those of C. gloeosporioides reference-isolates from other hosts. Moreover, olive isolates from Sicily were benomyl-sensitive (MIC <1 µg ml^-1) and grew fast (radial growth rate on potato-dextrose agar 24°C 12-14 mm day^-1), with an optimum growth temperature at 28°C, thus suggesting that they can be referred to as C. gloeosporioides sensu stricto, a species with a broad host-range. Although artificial inoculations of Sicilian isolates in olives induced symptoms similar to those caused by the host-specific Colletotrichum sp. responsible for anthracnose in other regions of southern Italy, C. gloeosporioides seems to be weakly pathogenic to olive in natural conditions.

MORPHOLOGICAL AND BIO-MOLECULAR CHARACTERIZATION OF SPHAEROPSIS SPP. AND THEIR INTERACTION WITH HOST PLANTS. M.A. Mannoni, L. Maddau, A. Schiaffi-no 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

Fungi of the genus Sphaeropsis have often been associated with the decay of fruit and forest plants, even though their pathogenetic role and taxonomic identity is not always clear. Because Sphaeropsis sapinea and S. malorum [teleomorph = Botryosphaeria obtusa] from Pinus radiata and fruit plants (Pyrus communis, Prunus dulcis and Vitis vinifera), respectively, have been repeatedly isolated in Sardinia, we believed that preliminary morphological, bio-molecular and pathogenetical studies should be carried out to characterise those isolates. Thus, isolates of S. sapinea and S. malorum from various parts of Sardinia were compared with strains of the same fungal species and with anamorphs of Botryosphaeria spp. collected from different hosts and from several Italian areas. Two distinct morphotypes which were common to S. sapinea and S. malorum were found. They were both able to induce cankers and discolorations of the xylem in wound-inoculation field experiments. Molecular analysis by RAPD-PCR using random primers, generated a dendrogram of genetic similarity. Sphaeropsis samples were distributed in two main clusters which corresponded exactly to both observed morphotypes. By contrast, no clear relationship was observed between strain distribution and host species or between this distribution and the geographical origin of the strains. These results confirm the close molecular relationship between strains of S. sapinea and S. malorum, which has often been reported in the literature. Further research on the bio-molecular aspects of these species and on the mechanisms involved in their pathogenicity is necessary.

MOLECULAR ANALYSIS OF V6 AND V9 MITOCHONDRIAL DOMAINS OF TWO TAXA OF THE PLEUROTUS ERYNGII COMPLEX, ISOLATED FROM ERYNGIUM SPP. AND FERULA COMMUNIS. P. Marongiu, L. Maddau 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: fmarras@uniss.it

From a collection of Pleurotus eryngii (DC.:Fr.) Quél. isolates owned by the Plant Protection Department of the University of Sassari we selected 10 from Eryngium spp., and 10 from Ferula communis. These two groups show several morphological, biochemical and molecular differences and therefore were previously classified into two different species. With the aim of finding a new molecular marker capable of distinguishing the two species, we carried out a comparative study of the V6 and V9 variable domains of the mitochondrial small-subunit (SSU) rRNA which have been previously used to discriminate other species of Pleurotus. The PCR analysis of total DNA extracted from vegetative mycelia, showed amplimers of identical size among the 20 isolates, for the V6 and V9 mitochondrial domains respectively. The nucleotide sequence of the two domains was determined in the 20 isolates and aligned to each other and with the NCBI database using the CLUSTAL W (ver. 1.82) software. The secondary struc-
ture of both V6 and V9 domains was simulated and analysed. It was found that there is a 100% identity between all analysed isolates within V6 and V9 domains as well as a complete homology with those of P. eryngii reported in GenBank. In conclusion, these two domains did not appear to be useful for distinguishing P. eryngii eryngii from P. eryngii fenulæ.

**QUANTITATIVE REAL TIME PCR FOR THE DETECTION OF TOMATO YELLOW LEAF CURL SARDINIA VIRUS IN PLANTS AND IN ITS INSECT VECTOR.** M. Mason, G.P. Accotto, P. Caciagl0, D. Marían and E. Noris. Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. Fax: +39.011.343809; E-mail: e.noris@ivv.cnr.it

Tomato yellow leaf curl Sardinia virus (TYLCSV) causes severe crop losses in tomato, frequently limiting its production in the Mediterranean basin. TYLCSV belongs to the Geminiviridae family, genus Begomovirus. It is transmitted by the whitefly *Bemisia tabaci* in a circulative persistent manner. Although rapid and reliable diagnostic protocols are available for the detection of TYLCSV using PCR and hybridization techniques, viral load in plant and insect tissues have not been investigated to date. We have developed a Real Time PCR-based method for the detection and quantification of TYLCSV in plant tissue and in the insect vector. The TaqMan assay proposed is based on the quantification of viral DNA using primers and probes targeted to the viral replication-associated protein gene. Relative viral quantification is achieved performing parallel reactions that specifically target either plant or insect endogenous genes. This approach provides a rapid and accurate diagnostic tool for determining the presence of TYLCSV. Moreover, the higher sensitivity of the technique compared to conventional PCR-based methods will be useful for virus-vector interaction studies, while the possibility of determining viral load will be exploited in studying the distribution of the virus within the plant, and in evaluating tolerant/resistant tomato varieties.

**FUNGAL ENDOPHYTES MONITORING IN OAK STANDS IN CENTRAL ITALY.** A. Mazzaglia, I. Librandi and N. Anselli. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. Fax: +39.0761.357473; E-mail: anselli@unitus.it

This research is part of the studies on fungal endophytes involved in oak decline. Analysis of endophytic microflora in oak stands grown at different altitudes in central Italy was carried out. Our aim was to relate these data to the health status of the plants, site features, and Quercus species. Four healthy plants were selected in each of 16 sites in 5 Italian regions: Abruzzo, Molise, Lazio, Umbria and Marche. One hundred samples were collected from each plant. After surface sterilisation, they were transferred to proper substrate. The recovered fungi were subcultured and the species morphologically determined. The most common species isolated in the different sites were: *Dysidea umbrinella* (36,5%), *Biscognia xuxia mediterranea* (10,8%), *Aureobasidium pallidum* (5,5%), *Cladosporium spp.* (5,4%), *Botrytis spp.* (1,6), *Trichoderma spp.* (1,1), followed by few other species with sporadic presence (20,6%). The remaining 17,0% were negative isolation attempts. Except for heavy declining stands, *Biscognia xuxia mediterranea* was less abundant than expected, probably due to the rainy year. However, the pathogen was detected in all the sampled sites, both in mixed and in pure stands at altitudes ranging from 0 to 1200 m asl. Its incidence varied from 1% in a *Quercus cerris* pure stand at Carpegna (PU), to 42% at Monte Rufeno (VT) in a mixed oak stand bordering areas with a severe decline condition. *Dysidea umbrinella* was quantitatively the most important endophyte in all *Quercus* species and in most of the sites. Its pathogenic role, related to plant weakening, is being investigated. The presence of weakness parasites in oak forests, their relative abundance and their role in decline is discussed.

**INVESTIGATIONS ON THE ETIOLOGY OF BARK CANKERS ON WOODY WALNUT TREES.** A. Mazzaglia and A. Fabi. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. Fax: +39.0761.357473; E-mail: anmgzza@unitus.it

Several diseases are reported from walnut (*Juglans regia*), an important economic resource for Italian agriculture. One of these is the presence of typical cankers with black essudates on the trunk. The causal agents of this symptomatology have not yet been identified. In this work several isolations were attempted, both for fungi and bacteria, in order to improve our knowledge of this problem. Two walnut stands, severely interested by the disease in S. Matteo (MN) and Loreto (AN), were investigated. To study fungal involvement, 378 isolations in the first site and 664 in the second were attempted from woody fragments bordering lesions of the bark. Even if the number of positive isolations were considerably low in both sites (88.9 and 74.4%, respectively), it should be noticed that three fungal species were mainly retrieved: *Botryosphaeria sp.*, *Paraphaephaeria* sp. (an. *Coniothyrium* sp.), and *Nectria haematococca* (an. *Fusarium solani*). Their identification was carried out both morphologically and molecularly by comparing ribosomal sequences (ITS and a portion of 18S). These are all potential canker agents. Bacteria isolated from walnut tissues were characterized by Biolog® System their phenotypic characteritistics being typical of *Enterobacteriaceae*. Further studies of identification by Fatty Acid Profiling showed that *Brenneria nigerfliuens*, the bacterial agent of bark canker on walnut, was constantly present in walnut tissues in both sites, in association with other saprophytic *Enterobacteriaceae*. Even though *Brenneria nigerfliuens* was detected also in other sites and is considered the main causal agent of the disease, the simultaneous presence of both fungi and bacteria in walnut canker is very interesting and their potential role in the cankers, alone or in association, is discussed.

**PATHOGENICITY OF ARBILLARIA SPP. ON WATER-STRESSED MEDITERRANEAN OAK SEEDLINGS.** R. Metalaj, G. Sicoli and N. Luis. Dipartimento di Biologia e Patologia vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.3442906; E-mail: laiusin@agr.uniba.it

Pathogenicity tests using three *Armillaria* species (*A. mellea* (Vahl: Fr.) P. Kummer, *A. gallica* Marsxm. et Romagn. and *A. tabescens* (Scop.: Fr.) E. Emel.) were carried out on 1440 three-year-old potted seedlings of five *Quercus* species (*Q. cerris* L., *Q. ilex* L., *Q. pubescens* Willd., *Q. robur* L. and *Q. trojana* Webb.) distributed into three groups subjected to different water supplies in a screenhouse. The inoculation was made by attaching a 2.5-3.0x6.0-cm sized piece of *Q. ilex* branch previously colonised by the fungus at the unwounded main root of the oak seedlings. A sterile equal inoculum was used for the control seedlings. Water stress was assessed monthly by measuring the Midday Water Potential on non-inoculated equally-watered seedlings during the vegetative season by means of a pressure chamber. Data, based on infection extent and plant decline symptoms, were collected one year after inoculation and statistically analysed by means of ANOVA and Duncan’s test. Signs of infections were detected in 63,1% of the least and in 44,2% of the most watered seedlings. Although all three *Armillaria* species were able to induce infections, *A. mellea* proved to be the most pathogen in all cases. Based on symptoms severity, *A. gallica* showed to be statistically as pathogenic as *A. mellea* only on the least watered
seedlings. As to *Quercus* species, the highest number of infected seedlings belonged to *Q. ilex* (64.8%), the least to *Q. robur* (50.6%). *Q. ilex* proved also to be the most statistically severely attacked species. Water stress in oak species appears to be a significant tool for assessing differences in pathogenicity among *Armillaria* species.

**CHARACTERIZATION OF ISOLATES OF PHOMA TRACHEIPHILA BY RAPD-PCR, MICROSATELLITE-PRIMED PCR AND RNDA ITS1/ITS2 SEQUENCING.** V. Balmas, B. Scherm, Q. Miglieli. Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; E-mail: miglieli@uniss.it

The mitosporic fungus *Phoma tracheiphila* is the incitant of the “mal secco” disease of lemon and other *Citrus* species. The pathogen invades the xylem and causes progressive clogging of vascular elements, leading to wilting of branches and eventually to plant death. When grown in liquid substrate, the fungus produces a complex of phytotoxic glycoproteins, among which a 60 kDa toxin coded Pt60 was shown to reproduce disease symptoms when injected into plant leaves. Moreover, pectolytic enzymes are produced by *P. tracheiphila* and their role as pathogenetic or virulence factors is being currently evaluated. Aiming at elucidating whether the variability in virulence observed among *P. tracheiphila* isolates may be related to differences at the genome level, a collection of 35 isolates representing Italian populations of this pathogen was characterised by different molecular tools. These included analysis of the distribution of RAPD and microsatellite markers and sequencing of the internal transcribed spacer region of the nuclear rDNA genes. Preliminary results obtained with 10 RAPD primers and 8 microsatellite primers indicate that *P. tracheiphila* presents a high level of genetic homogeneity, leading to overlapping patterns upon amplification with most of the tested primers. Accordingly, ITS1 and ITS2 sequences were very similar among the tested isolates, except for two diverging isolates from lemon cv Femminello.

Research supported by a grant of the Italian Ministry of University (“Studio biomolecolare della variabilità delle popolazioni di *Phoma tracheiphila*”) and by the University of Sassari.

**DETECTION OF TRANSCRIPTS OF THE AFLATOXIN GENES NOR1, OMTPB, AND OMTPA BY RT-PCR ALLOWS DIFFERENTIATION OF AFLATOXIN-PRODUCING AND NON-PRODUCING ISOLATES OF ASPERGILLUS FLAVUS AND ASPERGILLUS PARASITICUS.** B. Scherm, M. Palomba, F. Brundu, D. Serra, A. Marcelli and Q. Miglieli. Department of Plant Protection - Center for Biotechnology Development and Biodiversity Research, University of Sassari, Via De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; E-mail: miglieli@uniss.it

An RT-PCR (reverse transcription-polymerase chain reaction) technique was applied to differentiate aflatoxin-producing from aflatoxin-non-producing strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Total RNAs of 13 strains grown under inducing yeast-extract-sucrose (YES) and non-inducing yeast-extract-peptone (YPE) media, respectively, were analyzed by using specific primers based on the conserved regions of 9 structural genes (*nor1*, *avuA*, *adhA*, *asfA*, *sfb*, *nor1*, *omtpB*, *omtpA*, and *orda1*) and two regulatory genes *affl* and *affR* of the aflatoxin B1 biosynthetic pathway. Transcription was confirmed by the expression of the β-tubulin gene. The expression of the majority aflatoxin biosynthetic genes including *affR* and *affl* of all strains varied with regard to the aflatoxin-producing ability and growth conditions. Nonetheless, we found that the expression profile of the three genes *nor1*, *omtpB*, and *omtpA* was consistently correlated with the ability to produce aflatoxins or not in YES as well as the inability to produce aflatoxins in YEP. The devised RT-PCR profiling method reflects aflatoxin concentrations ranging from 0.1 to 60 mg per ml of the culture filtrates as determined by fluorescence HPLC. These results open the perspective to adopt RT-PCR as well as cDNA-based micro- and macroarray techniques to rapidly identify toxigenic isolates of *Aspergillus* species.

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**PRELIMINARY SURVEY ON THE DISTRIBUTION OF CITRUS TRISTEZA VIRUS IN SARDEGNA CITRUS ORCHARDS.** M.M. Muresu1, W. Ramassini2, S. Buccioli2, D. Sedi2, D. Serra2 and F. Marras1. Dipartimento di Protezione delle Piante – Sezione di Patologia Vegetale, Università degli Studi di Sassari, 07100 Sassari, Italy. E-mail: fmarras@uniss.it. 2Servizio Fitosanitario e Difesa dai Parassiti dell’Uomo e degli Animali, Assessorato alla Difesa dell’Ambiente, Regione Autonoma della Sardegna, 09100 Cagliari, Italy.

The most important commercial orchards and nurseries in Sardinia were monitored in the last four years for the presence of *Citrus tristeza virus* (CTV). This was carried out in compliance with the regulations of D.M. 22/11/1996, which established mandatory control measures against this virus. Nursery plants were tested individually for the presence of CTV by DAS-ELISA while for field plants a preliminary analysis was done on a composite sample. If the sample was positive, individual plants were analysed by RT-PCR. The survey focused for the first three years on 27 citrus groves, examining, 3,212 samples from 2000 to 2002. Twenty five trees were positive, thus were uprooted and burnt, in compliance with by-lows. In 2003, 22 samples were positive out of 2,830 analysed, from 41 groves in 15 localities in Sardinia. Further analyses by RT-PCR are currently being performed. Monitoring will be carried out until every main citrus stand has been examined. The data above show the incumbent threat of CTV for the Sardinian citrus industry since most of the groves are grafted on sour orange.

**HIGH TECHNOLOGY INSTRUMENTS FOR DETECTION OF DECAY IN TREES: ELECTRIC AND ULTRASONIC TO-MOGRAPHIES.** G. Nicolotti1, R. Martinis1, P. Gonthier1 and L. Sambuelli2. 1Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via Leonardodda Vinci 44, 10095 Grugliasco (TO), Italy; 2DIGET - Politecnico di Torino, C.so Duca degli Abruzzi 24, 10129 Torino, Italy. Fax: +39.011.2368544; E-mail: giovanni.nicolotti@unito.it

During wood degradation processes fungi release a noticeable quantity of ions: brown-rot increases acidity by releasing H-ions and white-rot accumulates cations (especially Ca++ and K+); both decrease wood resistivity and increase the dielectric constant. The fungal action also influences wood moisture, density and modulus of elasticity. Most of the instruments, designed for wood investigation, measure its physical properties, besides they only give local information of the material surrounding the sensors. On the contrary, the tomography can supply information on different physical and chemical properties, by different types of energy, allowing to reconstruct a cross section through the trunk, with a distribution of the investigated wood properties. This paper reports the applications of electric and ultrasonic tomographies on standing trees for detection of decay; possibly broader application is also evaluated, critically considering some “open problems”. The experiments were carried out on different broadleaf trees before cut...
ELEPHANTIASIS OF KIWIFRUIT: BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SOME DISEASE-ASSOCIATED FUNGI. A. Prodi, I. Gonzalez, S. Sandalo, F. Telfizz and P. Nipoti. Dipartimento di Scienze e Tecnologie Agroambien- tali, Università di Bologna, via Fanini 42, 40127 Bologna, Italy. E- mail: pnipoti@agrsci.unibo.it

Elephantiasis is a new wood disease of kiwifruit that occurs in most Italian growing areas. In affected plants the main symptom consists of an abnormal hypertrophy of the entire trunk, so “elephantiasis” was proposed for naming the disease. Studies have been carried out to elucidate the etiology of this disorder. The most frequent fungi, isolated from cross sections of the trunk of affected plants in Emilia-Romagna and Lazio orchards, were Fusarium spp., Cylindrocarpon spp., Phaeoacremonium spp., Acre- monium spp. and Phialophora spp. Some isolates of Phaeoacre- monium and similar fungi were studied using biological assays. Phenotype, growth temperature and in vitro pathogenicity were evalu- ated for each strain. Fungal isolates showed a great variability, a different level of pathogenicity, and only a few were able to grow

OBSERVATIONS ON PETRI AND ESCA DISEASES AND DEVELOPMENT OF MOLECULAR DIAGNOSTIC METHODS FOR PHAEOMONIELLA CHLAMYDOSPORA. A. Abbatecola1, C. Dongiovanni2, S. Pollastro1, P. Natale1, H. Hahie1 and F. Faretra1, 1Dipartimento 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@agrunic- ha.it. 2Centro di Ricerca e Sperimentazione in Agricoltura “Basil e Carasa”, Via Cisternino 281, 70010 Locorotondo (BA), Italy. Fax: +39.080.4313071; E-mail: crsa@libero.it.

In the last years, Petri and esca diseases showed an increased recrudescence also in young vineyards. Effective methods for the control of the diseases are so far lacking. It was suggested that fungi involved in the diseases can be transmitted through grapevine propagation materials. A better understanding of this aspect is essential to implement actions suitable for preventing disease spreading in vineyards. Field surveys were carried out in 11 (3 to 11-year-old) vineyards from 1997 to 2002. Over 15,200 vines were observed and mapped. A progressive increase of the number of vines showing symptoms was recorded. Frequently, symptomatic vines showed an aggregate distribution in the vine- yards, and symptoms appeared on neighbouring vines in next years. The most common wood discolorations were black or brown wood-streaking (90%), while white rot was much less frequent (10%). Pomitoria mediterranea was isolated only from white rot. The following fungi were isolated from brown wood-streaking: Botryosphaeria spp. (9%) of analysed vines, Phaeoconiella chlamydospora (33%), Phialophora spp. (17%), Cylindrocarpon spp. (11%), Eutypa lata (8%), Phomopsis viticola (6%), and Phaeocercosporiella spp. (1%). Artificial inoculation of cuttings, rootstocks and grafted rootlings with P. chlamydospora showed that the fungus is able to colonize young plants in the nursery. SCAR (Sequence Characterized Amplified Regions) primers specific for P. chlamydospora were designed starting from species-specific RAPD markers. A diagnostic protocol based on nested-PCR (primer pairs OPA13s4d and OPA13s4dF) allowed fast and sensitive detection of P. chlamydospora in soil samples and in grapevine propagation material.

TOBACCO MILD GREEN MOSAIC VIRUS IN NICOTIANA GLAUC A IN SOUTH ITALY. G. Pararella1, A. De Stradis2 and C. Vovlas3. 1Istituto per la Protezione delle Piante del CNR, Sezione di Portici, Via Università 133, 80055 Portici (NA), Italy. 2Istituto di Virologia Vegetale del CNR, Sezione di Bari, and Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126, Bari, Italy. Fax: +39.81.7758122; E-mail: giuseppe.pararella@ipp.cnr.it.

Tobacco mild green mosaic virus (TMGMV; genus Tobamovirus) occurs probably in all tropical and subtropical regions where Nicotiana glauca (tobacco tree) is distributed (North America, Australia, many European and African countries, Ca- nary and Mediterranean islands). In Italy, TMGMV has been re- ported in pepper in 1970. During spring 2003 several plants of N. glauca growing in south Italy (Naples), were found to be infected by a tobamovirus-like virus based on preliminary electron-microscope observation of leaf samples. In immuno-diffusion agar tests sap extracts from symptomatic leaves of N. glauca formed a clear precipi- tating line with a serum to an American isolate of TMGMV (kindly provided by H. Elliot), while no reaction was observed with a serum to a common isolate of Tobacco mosaic virus (TMV). Molecular hybridization confirmed the absence of TMV in symp- tomatic N. glauca plants. The CP and the 3’ UTR sequence of a TMGMV isolate (TMGMV-GNA) were determined from a single symptomatic N. glauca plant. The CP sequence confirmed that TMGMV-GNA is an isolate of TMGMV while 3’ UTR sequence revealed the presence of an additional repeat sequence of 147 nt

BIological AND MOLECULAR CHARACTERISTICS OF A CHIMERIC CUCUMBER MOSAIC VIRUS. M. Nuzzaci, A. Natilla, A. Vitti and P. Piazzolla. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilica- ta, C/dà Macchia Romana, 85100 Potenza, Italy. Fax: +0039-0971- 205503; E-mail: piazzolla@unibas.it.

Cucumber mosaic virus (CMV) was used as a carrier of an epi- tope of the hepatitis C virus (HCV), the major etiological agent of a worldwide parenterally transmitted hepatitis. Presently, there is no effective vaccine against this human virus. A continu- ous epitope of 27 aminoacids, the so called R9, present in the hyper-variable region 1 (HVRI) of the putative envelope protein E2 of HCV, was inserted in CMV-S RNA3, carrying the CMV coat protein (CP) gene. The CMV-S chimeric RNA 3 was then supplement- ed with RNA 1 and RNA 2 of CMV-D and inoculated in Nicotiana tabacum cv. Xanthi. The resulting chimeric virus was stable in several serial passages in this host. Symptoms elicited by chimeric CMV and original CMV strains were different. This behaviour was confirmed in other hosts. Serum samples from patients with chronic hepatitis C displayed a significant im- munoreactivity to crude plant extracts, thus demonstrating the exposure of the foreign epitope. These results suggest the capa- city of a possible vaccine function for the CMV-HCV mimo- tope system.
in the part of 3' UTR that is involved in the formation of six pseudoknots in the Large-type TMGV isolates (three pseudoknots=Small-type TMGV isolates). The host range of TMGV-GNA was in agreement with that reported for TMGV Large-type isolates. Dot-blot hybridization tests with a specific riboprobe for Large-type isolates showed that all symptomatic N. glauca plants harbor exclusively Large-type isolates of TMGV.

MOLECULAR AND BIOLOGICAL FEATURES OF AN ISO­LATE OF MALVA VEIN CLEARING VIRUS. G. Parrella1, B. Delecolle2 and C. Vovlas3, 1Istituto per la Protezione delle Piante del CNR, Sezione di Portici, Via Università 133, 80055 Portici (NA), Italy. 2INRA - Station de Pathologie Vegetale, BP94, 84143 Montfavet Cedex, France. 3Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.81.7758122; E-mail: giuseppe.parrella@ip.cn.it

A potyvirus causing vein clearing in Malva sylvestris was identified as an isolate of Malva veinal clearing virus (MVCV) by biological, cytological and molecular studies. In mechanical transmission tests this isolate (MVCV-DS) was able to infect plants of Malva parviflora, M. neglecta, M. sylvestris, M. nicaenis and Lavatera trimestris, inducing in all instances vein clearing followed by yellow mosaic. MVCV-DS did not infect Chenopodium amaranticolour, Cacumis sativius, Datura stramonium and Nicotiana tabacum cv. Xanthi nc. Electron microscope observations of ultrathin section from infected tissues revealed the presence of cylindrical inclusions consisting of pinwheels and scrolls (potyvirus subdivision-I). These results are in agreement with those reported earlier from Italy and Hungary for MVCV. Sequence comparison of the coat protein (CP) gene showed the highest identity at the aminoacid level (56% and 52%) with comparison of the coat protein (CP) gene showed the highest identity at the aminoacid level (56% and 52%) with

MOLECULAR DETECTION OF STOLBUR PHYTOPLASMAS IN TRIALEURODES VAPORARIORUM (WESTW.) COLLECTED IN STRAWBERRY FIELDS WITH PHYLLODY DISEASE. M. Pastore, S. Paltrinieri, V. Graziano, R. Priore, P. Nuges and A. Bertaccini, Istituto Sperimentale per la Frutticoltura, Section of Caserta. MiPAF, Via Torrino 3, 81100 Caserta, Italy. Fax: +39.0823.256239; E-mail: Pastore2000@inwind.it

Yellow sticky traps were exposed and replaced each ten days, from October 2002 to June 2003, in two strawberry fields in Caserta province near plants with phyllody and virescence of cvs Camarosa and Chandler. The leafhopper Empoasca spp. (Ho­moptera-Typhlocybinae) and the greenhouse whitefly Trialeu­rodes vaporariorum (Westw.) (Homoptera-Aleyrodinae) were found on traps. It was also possible to collect Trialeurodes vap­orariorum alive. Molecular analyses, carried out only on the insect samples collected in November, when the phyllody symptoms were very evident, showed that seven T. vaporariorum batches, out of the nine collected on cv Camarosa, and five T. vaporaria­rum batches, out of the seven collected on cv Chandler, contained 16SrXII-A phytoplasma subgroup. The ten batches of Empoasca spp., collected on cv Camarosa and the other T. vaporaria­rum samples did not contain phytoplasmas. Eight symptomatic strawberry plants, collected in November, near traps on which phytoplasma positive insects had been found, were analysed: four were phytoplasma-negative, three were infected by 16SrI-C and one by 16SrXII-A. The latter phytoplasma was detected recently in France in strawberry plants showing marginal chlorosis symptoms that were undistinguishable from those associated to Candidatus Phytoplasma fragariae. This is the first case of association of this phytoplasma to phyllody symptoms in strawberry even if different phytoplasms have been reported to be associated with similar symptoms worldwide. Further molecular analyses of plants and insects collected till June will provide useful information to define the role of phytoplasmas in the phyllody epidemics, typical of autumnal months in southern Italy.

BIOCHEMICAL CHARACTERIZATION OF THE SUN­FLOWER STEM CANKER FUNGUS DIAPORTHE HELIANTHI. L. Guidi, S. Pecchia, E. Degl’Innocenti, L. Patti, G.F. Soldatini and G. Vannacci. Dipartimento di Coltivazione e Difesa delle Specie Legnose “G. Scarruzzo”, Università degli Studi di Pisa, Via del Borghetto 80, 56124 Pisa, Italy. Fax:+39.050.543564; E-mail: geanna@agri.unipi.it

Diaporthe helianthi is known to cause stem canker and leaf necrosis of sunflower resulting in significant yield losses in many European and non European countries. Biochemical characterization of the pathogen was assessed using a collection of D. helianthi isolates of different geographic origins (Argentina, France, Italy, Romania, Yugoslavia). Four key enzymes of primary metabolism were analyzed: malate dehydrogenase (NAD-MDH; EC 1.1.1.37), malic enzyme (NADP-ME; EC 1.1.1.40), glucose 6-phosphate dehydrogenase (NADP-G6PDH; EC 1.1.1.49), and isocitrate lyase (ICL; EC 4.1.3.1). Isoenzyme analysis on polyacry­lamide gels with a discontinuous buffer system, and determination of in vitro enzyme activities expressed as mmoles min⁻¹ mg⁻¹ of proteins were carried out. Data were subjected to multivariate statistical analysis using the program PAST version 1.06. The activity of MDH, ME and G6PDH on the whole showed the same behaviour, whereas ICL activity was completely divergent. These biochemical traits showed a large variability among the isolates tested. Isoenzymes were polymorphic among strains and these markers revealed distinct groups that correlated with the geographic origin of D. helianthi isolates. Isolates originating from countries (France, ex Yugoslavia) where severe outbreaks of the disease are reported yearly formed a homogeneous group, char­acterized by relatively low variability. This group was distinct from the group formed by isolates originating from Italy, Rom­ania and Argentina, whose variability is relatively much higher.

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PARTIAL CHARACTERIZATION OF AN ABC-TRANS­PORTER ENCODING GENE IN AUREOBASEDIUM PULLU­LANS, STRAIN L47, AND DEVELOPMENT OF A QUANTI­FICATION METHOD OF THE RELATIVE TRANSCRIPT LEVEL. I. Pentimone, L. Schena, A. Ippolito and F. Nigro. Dipar­mento di Protezione delle Piante e Microbiologia Applicata, Universita degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: nigro@agr.uniba.it

Aureobasidium pullulans, strain L47, is an effective biocontrol agent of postharvest diseases of several fruits and vegetables. Moreover, preharvest application in combination with low doses of fungicides further enhances the protection level during stor­age. Usually, ABC-transporter encoding genes (ABC-teg) play an important role in the resistance of microorganisms towards toxic compounds. Studies were conducted to characterize an ABC-teg from A. pullulans, strain L47, and to develop a molecular method
for monitoring its expression in response to fungicides. Specific primers were designed, on the basis of a known ABC-teg region of *A. pullulans* (accession number: APU62928), enabling the amplification of a 701 bp DNA fragments from strain L47. This fragment was cloned, sequenced, and analysed by means of basic local alignment search tool to evaluate the homology with similar sequences reported in the GenBank. Two specific primers, amplifying a 173 bp amplicon suitable for the development of real time PCR detection, were designed to quantify the relative transcript level of the ABC-teg by quantitative RT-PCR. Since relative quantification requires a nonregulated housekeeping gene to normalise results, a portion of L47 β-tubulin gene was amplified with universal primer, cloned, sequenced, and utilised to design two primers amplifying a 175 bp amplicon. Preliminary results indicated a good correlation between fluorescence intensity and the relative transcript level in *A. pullulans* cells growing in fungi- cides-amended media. The study of ABC-teg expression may provide useful information on the use of biocontrol agents in combination with low doses of fungicides.

**VARIATION IN PHOMOPSIS VITICOLA AND DEVELOPMENT OF MOLECULAR DIAGNOSTIC TOOLS.** S. Pollastro, A. Abbatecola, P. Natale, 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@agri.uniba.it

Phomopsis viticola (Sacc.) Sac. is the causal agent of Phomopsis cane and leaf spot of grapevine. The disease causes a severe decline or even the death of arms or of whole vines. In last years, new table-grape cultivars very susceptible to the disease, such as ‘Victoria’, ‘Red Globe’ and many seedless cultivars, were introduced in Italy causing a re-eruption of the disease. The microbial composition of the rhizosphere and the aerial organs further underlines the complexity of the decline phenomenon. Once this has started, it progresses in a conspicuous manner, altering the equilibrium between the microbial communities and the host tree.

**MICROBIAL POPULATIONS ON QUERCUS ROBUR: ANALYSIS OF THE RHIZOSPHERE AND AERIAL ORGANS.** A. Ragazzi1, L. Giovannetti2, C. Viri2, A. Messini3, S. Moricca1, L. Manielli1, E. Tuccio1 and I. Dellevalle1. 1Dipartimento di Biotechnologia Agraria, Sezione di Patologia vegetale, Università degli Studi di Firenze, P.le delle Cascine, 50144 Firenze, Italy. 2Dip. di Biotecnologia Agrarie, Sezione di Microbiologia. 3Istituto per la Protezione delle Piante, CNR, Firenze, Italy. E-mail: alessandro.ragazzi@unifi.it

The microbial composition of the rizosphere and the endophytic population from aerial organs of healthy and declining 20-year-old *Quercus robur* trees in a stand at San Rossore (Pisa) were studied during 2002. The rhizosphere composition was studied with a traditional method, by counting the number of cultivable heterotrophic and calcium oxalate-degrading bacteria, and with the Biolog system, by determining the metabolic profile of bacterial communities. The isolation frequency of endophytic fungi was determined using the formula IF= Ni/Nt x 100. All data were statistically processed. The Biolog test revealed no significant differences (P>0.05) in bacterial metabolic profile between healthy and declining trees. The count of cultivable heterotrophic bacteria was greater in the rhizosphere of healthy trees; similar results were obtained for the oxalate-degrading bacteria. As to the endophytic population, a total of 19 species in 18 genera were found in healthy trees, and 17 species in 16 genera in declining trees. In declining trees, 7.83% of the total isolates were represented by fungi that become pathogenic when the tree is under stress, such as *Phomopsis quercina*, *Colpoma quercinum* and *Pestalotiopsis* sp., while in healthy trees they were only 3.80%. By contrast, fungi such as *Trichoderma viride* and *Cladosporium cladosporioides* were more common in healthy (25.3%) than in declining trees (15%). The variations between the oxalate-degrading microbial and the endophytic populations of healthy and declining trees further underline the complexity of the disease and it plays an important role in the disease management.

**CHARACTERISTICS OF ENDOPHYTIC AGROBACTERIA FROM FICUS BENJAMINA.** A. Raio, R. Peluso, G. Popolo and A. Zoina. Dipartimento di Agrarave, Università degli Studi di Napoli “Federico II”, Università “Federico II”, Via Università 100, 80055 Portici (NA), Italy. Fax: +39.081.7755320; E-mail: zoina@unina.it

Fifty *Agrobacterium* isolates were obtained from epi- and hypogeous tumors of *Ficus benjamina* plants grown in Italy and The Netherlands. Biochemical and physiological tests for biovar identification and the sensitivity assays to 14 different antibiotics showed that the strains were sharply distinguishable into two groups. Carbohydrate assimilation profiles (BIOLOG) and the analysis of 16S ribosomal region by PCR-RFLP revealed that one group of agrobacteria belonged to biovar 1 (ex *Agrobacterium tumefaciens*) and the other to the new species *Agrobacterium larrynoorei*. Both species were responsible for the induction of tumors on *F. benjamina* plants. Genetic variability of the two species was examined by analysing the ribosomal region 16S-IGS, the plasmid profiles and the Ti plasmid. Most of the strains harboured a “nopaline type” Ti plasmid, while some of the Dutch strains harboured an “atypical” Ti plasmid. No correlation was found between chromosomal and plasmid characteristics of the strains analysed. Biovar 1 agrobacteria and those identified as *A. larrynoorei* were found constantly associated in weeping fig tumors. Virulent and non virulent agrobacteria belonging to both species were able to move in the vascular system of host plants. Endophytic behaviour of tumorigenic agrobacteria is a key point in the epidemiology of the disease and it plays an important role in the disease management.

**A MULTIPLEX RT-PCR ASSAY TO DISTINGUISH AND CHARACTERIZE SUGARBEET SOIL BORNE VIRUSES.** C. Ratti, G.R.G. Clover, C. Rubies-Autonell, R. Resca, A. Pisi, VA. Harju and C.M. Henry. DSTA, Università degli Studi di Bologna, Via Filippo Re 8, 40126-Bologna, Italy. E-mail: crubie@agrsci.unibo.it

Six different primer sets were developed to identify *Polymyxa betae*, *Beet soil borne virus (BSBV)* and *Beet virus Q (BVQ)* and distinguish between A, B and P types of *Beet necrotic yellow vein virus (BNYVV)* using a multiplex reverse-transcription poly-
merase chain reaction (RT-PCR). RNA was extracted from 80 infected samples from Asia, Europe and North America. Virus infection was determined using single step RT-PCR and the BNYVV type using an established single strand conformation polymorphism (SSCP) detection method. Partial sequences of RNA 2 from 16 isolates of the BNYVV A and B type were determined and aligned and two sets of PCR primers were designed which yielded a single 323 base-pair RT-PCR fragment from samples containing the A type and a 157 base-pair product from samples containing the B type. Published sequences from P. betae, BSBV RNA 2, BVQ RNA 3 and BNYVV RNA 5 were also aligned and specific RT-PCR assay for P. betae (261 base-pair), BSBV (456 base-pair), BVQ (521 base-pair) and BNYVV RNA 5 (593 base-pair) were designed. Fragment length differed sufficiently to allow all tests be run in a single PCR tube. Results obtained using the new multiplex RT-PCR assay were consistent with those from the established SSCP method and from single RT-PCR method for all 80 reference samples. Future work will identify primers to detect Beet soil borne mosaic virus (BSBMV) isolates as part of the same multiplex system.

ENVIRONMENTAL FACTORS INFLUENCING THE LIFE CYCLE OF TAPHRINA DEFORMANS. V. Rossi, M. Bolognesi, S. Giosué, G.L. Spada and F. Mazzini. Istituto di Entomologia e Patologia vegetale. Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: ist.patologia-pc@unicatt.it

Taphrina deformans can cause severe damages when prolonged conditions of humid and cool climate are present during the plant susceptible stage that begins with bud break. The disease is primarily controlled by fungicides applied during winter and once or more times after bud break; disease control is not always efficient because some key elements of T. deformans epidemiology are not sufficiently known. In order to optimise fungicide applications, some aspects of the disease cycle were investigated by laboratory and field experiments: (i) environmental conditions favourable to infection; (ii) length of incubation and disease appearance; (iii) latency and spore dispersal; (iv) spore production and dispersal. Results from these experiments pointed out that a successful infection depends on air temperature and leaf wetness; temperature influences both fungal growth and rate of leaf development and, consequently, leaf susceptibility. Rainfall is not necessary for infection but it can cause wetness and determine spore dispersal to the shoot apaxes. Some infections can occur during the period of host susceptibility, but one or two of these are significant. Incubation length depends mainly on the temperature; symptoms appear within 2-3 weeks. For each infection, latency ranges between 45 and 60 days and the infectious period is of about 10-14 days; temperature and raindrops are the main factors affecting spore dispersal. All this information was used to elaborate the infection cycle of T. deformans and to improve a model simulating infection establishment and disease appearance previously elaborated. This model can be used in optimising fungicide sprays.

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC TO GRAPEVINE LEAFROLL ASSOCIATED VIRUS 3 AND EPITOPE MAPPING OF THE COAT PROTEIN GENE. Z. Zhou, C. Turturo, O. Potere, P. Saldarelli, D. Boscia and G.P. Martelli. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi e Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: cssvps04@area.ba.cnr.it

Grapevine leafroll-associated virus 3 (GLRaV-3), the type member of the genus Ampelovirus, is the most widespread and economically important virus species among grapevine leafroll-associated viruses. For raising monoclonal antibodies (Mabs) specific to GLRaV-3: Cortical scrapings from an Italian grapevine with mixed infection of GLRaV-3 and Grapevine leafroll-associated virus 6 (GLRaV-6) were used as antigen source for immunization of BALB/C female mice. Four hybridoma lines secreting GLRaV-3 specific antibodies were selected by DAS-ELISA and used for in vivo mass production. All four Mabs were able to decorate viral particles in immunosorbent electron microscopy (IEM), indicating that they were elicited from surface epitopes. These Mabs detected GLRaV-3 in 280 infected grapevines in DAS-ELISA. For localizing surface epitopes, a full-length coat protein (CP) gene of an Italian GLRaV-3 isolate was cloned in the expression vector pMALC2x as fusion with a maltose binding protein. Different deletions of the full-length CP-containing plasmid were produced and assayed in immune Western blot with all four 4 Mabs and GLRaV-3 rabbit polyclonal IgGs. Both IgGs or the four Mabs identified the same epitope on GLRaV-3 CP, which was readily recognized in denatured conditions, thus appearing to be linear. This major epitope was likely located between nucleotides 183 and 484 of GLRaV-3 CP. Analysis of CP amino acid sequence by the Peptide Structure software (GCG package) showed this region to contain the highest density of residues with the highest surface probability and antigenic indexes along the entire CP.

VEGETATIVE COMPATIBILITY GROUPS IN CRYPTO- NECTRIA PARASITICA POPULATIONS IN SICILY. D. Spica, A. Chimento, G. Sammarco and S.O. Cacciola. Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Sezione di Patologia vegetale e Microbiologia agraria, Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy. Fax:+39.091.423238; E-mail: gisammar@unipa.it

Blight caused by Cryphonectria parasitica (Murr.) Barr. is a serious chestnut disease in Sicily. The number and relative prevalence of vegetative compatibility groups (VCGs) were determined by the Powell’s method within a population of 240 C. parasitica isolates from 15 geographical locations in Sicily. Three VCGs, EU-2, EU-10 and EU-12, were detected. VCG EU-12 occurred in 13 locations and comprised 85% of all the isolates, while VCG EU-2 occurred in seven locations and included 14% of all isolates. VCG EU-10 occurred in one location in Palermo province and it was represented only by two isolates. All VCGs included isolates with normal and albino colony morphology. Moreover, VCGs EU-12 and EU-2 comprised isolates with an intermediate colony morphology (1 isolates). Pathogenicity of isolates was tested on chestnut trees. Isolates with normal colony morphology (N isolates) induced evolutive virulent cankers, irrespective of their VCG. Conversely, all isolates with albino colonies induced healing cankers, proving to be hypovirulent (H isolates). About 70% of the I isolates induced healing cankers whereas the remaining were virulent. H isolates occurred in 11 locations. In locations where isolates with all these three phenotypes occurred, the ratio between H, I and N isolates ranged from 0.3 to 1.8, but in many locations it was near or greater than 1, indicating a natural spread of virulence. The analysis of the diversity of VCGs in natural populations of C. parasitica in Sicily would suggest that biological control of chestnut blight by hypovirulent strains may be effective.

IDENTIFICATION, DETECTION, AND QUANTIFICATION OF ERWINIA AMYLLOVORA BY REAL-TIME SCORPION-PCR. P. De Bellis, L. Schena and C. Cariddi. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli
A specific pair of primer (E3-E4) amplifying a single DNA fragment of 110 bp from plasmid pE29 was designed to identify, detect, and quantify Eruinia amylovora by real-time PCR. Primer E3 was modified to obtain a Scorpion probe for detecting the specific 110 bp amplicon by florescence emitted from a fluorophore through a self-probing PCR assay. Specificity of primers and probe was assessed by means of BLAST analysis, to exclude the presence of similar sequences among available DNA databases (GenBank) and by using genomic DNA from a large number of E. amylovora isolates and other bacteria from several hosts and different geographic areas. In Scorpion-PCR, with a 10-fold dilution series of E. amylovora DNA, the limit of detection was 1 pg ml\(^{-1}\). A high and significant correlation (\(r^2 = 0.995\)) was obtained between target DNA quantity and cycle threshold (Ct). Combining two sequential amplifications with conventional reported primers (PEANT1-PEANT2) and Scorpion primers (E3 Scorpion-E4) the detection limit was 1 fg ml\(^{-1}\) (nested Scorpion-PCR). Using serial dilution of bacterial suspensions the limit of detection was 10\(^4\) CFU ml\(^{-1}\) in Scorpion PCR and 10\(^5\) CFU ml\(^{-1}\) in nested Scorpion PCR. Real-time PCR combined with simple, rapid, and effective procedures for DNA extraction enabled the detection and the quantification of the epiphytic population of E. amylovora in the washings of flowers and leaves of artificially inoculated pear. A significant correlation (\(r^2 = 0.91\)) was found between pathogen CFU on semi-selective media and the corresponding target DNA concentration evaluated by real time PCR.

CHARACTERISATION OF SARDINIAN ISOLATES OF CITRUS TRISTEZA VIRUS BY SINGLE-STRAND CONFORMATION POLYMORPHISM ANALYSIS OF THE COAT PROTEIN GENE. A. Schiaffino, R.I. Pinna 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: fmarras@uniss.it

Citrus tristeza virus (CTV) was detected in Sardinia since 2001. Lately the infection was found in three different sites on Citrus spp., mainly on symptomless plants of orange (C. sinensis) and mandarin (C. reticulata) grafted on sour orange (C. aurantium) rootstocks. CTV detection was carried out by DAS-ELISA and confirmed by means of RT-PCR using primers for the coat protein gene of the Florida B3 isolate of the virus. Positive samples showed an amplification product of 670 bp, which was confirmed to be the CTV coat protein gene by digestions with restriction endonucleases BstEII and CclI. Isolates were compared for variation in their coat protein gene sequence by single-strand conformation polymorphism analysis (SSCP). Six distinct SSCP profiles were found for the isolates examined. Samples from the province of Oristano showed a common SSCP profile which was different from the five mobility patterns found among isolates in the province of Cagliari. In particular one pattern was common to most samples from two different sites in this area: S. Vitro and Muravera. The remaining four mobility patterns were characteristic of four isolates, two from each site. These results suggest introduction in Sardinia of CTV from different origins.

BISC OgNIAUXIA NUMMULARIA PRIMARY PATHOGEN ON BEECH. A. Sidoti\(^1\) and G. Granata\(^2\). \(^1\)Regione Siciliana, Assessorato Agricoltura e Foreste, Dipartimento Interventi Strutturali, Servizio IV, UO 21-OMP di Acireale, Corso Umberto 114, 95024 Acireale, Italy. E-mail: asidoti@omp-acireale.org. \(^2\)Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: granatag@unict.it

Some beech (Fagus sylvatica L.) stands in the Nebrodi Mountains and Mt. Etna are known to be affected by a decline associated with the fungus Biscogniauxia nummularia (Bull. Fr.) O. Kuntze since 1990. The disease determines yellowing, leaf drop, cankers and tree death. A similar decline condition has also been observed in the Ferdinandea beech wood (Calabria, Italy). Artificial field inoculations were performed to assess the effective pathogenic capacity of Sicilian and Calabrian isolates, and whether the B. nummularia populations are heterogeneous and if they possess a different degree of virulence. Field trials showed that B. nummularia has a primary pathogenic role under the environmental conditions studied. Isolates from the declining beech woods in Sicily and Calabria were heterogeneous in characteristics and pathogenic behaviour. The repeated periods of drought and high temperatures, the soil conditions that do not favour good water retention and the effects of coppice management may have determined stress and reduced the resistance of trees to the fungus. Reduced ammonification and nitrogen fixation processes in the soil under declining trees confirmed the existence of environmental degradation. It was also observed that the fungus can adopt endophyte-like behaviour and show its pathogenic capacity on stressed trees. Felling suckers and infected stumps are necessary for controlling the spread of the disease. On the contrary, the disappearance of vegetation may determine soil degradation, erosion and desertification.

A MOLECULAR ASSAY TO INVESTIGATE THE POSSIBLE ASSOCIATION BETWEEN THE CHESTNUT WEEVIL CURCULIO PROPINQUUS AND THE BLACK ROT FUNGUS RA- CHO DIELLA CASTANEAE. A.M. Vettraino, S. Speranza, B. Papparati, C. Pucci and A. Vannini. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: vettrain@unitus.it

Black rot, induced by Rachodiella castaneae Pyr. (teleomorph: Sclerotinia pseudotuberosa Belm.; syn. Ciboria batschiana (Zopf) Buchw.), and the chestnut weevil [Curculio propinquus (Desbr.)] are among the most serious phytosanitary problems of the chestnut (Castanea sativa Mill.) fruit industry in Italy, causing relevant economic losses at harvest and during storage. Symptoms of black rot are frequent in nuts infested by C. propinquus, and hence an association between the fungus and the insect has been hypothesised. To verify this hypothesis, clusters of immature burrs from three trees in a chestnut area in Central Italy were covered with a net and treated as follow: (i) infested with C. propinquus, and hence an association between the fungus and the insect has been hypothesised. To verify this hypothesis, clusters of immature burrs from three trees in a chestnut area in Central Italy were covered with a net and treated as follow: (i) infested with C. propinquus adults that had been artificially contaminated with R. castaneae; (ii) infested with C. propinquus uninfested adults; (iii) uninfested negative control. At harvest time, the nuts were removed and the adults of the insects and chestnut fruits were collected. Due to the inefficacy of traditional diagnostic methods, the detection R. castaneae in C. propinquus and in chestnut fruits was performed by PCR. Two sequences specific for R. castaneae were identified in the ITS region of rDNA, and two species-specific primers (RAC1 and RAC2) were designed. R. castaneae was detected in more than 90% of the chestnut weevils analysed, regardless the treatment, and in 77, 73, and 92% of nuts following the treatments (i), (ii), and (iii), respectively. These findings suggest an endophytic habitus of R. castaneae in chestnut fruits and that C. propinquus is a potential vector of the pathogen.

A TWO-YEAR MONITORING OF AN EPIDEMIC OF TOMATO SPOTTED WILT VIRUS IN SPECIALIZED LETTUCE PRODUCTION AREA. M. Tessitori, A. Reina, S. Rizza, P. Roggero and R. La Rosa. Dipartimento di Scienze e Tecnologie Fr-
Tomato spotted wilt virus (TSWV), genus Tobrivirus, family Bunyaviridae, has one of the widest host range (over 1000 species) in comparison with any other plant virus. In spring 2002 a high incidence of severe virus-like symptoms was observed in different cultivars of lettuce (Lactua sativa L.) in the Adriano area (Catania, Italy). This area of about 1300 ha is cultivated with at least three production cycles per year. Infections had an incidence of 50-70% and were characterised by small necrotic areas in young leaves resulting in distortions and necrosis of foliar tissues. High infestation of Frankniella occidentalis was recorded in the same period. Ten percent of symptomatic plants in each field and a small number of symptomless plants were assayed by DAS-ELISA to check the presence of TSWV. Only samples showing symptoms were positive in ELISA. RT-PCR with L1 and L2 TSWV specific primers was applied on DNA from several bacterial isolates. The expected amplification product of 519 bp was obtained from all the 25 isolates of Xcc. tested. No amplicons were obtained from about 30 isolates of other pathogenic bacteria and fungi were done. Only thirteen isolations gave fungi as Cladosporium spp., Alternaria spp. and, rarely, Epicoccum spp., Penicillium spp. and Stemphylium spp. Conversely, all the isolations gave plenty of bacterial colonies. Over 100 bacterial isolates were purified and characterized for morphology, gram reaction, fluorescence and hypersensitive reaction in tobacco leaves. The majority of the colonies were mucous, yellow and gram negative but morphologically different from Xanthomonas spp. One fourth of the colonies were fluorescent and morphologically similar to Pseudomonas spp., and most of them gave hypersensitive reaction in tobacco leaves. Pathogenicity tests on young tomato plants and molecular analysis by PCR, confirmed that these colonies belonged to Pseudomonas syringae pv. tomato. Pathogenicity tests to reproduce the typical symptoms observed in the field on ripe tomato fruits, are in progress.

DETECTION OF XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS BY PCR: PRELIMINARY RESULTS. M. Zaccardelli, M. Merighi, A. Spasiano and A. Del Galdo. Istituto Sperimentale per le Colture Industriali, Mi.P.A.F., Battipaglia (SA), Italy. E-mail: m.zaccardelli@tiscali.it

Xanthomonas campestris pv. campestris (Pammel) Dowson (Xcc) is the causal agent of the vascular blackening of crucifiers. The symptoms consist of discolorations of vascular tissues and marginal chlorosis of the leaves. The detection of Xcc is based on time-consuming and laborious direct isolations on SP or YDC-agar and IFAS/ELISA tests. Therefore a rapid and reliable test to detect Xcc would be desirable. In this work, preliminary results on detection of Xcc by PCR are presented. Two pathovar-specific oligonucleotide primers, designed on the brc gene of the HrpXcc pathogenicity island, were designed and a PCR protocol was applied on DNA from several bacterial isolates. The expected amplification product of 519 bp was obtained from all the 25 isolates of Xcc. tested. No amplicons were obtained from about 30 isolates of the phytopathogenic bacteria Xanthomonas campestris pv. juglandis, Xanthomonas campestris pv. pelargonii, Xanthomonas campestris pv. vitians, Xanthomonas axonopodis pv. phaseoli, Xanthomonas axonopodis pv. vesicatoria, Xanthomonas arboricola pv. pruni, Xanthomonas vesicatoria, Pseudomonas syringae pv. phaseolicola, Pseudomonas syringae pv. tomato, Pseudomonas fluorescens, Pseudomonas marginalis, Erwinia carotovora subsp. atroseptica, Erwinia carotovora subsp. carotovora. Further analyses are in progress on a larger collection of phytopathogenic and epiphytic bacteria to establish a PCR detection method of Xcc in crucifer plants and seeds.

ASPECTS OF MOLECULAR EPIDEMIOLOGY OF PSEUDOMONAS SYRINGAE PV. TOMATO. M. Zaccardelli, F. Campanile and A. Del Galdo. Istituto Sperimentale per le Colture Industriali, Mi.P.A.F., Battipaglia (SA), Italy. E-mail: m.zaccardelli@tiscali.it

Pseudomonas syringae pv. tomato (Pst) is the causal agent of bacterial speck of tomato. Pst grows as epiphyte on tomato leaves and when condition of high humidity persist, bacterial cells penetrate in the leaves through stomata, developing typical disease symptoms if temperature ranges from 13 to 20°C. The penetration of bacterial cells in the fruits occurs also through lesions. In this work, the genetic variability of a population of Pst, isolated in a same field located in southern Italy and heavy infected in the year 2002, was characterized for DNA polymorphism. This population, included groups of isolates each obtained from a same fruit or from a same lesion. The characterization, performed by M13-PCR, showed high genetic uniformity of this Pst population. The aptotypes observed altogether were three. From a same...
fruit, two aplotypes and sometimes three aplotypes were isolated. From a same lesion, the aplotypes isolated were two or, more rarely, three.

**BIOMOLECULAR APPROACH TO THE TAXONOMY OF TERVERTICILLATE PENICILLIUM SPECIES BELONGING TO THE AURANTIOTRIGOSEUM GROUP** M. Zaccardelli, F. Campanile, E. Lahoz, A. Carella and R. Nicoletti. Istituto Sperimentale per le Colture Industriali, Battipaglia (SA), Italy. E-mail: m.zaccardelli@tiscali.it

Taxonomy of terverticillate Penicillium spp. (subgenus *Penicil- lium*) assigned to the aurantiotrigoseum group is quite controversial. Many species have been better characterized, or reported in synonymy, or separated from other taxa on the account of both morphological and biochemical features. However, these criteria have often proved to be unsatisfactory, and it can happen that different laboratories provide different reports on classification of isolates in this group. Of course the absence of perfect stages complicates the task. Therefore, the application of methods able to provide phylogenetic information on relatedness between different isolates or taxa is desirable. We evaluated genetic relatedness within a sample of 41 isolates belonging to the species *P. aurantiotrigoseum*, *P. commune*, *P. crustosum*, *P. echinulatum*, *P. polonicum*, *P. solitum*, *P. verrucosum*, *P. viridicatum*; isolates of *P. chrysogenum* were used as an outgroup. For each isolate, homology sequence analyses were performed on the 5.8 rDNA-ITS genes and on 5’ end of the tubulin gene sequences deposited in GeneBank. Nucleotide variability was 1-2% for 5.8 rDNA plus ITS regions and 4-10% for t-tubulin gene. RFLPs performed by software-analyses on these sequences allowed the selection of some restriction enzymes potentially useful for diagnosis. PCR-RFLP with Smal on ribosomal regions, permitted only to distinguish *P. chrysogenum* from all other isolates of the aurantiotrigoseum group. Using M13-PCR, *P. chrysogenum* fell in an unique cluster whereas the isolates of the aurantiotrigoseum group fell in different clusters, confirming in part the distinction in different Penicillium spp. for this group.

**CYPHONECTRIA RADICALIS A NEW PATHOGEN OF CARPINUS BETULUS** E. Dallavalle, M. Iotti and A. Zambonellli. Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, Via Fanin 46, 40127 Bologna, Italy. Fax: +39.051.2096579; E-mail: zambone@agrsci.unibo.it

During attempts to isolate the pathogenic ascomycete *Cryphonectria parasitica* from hosts other than chestnut six strains of *Cryphonectria parasitica* (Diaportales, Valascaceae) were isolated from *Carpinus betula* bark cankers that had caused a severe wilting of distal foliage. The identification of our isolates was supported by morphological and molecular analyses. On potato dextrose and corn meal agar our *C. radicalis* isolates were very similar to hypovirulent strains of *Cryphonectria parasitica* but with some distinctive characteristics such as the presence of small droplets of a purple exudate giving the mycelium and the substrate an orange-pink colour. On corn meal agar a few but large picnidia were produced whereas virulent and hypovirulent strains of *Cryphonectria parasitica* produced numerous small picnidia and no picnidia, respectively. The analysis of the ITS sequence of our *C. radicalis* isolates and their direct BLAST comparison in Genebank database confirmed our identification. These results show that *C. radicalis*, which is reported to be saprotrophic on chestnut, can be a potential pathogen of *Carpinus betula*.

**RELATIONSHIP AMONG DIEBACK OF HAZELNUT IN THE PROVINCE OF VITERBO, THE INSECT *ANISANDRUS DISPAR* F. AND BACTERIAL POPULATIONS. G.M. Balestra1, C. Pucci1, B. Paparattia, D. Bucini1, J.L. Vanneste1, D. Cornish2 and L. Varvaro1. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de’ Lelis, 01100 Viterbo, Italy. Fax: +39.0761.357473; E-mail: balestra@unitus.it. 2Hort Research, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand.

Insects have always been suspected to be involved in the spreading of dieback of hazelnut in the province of Viterbo (Italy), however their role has never been confirmed. Since 2002, the Latium region administration is funding a project on different aspects related to the dieback of hazelnut in the province of Viterbo and relationship with the populations of the insect *Anisandrus dispar* F. During 2003, 4123 females of *A. dispar* were caught from two areas with diseased hazelnuts using the chromotrophic traps “Rebell red®”. Higher catches of *A. dispar* females per trap were recorded during spring. Other procedures were developed to catch the *Scotilidae A. dispar* from diseased hazelnut trees, and to isolate and identify the bacterial populations present on and inside the insects. These were washed or homogenized to isolate bacteria present either on their outside or inside. Identification of the bacterial isolates was carried out by morphological, physiological, biochemical and molecular techniques. So far the main bacterial populations identified belong to *Erwinia* spp. and *Pseudomonas* spp.. Analysis of the DNA sequence of the 16S rDNA gene indicate that the bacteria are most probably *Erwinia billungeri*, *Erwinia quercina* and *Pseudomonas syringae* of an undetermined pathovar. The phytopathological and epidemiological implications of these results are discussed.

**SPECIFIC SCAR PRIMERS FOR DIAPORTHE HELIANTHII ISOLATES OF DIFFERENT GEOGRAPHICAL ORIGIN. M.A. De Guido, A. Abbatecola, S. Pollastro, P. Natale, R.M. De Nicolis Angelini and F. Faretra. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39 080 3442911; E-mail: faretra@agr.uniba.it**

*Diaporthe helianthii* Munt.-Cvet. et al. is the causal agent of stem canker of sunflower. The disease causes heavy yield losses in most sunflower-growing areas, including France and ex-Yugoslavia. The pathogen is present in Italy, but the disease occurs only in mild form. A possible explanation of this different harmfulness of the disease in neighboring areas, under similar environmental and cultural conditions, is the presence and spreading of *D. helianthii* isolates with different virulence. RAPD (Random Amplified Polymorphic DNA) analysis clustered together isolates from France, ex-Yugoslavia and Argentina showing a high genetic similarity; Italian isolates were separately grouped and showed a broader variation. Long-distance dissemination of the pathogen can occur through infected or contaminated sunflower seeds. Hence, there is a high risk of accidental introduction in Italy of highly virulent *D. helianthii* isolates, and diagnostic assays on imported seeds are highly recommended. Rapid and sensitive methods for detecting highly virulent strains of the pathogen are needed for this purpose. RAPD markers specific for isolates from France and ex-Yugoslavia were selected and sequenced. SCAR (Sequence Characterized Amplified Regions) primers were designed and tested for specificity and sensitivity in PCR reactions. Two primer pairs (OPB48B, OPD12135D) proved specific for both French and ex-Yugoslavian isolates and three primer pairs (OPD1175A, OPD1357B, OPD1376C) were specific for French isolates only. These SCAR primers makes feasible to recognize highly virulent isolates of *D. helianthii* and will be important tools for developing molecular diagnostic protocols.
STUDIES ON THE VARIABILITY OF APPLE CHLOROTIC LEAF SPOT VIRUS. M. Al Rwahnih1, C. Turturo1, A. Minafra1, A. Myrta2, V. Pallás3, O. Potere1, D. Boscia1 and V. Savino1. 1 Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. 2 Istituto Agronomico Mediterraneo, Bari, Italy. 3 Instituto de Biologia Molecular y Celular de Plantas, Universidad Politecnica de Valencia-CSIC, Avenida de los Naranjos s/n 46022 Valencia, Spain.

Apple chlorotic leaf spot virus (ACLSV) is the type species of the genus Trichovirus. High molecular variability was previously reported to occur among ACLSV isolates. However most of the available information on this variability comes from a few isolates. The aim of this study was to extend the analysis of the molecular and serological variability of more characterised ACLSV isolates from a number of countries, mainly Mediterranean. Phylogenetic relationships between ACLSV isolates from different host species and geographical origins were established and the electrophoretic mobility of some representative isolates was determined. Coat protein (CP) genes of thirty-five isolates were studied and compared with the available sequences found in GenBank database. Alignment of the amino acids sequences confirmed that variability is higher in the N-terminal than in the C-terminal region, although not for all isolates. Four isolates (EA5, PE-T, PE154 and PE297) showed also high variability throughout the CP gene. According to the phylogenetic analysis, isolates clustered into two groups: group A, which contains the great majority of the isolates, including all those previously sequenced, and group B, containing the four diverging isolates. Three isolates of group B were from peach and one from apricot; although this may indicate that this molecularly very different isolates could be typical of stone fruits, more pome fruit isolates need to be investigated to substantiate this hypothesis. Monoclonal antibodies were raised to representative isolates from both groups to investigate the serological relationships among and within groups.