SURVEY ON THE PRESENCE OF PHAEOMONIELLA CHLAMYDOSPORA IN GRAPEVINE ROOTSTOCKS. A. Abbatecola, S. Pollastro, A. Pichiari and F. Faretra. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it

Phaeonomiella chlamydospora (W. Gams, Crous, M.J. Wingf. et L. Mguni) Crous & Gams. is supposed to be involved in brown streaking of rootstock, Petri disease and esca disease of grapevine. The clarification of the role of P. chlamydospora and its mode of transmission is crucial for planning suitable approaches to the management of these important diseases of grapevine. Diagnostic protocols based on nested-PCR for the detection of P. chlamydospora in grapevine tissues and in soil were used in a large-scale survey for the presence of the fungus in grapevine propagation materials from Italian nurseries. At uprooting, grafted and ungrafted rootstocks were collected, cut longitudinally and transversally, and inspected for wood alterations the severity of which was recorded according to an empirical scale with five classes of severity. Wood discolorations were detected in more than 90% of the inspected samples. Symptoms were particularly intense at the level of the crown, the grafted union, and the upper part of the stem, but much less severe or absent in the middle portion of the rootstocks. DNA was extracted from wood fragments collected from these sites and submitted to nested-PCR. P. chlamydospora was detected both in grafted (44%) and in ungrafted rootstocks (21%), generally at the extremities of the stem, where wood discolorations were more severe. This finding confirms the crucial role of wounds in the penetration of the pathogen. A strong positive correlation (0.94) was found between the severity of wood discolorations and the presence of P. chlamydospora. Nevertheless, a simple observation of wood discolorations alone proved to be insufficiently informative for evaluating the potential presence of the pathogen.

ISOIATION AND SEQUENCING OF AN ENDOPOLYGALEAC-TURONASE GENE FROM TWO BIOCONTROL STRAINS OF TRICHODERMA HARZIANUM. P. Ambrosino1, E. Morán2, M. Ruocco1, R. Hermosa2, E. Monte2 and M. Lorito1,2,3. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. 2Dipartimento di Botanica, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università n. 100, 80055 Portici (NA), Italy. 3Istituto di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Università del Molise, Via F. De Sanctis, 86100 Campobasso, Italy

Strains of Trichoderma spp. secrete proteins to penetrate fungal hosts and plant cell wall, and eventually induce ISR. Using a proteomic approach, we have analysed the secretome of the biocontrol strain T. harzianum strains T22 and T34, in order to identify differentially expressed proteins. 2DE maps of extracellular proteins were generated from culture filtrates obtained by growing Trichoderma in mycoparasitic, nutrient stress, or with a fungal cell walls (Rhizoctonia solani and Pythium ultimum). Over-expression in Arabidopsis of the corresponding PG gene of the T. harzianum is being done.

ANTIFUNGAL ACTIVITY OF FIVE NOVEL SAPONINS FROM ALLIUM MINUTIFLORUM. V. Antignani1, G. Bonomi1, E. Barile2, V. Lanzotti2 and F. Scalì1. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, sezione di Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università n. 100, 80055 Portici (NA), Italy. 2Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Università del Molise, Via F. De Sanctis, 86100 Campobasso, Italy

Plants synthesize a broad range of secondary metabolites, including alkaloids, terpenoids and saponins that are toxic to pathogens. In this study, we analyzed the effects of five novel saponins (ABSg12h, ABSg12g, ABSg131, ABSg12e and ABS5m) isolated from Allium minutiflorum on soil-borne pathogens (Fusarium oxysporum, F. oxysporum f. sp. lycopersici, F. solani, Pythium ultimum and Rhizoctonia solani), air-borne pathogens (Botrytis cinerea, Alternaria porri and A. alternata) and the antag-onistic fungus Trichoderma harzianum (strains P1 and T39). All five saponins showed a significant antifungal activity depending on their concentration and with the following rank: ABSg12h > ABSg12g > ABSg131 > ABSg12e > ABS5m. Several permanent alterations in the tested fungi, such as hyphal swelling and changes in the sporulation rate were evident. The two T. harzianum strains resulted much more sensitive than pathogens, being completely inhibited at the lowest concentration (10 p.p.m.) of ABSg12h, ABSg12g and ABSg131. The possible role of these saponins in plant-microbe interactions is discussed.

RESPONSE OF THE LESION MIMIC V20368 TOMATO MU-TANT TO FENTHION AND XANTHOMONAS CAMPESTRIS PV VESICATORIA. M. Antonelli1, E. Santangelo2, G.P. Soressi2, and L. Varvaro1. 1Dipartimento di Protezione delle Pianta, Università degli Studi della Tuscia, Via S. Camillo de Lellis 01100 Viterbo, Italy. 2Dipartimento di Agrobiologia ed Agrochimica, Università degli Studi della Tuscia, Via S. Camillo de Lellis 01100 Viterbo, Italy. E-mail: varvaro@unitus.it

In the study of programmed cell death (PCD) phenomenon in plants, the use of “lesion mimic mutants” (LMM) plays an important role. LMM are characterized by spontaneous development of lesions of varying size, shape and colour on the leaves, resembling a hypersensitive response (HR). In some cases, the appearance of lesions was caused by the interaction between the pathogen and a lesion mimic mutant (LMM). In the present study, the two T. harzianum strains resulted much more sensitive than pathogens, being completely inhibited at the lowest concentration (10 p.p.m.) of ABSg12h, ABSg12g and ABSg131. The possible role of these saponins in plant-microbe interactions is discussed.
National Collection of Microorganisms of Agricultural and Agroindustrial Interest. M. Barba.
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Culture collections have the crucial role of providing authentic biological material upon which high quality research is based. The project “National Collection of Microorganisms of Agricultural and Agroindustrial Interest” was financially supported by the Italian Ministry of Agriculture and Forestry (Ministero delle Politiche Agricole e Forestali) and coordinated by the Plant Pathology Research Institute of Rome (ISPaVe) during a four-year period. Thirteen public research institutions were involved. The peculiarity of the project was to organize and maintain in situ collections of microorganisms of different interest and applicability for agriculture. The aims of the project were to improve the collection of characteristic microorganisms, to promote an extensive identification and characterizations of the isolates/strains, to create a database liable to be implemented by each research unit and freely consultable via internet, to favour exchange of information and isolates. For each isolate/strain, informative cards were filled by each Institution involved in the project. Each record contains useful information on each isolate, i.e. taxonomy, year and place of isolation, identification methodology (morphological, biochemical and/or molecular), conservation media used according to the type of organism, along with references of the papers in which the isolates have been described. A total of 1,458 fungi, 26 wine yeasts, 148 dairy bacteria, 184 plant pathogenic and entomopathogenic bacteria, 216 soil bacteria, 68 table olive processing bacteria, and 59 wine bacteria are described in the cards available at the website http://www.collezioneedimicroorganismi.com/.

Comparative Sequence Analysis of Coat Protein Gene of Apulian Citrus Tristeza Virus Isolates. L. Barbarossa 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: l.barbarossa@ba.ivas.cnr.it

Three isolates of Citrus tristeza virus (CTV) collected from different loci of tristeza disclosed in spring 2002 and 2003 in Apulia (Southern Italy) were characterized based on coat protein (CP) gene sequences. The isolates CTV-0032, CTV-0036 and CTV-0038 were recovered from Navelina orange trees showing different CTV symptoms. The CTV CP gene of all isolates was amplified by RT-PCR using CP degenerate primers that yielded a 672 bp amplicon. The restriction fragment length polymorphism (RFLP) profile, nucleotide and deduced amino acid sequences were analysed and compared to each other and also to some other CP gene sequences of exotic CTV isolates available in databases. Results showed that Apulian isolates CTV-0032 and CTV-0036 have high similarity to Florida T30 and Spanish T385 mild isolates, while CTV-0038 is closely related to the severe Argentinian isolate C269-6. Moreover the RFLP analysis showed that CTV-0038 could not be placed in any previously defined HinfI group, although in an earlier study based on molecular genotype characterization, this isolate was assigned to the T36 genotype. Additional work on different genomic regions of CTV-0038 may contribute to the study of the variability of Apulian CTV populations.

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During a survey of ancient local fruit trees, the occurrence of Apricot pseudo chlorotic leaf spot virus (APCLSV), was investigated. Samples were collected from 361 trees and analyzed by nested RT-PCR. APCLSV was detected in 7 of 112 apricots, 1 of 29 peaches and 2 of 26 plums. All apple, pear and cherry trees tested negative. The sequence of PCR products compared with those in databanks showed an average pairwise divergence between isolates of 11.6 (nt) and 4.5% (aa), respectively. The majority of the isolates clustered with those in databanks but three additional divergent clusters of isolates could be identified. These results provide the first evidence for the infection of peach by APCLSV and significantly extend our knowledge of its variability. They suggest that contrary to ACLSV, APCLSV could be restricted to stone fruit hosts. Forty-eight samples were also screened for the presence of tricho-, fovea- and capilloviruses by PDO Nested RT-PCR. Results showed a very high prevalence of ACLSV in all species tested. Additional viruses detected were Cherry green ring mottle virus (CGRMV), Apricot latent virus (ALV), Cherry virus A (CVA), Apple stem pitting virus (ASPV) and Apple stem grooving virus (ASGV). Detailed phylogenetic analyses of ACLSV and CVA provided for the first time evidence that the populations of these two viruses are, to some extent, differentiated by the host plant.

Geostatistics and Mycotoxin Producing Fungi. P. Battilani, C. Barbano, V. Rossi. Istituto di Entomologia e Patologia vegetale, Universita Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: paola.battilani@unicatt.it

Geostatistics focuses on the analysis of spatially distributed variables and the prediction or estimation of values at unsampled locations. It gives the elements to differentiate variables on a geographical basis and, as user-friendly output, to draw maps in which the gradient of the variables is highlighted. This approach has been usefully applied in plant pathology but it could be particularly useful in the management of mycotoxins and their producing fungi, strictly related to climate. Three main kinds of data can be analysed: (i) meteorological data; (ii) incidence of fungi or mycotoxins; (iii) results of predictive models. A first application was done for deoxynivalenol on small grain cereals in the Emilia Romagna region (Northern Italy): data from a wide monitoring
were elaborated with a geostatistical approach and a detailed risk map was drawn. Further interesting results were recently obtained regarding Aspergillus carbonarius and related ochratoxin, a contaminant of grape bunches in the Mediterranean basin. This study suggested that it may be possible to develop accurate risk maps on a wide regional basis where the potential for non-compliance levels of ochratoxin can occur. Meteorological conditions can contribute to explain variations in the spatial distribution of black aspergilli within the Mediterranean basin. Preliminary results were also obtained for maize, a crop where different mycotoxin-producing fungi can grow, among which the prevalence of the most represented species is essentially related to meteorological conditions. Geostatistical analysis of aridity indexes, computed with data collected in Northern Italy, made it possible to draw with a good reliability the risk areas for aflatoxins and fumonisins.

DETECTION OF ILARVIRUSES IN DORMANT MATERIAL OF STONE FRUIT TREES. A. Bazzoni, D. Boscia, A. Didonna and V. Savino. Dipartimento di Protezione delle Piantine e Microbiologia Applicata, Università degli Studi di Bari, and Istituto di Virologia Vegetale del CNR, sezione di Bari, Via Amendola 156/A, 70126 Bari, Italy. E-mail: d.boscia@ha.issv.cn.it

Infections of Prunus necrotic ringspot virus (PNRSV) and of Prunus dwarf virus (PDV) are not compatible with the minimum sanitary status (CAC) currently required by European and National rules for propagating materials of stone fruits. Current ELISA diagnostic protocols consider young leaves sampled in the spring as optimal tissues for the reliable detection of these viruses. To verify whether an equally reliable detection is possible in seasons other than spring, a study was carried out on 29 accesses of different Prunus species infected by PNRSV (8 cherry, 1 almond tree, 7 plums, 6 apricots, 6 almonds, 1 cherry, 1 mahaleb cherry) and 30 accesses infected by PDV (24 cherries, 2 mahaleb cherries and 4 almonds). Results pointed out that testing dormant materials (cortical scrapings and buds) by ELISA yields results that compare well with those obtained with extracts from young leaves. Therefore glasshouse forcing of dormant budsticks to push new flushes of vegetation to be assayed in winter is no longer necessary.

A CLONAL SUBPOPULATION OF ERYSPHE NECATOR OVERWINTERING AS MYCELIUM IN DORMANT BUDS. D. Bertocchi, C. Pizzatti, M.G. Milgroom, P. Cortesi. Institute of Plant Pathology, State University of Milan, Via Celoria 2, 20133 Milan, Italy. E-mail: paolo.cortesi@unimi.it

Erysiphe necator overwinters both as ascospores in cleistothecia and as mycelium in dormant buds of grapevines. We showed that a flag shoot subpopulation of E. necator, overwintering as mycelium, deviates from a strictly clonal or randomly mating mode of reproduction. In this study we expand the study to an isolated subpopulation overwintering as mycelium with the objective of (i) analyze its multilocus genetic structure, and (ii) determine whether there is genetic differentiation between the two flag shoot subpopulations. One vineyard in Lombardy, isolated from other vineyards, and one in Tuscany, in a grape-growing area, were intensively sampled for several years for flag shoot infections early in the epidemics. Flag shoot isolates of the subpopulation in Lombardy were of the same mating type, whereas those from the subpopulation in Tuscany were of two mating types, distributed in 1:1 ratio. All isolates from Lombardy had the same or nearly the same ISSR multilocus genotype with very low genotyp-ic diversity; this subpopulation contained only the flag shoot bio-type. In contrast, the subpopulation from Tuscany, which contained flag shoot and ascospore biotypes, had high genotypic diversity. Multilocus analysis of population structure was not consistent with the hypothesis of random mating in Tuscany where the subpopulation was genetically differentiated among sampling years and with the subpopulation in Lombardy. These results indicate that the E. necator overwinters as mycelium in dormant buds is genetically subdivided in subpopulations in which the fungus reproduces exclusively clonally, or clonally and sexually, with restricted gene flow between subpopulations. This is additional evidence that genetic differentiation does not correlate strictly with the overwintering strategies of E. necator.

SURVEY OF FRUIT TREE VIRUSES ON CERTIFIED PROPAGATION MATERIALS IN NORTHERN ITALY. L. Bianchi1, A.R. Babiò1, C. Ratti1, L. Giunchedi1 and C. Rubies Autonell2. 1Dipartimento di Scienze e Tecnologie Agroambientali, DiSTA, area di Patologia Vegetale, Università di Bologna, Via G. Fanin 40, 40127 Bologna, Italy. E-mail: crubies@agrsci.unibo.it

In Italy fruit tree nursery production is a very important activity regulated by a phytosanitary certification law (D.M 24/07/03). In 2005, we studied the incidence of viruses in candidate, nuclear and propagation mother plants, included in the virus-free certification programs of Emilia-Romagna and Piedmont regions. Samples (311) were first analyzed by indexing and serological assays. All accesses were virus-free when tested by DAS-ELISA, although uncertain results were obtained for 90 samples, while with indexing viral symptoms were observed in 8 samples. Molecular analysis (RT-PCR) was then made to investigate virus presence in DAS-ELISA doubtful samples and in symptomatic indicators. Eleven samples from candidate mother plants were infected, while one infected sample was from a propagation mother plant. In particular, 3 accesses (apple and plum) were infected by Apple chlorotic leaf spot virus (ACLSV), 7 (apple and pear) by Apple stem pitting virus (ASPV), 4 (apple and pear) by Apple stem grooving virus (ASGV), 3 (cherry) by Cherry green ring mottle virus (CGRMV), and 2 (apricot and plum) by Prunus necrotic ringspot virus (PNRSV). Additionally, one indicator was infected by ASGV. Mixed infection by ASPV, ACLSV and ASGV was only observed in one sample as was ACLSV/ASPV and ACLSV/PNRSV. Two samples had a mixed ASPV/ASGV infection. No Apricot latent virus (APLV), Prunus dwarf virus (PDV) or Plum poxvirus (PPV) infections were detected. This survey confirms the high sanitary quality (96%) of plant material produced in Northern Italy thanks to the regional phytosanitary service activity.

DECOMPOSITION OF MEDICAGO SATIVA RESIDUES AFFECTS PLANT GROWTH AND DISEASE INCIDENCE. G. Bonanomi, C. Pane, V. Antignani and F. Scala. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: giulianobonanomi@hotmail.com

Soil-borne plant pathogens that cause root and crown rots, wilt, and damping-off are among the major limiting factors for the productivity of many crops. Recent evidence pointed out that soil organic matter plays an important role in the outcome of plant-pathogen interactions. However, the mechanisms by which
organic matter influences soil microorganism communities and their interactions are not completely understood. In this work we studied the effects of the decomposition process of alfalfa (Medicago sativa) residues (leaves and stems) on the growth of alfalfa and common cress (Lepidium sativum) and saprophytic and pathogenic fungal species. The outcome of the interactions between alfalfa and fungal pathogens was also analyzed. Undecomposed alfalfa residues were phytotoxic and autotoxic, both in laboratory and greenhouse bioassays. Phytotoxicity and autotoxicity steadily decreased in aerobic conditions, but were fairly stable in anaerobic conditions. In addition, radial growth and hyphal density of almost all tested fungi were favoured by undecomposed alfalfa leaf extracts, but dramatically decreased during the decomposition process, both in aerobic and anaerobic conditions. Greenhouse bioassays showed that alfalfa seedling damping-off due to Pythium ultimum and Rhizoctonia solani increased when soil was amended with undecomposed residues.

BUD AND HEART ROT OF FOX TAIL AGAVE CAUSED BY PHYTOPHTHORA ASPARAGI. S.O. Cacciola1, A. Pane2, F. Raudino1, A. Chimento1, S. Scibetta1, S. Davino2 and G. Mangano di San Lio1. 1Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche agrarie e Zootecniche, Università degli Studi di Palermo, Viale delle Scienze 2, 90129 Palermo, Italy. 2Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95125 Catania, Italy.

Fox tail agave (Agave attenuata), native to central Mexico, is very popular as an ornamental in Italy. In December 2004, a new disease was observed in Sicily on agave plants grown outdoor under cool wet conditions. Symptoms included a black and water-soaked rot at the base of the central leaves extending rapidly to the base of outer leaves, with a dark brown exudate oozing from rotten tissues, an internal brown rot of the stem, and collapse of the entire plant. A homothallic Phytophthora sp. was consistently isolated from infected tissues. This Phytophthora sp. produced persistent, nonpapillate, internally proliferating sporangia and prevalently paragynous antheridia. On agar media, it had an optimum growth temperature of about 25°C and did not grow at 30°C. Electrophoretic patterns of total mycelial proteins and isoenzymes and the sequence analysis of PCR-amplified ITS regions of rDNA of two representative isolates from agave (IMI 393958 and IMI 393045) showed that this Phytophthora sp. could be referred to a new species previously identified as P. megasperma sensu lato and recently named P. asparagi, which is responsible for a rot of asparagus spears. Koch’s postulates were fulfilled by inoculating both IMI 393958 and IMI 393045 isolates on potted plants of fox tail agave and maguey (Agave americana). In contrast, reference-isolates of other related clade 6 Phytophthora species as well as asparagus isolates of P. asparagi were not pathogenic or only weakly pathogenic to agave.

EFFECT OF DOWNY MILDEW INFECTION ON GRAPE LEAF PHOTOSYNTHESIS. T. Caffi1, V. Rossi1, S. Giosuè1 and S. Ponz2. 1Istituto di Entomologia e Patologia Vegetale, 2Istituto di Fruttivetticolture, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it

It is well known that foliar diseases may reduce crop production throughout a decrease in photosynthesis and consequently in carbon uptake and biomass accumulation. This notwithstanding, few studies are available on the impact of downy mildew (caused by Plasmopara viticola) on grapevine leaf photosynthesis. Leaves of potted ‘Barbera’ and ‘Sangiovese’ plants were artificially inoculated with P. viticola sporangia at three different growth stages. Gas exchange measurements (assimilation, transpiration and stomatal conductance) were taken using a portable photosynthesis system on three different leaf samples: (i) infected tissue; (ii) healthy tissue of infected leaves; (iii) tissue of healthy leaves. Two more experiments were carried out to study the effect of incubation period and distance from the infection site (oil spots) on leaf gas exchange. Chlorophyll concentration in the different leaf tissues was also estimated by a SPAD chlorophyll meter to better investigate physiological changes related to leaf assimilation. Assimilation rates were not significantly influenced by the growth stage of the host and the leaf age. Leaf tissue of the oil spots did not show a net photosynthesis, while the green tissue of affected leaves had the same assimilation rate as unaffected leaves, apart from a few centimeter-wide band of green tissue around the infection sites. Reduction in gas exchange began three days after inoculation, when disease symptoms were not yet visible. In conclusion, losses in carbon uptake due to grape downy mildew were greater than expected based on visible disease severity on the leaves.

PHOMA EXIGUA AS CAUSAL AGENT OF STEM AND VEIN ROT DISEASE OF ARTICHOKE IN SOUTHERN ITALY. R. Caiazzo, P. Tarantino, A. Carella, F. Porrone and E. Lahoz. C.R.A. Scafati, Laboratory of Biology and Plant Pathology, Via P. Vitiello 108 I, 84018 Scafati, Italy. E-mail: ernesto.lahoz@entecra.it

In the course of surveys carried out in 2005 and 2006 at Pietrelcina (province of Benevento, Southern Italy) about 25% of the plants of artichoke fields showed necrotic spots on the main veins of the leaves and internal necrosis of the tissues at the crown level. Isolations made on different culture media yielded up to 70% colonies of a fast growing fungus with no pigmentation but producing a dark green yellow colour when treated with NaOH. Pycnidia contained prevalently single-celled conidia 4 to 9.0 μm × 2.3 to 3.4 μm in size, suggesting the fungus to be a Phoma sp. For the rapid and unambiguous identification of the fungal species, the internal transcribed spacers and the 5.8 rRNA gene (ITS1-5.8-ITS2) were amplified with the universal primers ITS1 and ITS2 from DNA extracted from the colonies. BLAST search disclosed that the best matching sequences aligned with those of Phoma exigua, thus confirming the tentative morphological identification. Four fungal isolates were used for pathogenicity tests made by inoculating young plants by at the crown with 15 ml of a conidial suspension at a concentration of 10^5 conidia/ml. All isolates reproduced the field symptoms and often induced death of the plants. The same fungus used for inoculation was consistently re-isolated from inoculated plants. It is likely that the P. exigua outbreak was due to the unfavourable climatic and growth conditions during artichoke cropping season.

MOLECULAR CHARACTERIZATION OF FUNGI ASSOCIATED WITH MELON COLLAPSE. I. Camele1, C. Marcone2, G. Flora1, A. Carlucci2, M.L. Raimondo3 and S. Frisullo3. 1Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale Ateno Lucano 10, 85100 Potenza, Italy. 2Dipartimento di Scienze Agro- Ambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. E-mail: camele@unitab.it

Melon the most frequently grown cucurbit in Italy is severely affected by a complex fungal disease known as collapse. Follow-
ing surveys of melon crops in open fields and greenhouses, a number of fungi were identified by PCR-based methods. PCR amplification using DNA extracted from Rhizopyenens vagum, Acremonium cucurbitacearum, Plectosporium tabacinum and Monosporascus cannonculus was made with primer pairs ITS5/ITS4, which amplify rDNA of many eukaryotes, and Ryv1-F/Ryv1-R, Acrecu1/Arecuc2, Plect1/plect2 and A/E, which specifically amplify rDNA from R. vagum, A. cucurbitacearum, P. tabacinum and M. cannonculus, respectively. The primer pair 5FWDACT/MIDREVACT directed to actin gene sequences of many plant pathogenic fungi, was also used. PCR products obtained with primer pair ITS5/ITS4 were sequenced, whereas 5FWDACT/MIDREVACT amplicons were digested separately with AlaI and MseI restriction endonucleases. With PCR assays, using universal and species-specific primer pairs an amplification product of the expected size was obtained from all isolates examined. With DNA template from M. cannonculus isolates, visible PCR products were only obtained by nested PCR. Sequence analysis revealed no differences in the nucleotide positions of all Italian isolates of M. cannonculus, R. vagum and P. tabacinum species examined, which proved identical to those available in GenBank. Nucleotide sequences of all A. cucurbitacearum isolates, except for one from Spain, also were identical. Sequences of three Italian M. cannonculus isolates were deposited in GenBank under accession numbers AM 167935, AM 167936 and AM 167937. Following digestion of PCR-amplified actin gene sequences, all M. cannonculus isolates showed the same restriction pattern with each endonuclease, whereas a small polymorphism was observed among the remaining isolates.

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SANITARY CHARACTERIZATION OF DURUM WHEAT KERNELS PRODUCED IN SICILY. V. Campanella, C. Miceli. Ente Nazionale delle Sementi Elette, sezione di Palermo, Viale Region Siciliana sud-est 8669, 90121 Palermo, Italy. E-mail: sezione-palermo@ene.it

Results are reported of investigations for assessing the presence of seed-borne fungi on durum wheat kernels collected in 2003-2005 from conventional and organic crops in the main cereal growing areas of Sicily. Fungi were isolated by the agar plate method with deep freeze seed treatment, whereas their identification was based on the colony characteristics on seeds observed under a stereomicroscope and examination with the light microscope of the fruiting structures from single spore cultures. A heterogeneous mycoflora was recorded, which was composed of ubiquitous and weakly pathogenic genera such as Alternaria, Cladosporium, Pencilium, and Stemphylium, and by saprophytic genera such as Gonatobotrys, Mortierella, Paecilomyces and Epicoccum. Contamination by Fusarium, Bipolaris, and Drechslera was restricted to a few samples from both cropping systems. The most common and aggressive Fusarium spp. associated with head blight, foot rot, and seedling blight were not found. However, a progressive increase of Fusarium spp. contamination of durum wheat kernels from conventional and organic crops was observed during the three years of investigation. Nonetheless, natural seed contamination was low and apparently did not affect germination level, which, for kernels from both conventional and organic crops reached the average of 92.3%, a value higher than legal limit of 85%.

Effectiveness of biological and chemical trade products used in the nursery to control Telluric pathogens of melon crops. A. Carlucci, F. Lops, M. Mucci, C. Lazzizera, L. Colatruglio, M.L. Raimondo and S. Frisullo. Dipartimento di Scienze Agro-ambientale, Chimica e Difesa Vegetale (DiSACD), Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. E-mail: f.lops@unifg.it

In this study the results of experimental trials carried out for three years are reported. The investigation was aimed at identifying measures differing from the traditionally used means for controlling Pyrenochaeta lycopersici, Macrophomina phaseolina, Rhizoctonia solani, Plectosporium tabacinum, Acremonium cucurbitacearum, Sclerotinia sclerotiorum, Rhizopus vagum, Fusarium spp., the casual agents of root diseases of melon crops. Thus, the commercial biocontrol agents Trichoderm a viride, T. harzianum and Glomus intraradices were tested in comparison with the fungicide Fosetyl-Al. All products controlled significatively root diseases of melon. T. harzianum, however, proved the most effective, especially when it was associated with Fosetyl-Al.

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Biological control of white root rot of onion. A. Carlucci. Dipartimento di Scienze Agro-ambientale, Chimica e Difesa Vegetale (DiSACD), Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. E-mail: a.carlucci@unifg.it

White root rot elicited by Sclerotium cepivorum Berck., causes heavy losses to onion crops in the Apulian coastal plains of Zap-
and two of *Pseudomonas* produced the infection rate in comparison with the controls. However, the results obtained showed that all antagonistic microorganisms reduced the infection rate in comparison with the controls. However, the best responses were given by *B. subtilis*, a strain of *Pseudomonas* and two of *T. viride*, all of which significantly protected the onion bulbs from fungal attacks.

**DIFFERENT MORPHOTYPES OF *PECTOSPORIUM TABACINUM* ASSOCIATED WITH COLLAPSE OF CUCURBIT IN APULIA.** A. Carlucci, M.L. Raimondo and S. Frisullo. Dipartimento di Scienze Agro-ambientale, Chimica e Difesa Vegetale (DISACD) Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. E-mail: a.carlucci@unifg.it

The results are reported of a five-year monitoring of cucurbit crops affected by collapse in the Apulian provinces of Foggia, Brindisi and Lecce (Southern Italy) where melon and watermelon are extensively grown. In the last ten years, there has been a consistent expansion of both crops and of the significance of the losses they suffer due to the increased attacks by soil-borne fungi. *Monosporascus cannonbatus*, *Acremonium cebitacearum*, *Fusarium oxysporum*, *Rhizopus vagum*, and *Plectosporium tabacinum* were most frequently isolated from collapsed cucurbit plants. *P. tabacinum* was the object of morphological, molecular and pathogenicity studies which involved 270 isolates of the fungus. Based on morphology and on the colour of the top and bottom of the colonies 10 different morphotypes of *P. tabacinum* were identified. These, however, differed very little from one another when their conidiogenesis was analyzed. Molecular analyses by PCR-based methods showed that all morphotypes belonged to the same species. Further morphological and genetic studies are in progress, together with investigations on the origin and geographical distribution of this fungus.

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**OPHIOSTOMA QUERCUS PRODUCES A CERATO-ULMIN-HOMOLOGOUS PROTEIN.** L. Carresi1, C. Comparini1, F. Fontanat1, R. Bernardi1, L. Pazzaglil, G. Cappugi1, F. Scala4 and A. Scala1. 1Dipartimento di Biotecnologie Agrarie, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 10, 50101 Sesto Fiorentino, Firenze, Italy. 2Dipartimento di Biologia delle Piante Agrarie, Sezione di Genetica, Università di Pisa, Via Matteotti 1b, 56100 Pisa, Italy. 3Dipartimento di Scienze Biochimiche, Università di Firenze, Viale Morgagni 50, 50134 Firenze, Italy. 4Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: anicello.scala@unifi.it

Cerato-ulmin (CU) is a class II hydrophobin protein of about 7600 Da, produced by the Ascomycota *Ophiostoma ulmi* (Buissman) Nannf., *O. novo-ulmi* Brasier and *O. bimodal-uli* Brasier et M. D. Mehrotra. *O. ulmi* and *O. novo-ulmi* are responsible for the Dutch elm disease (DED) that in the 20th century has destroyed most of the elms native to Europe and North America (*Ulmus minor*, *U. glabra*, *U. procera*, *U. americana*, and *U. rubra*), whereas *O. bimodal-uli* is present in Asia. CU accumulates in the cell walls of DED fungi and is abundantly released in liquid culture media except for *O. ulmi*, which is known to be a scarce or nil CU producer. It was suggested that CU plays a key role in different phases of DED. However, the relationship between CU production and virulence of DED pathogens is still debated. In another ophiostomatoid species, *O. quercus*, non pathogenic to elm, an immunologically CU-related protein is present in the mycelial cell wall, but not in the culture medium. Moreover, a DNA sequence that cross-hybridize an *O. novo-ulmi cu* gene fragment was detected, thus suggesting the presence of a *cu*-orthologous gene in *O. quercus*. In the present work we report the cloning and sequencing of the *cu* gene from *O. quercus*, as well as the partial purification and characterization of the protein it encodes.

**PATHOGENICITY ASSAYS OF SOME SYSTEMIC FUNGAL ISOLATES AS PUTATIVE STARTER OF YOUNG ESA DIS-EASE OF OLIVE.** A. Carlucci, C. Lazzizera, F. Lops, M.L. Raimondo, L. Colatruglio and S. Frisullo. Dipartimento di Scienze Agro-ambientale, Chimica e Difesa Vegetale (DISACD) Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. E-mail: s.frisullo@unifg.it

In previous studies on the fungal population inhabiting the xylem tissues of small apparently healthy olive branches, several species were identified. Thus, *Phaeoacremonium rubrigenum*, *P. aleophilum*, *P. mortoniae*, *P. inflatipes*, *P. angustius*, *Phoma incepta*, *Phialophora richardiae*, *Lecythobasidium lignicolae*, *Acremonium curvulum* and *Verticillium nigrescens* were used for inoculating four-year-old olive plants of cvs Carolea, Leccino and Coratina with three different methods: (i) introduction of an agar plug from fungal colonies into a U-shaped cut; (ii) introduction cylindrical agar plug into a hole made with a small hand drill; (iii) introduction of a small woody stick colonized by each of the fungi into a hole made with a small hand drill. After one and six years, the plants were subjected to visual and laboratory inspections for evaluating the presence, type and extension of black streaks of the wood extending below and above the inoculation site. Attemps were also made to re-isolate the fungi from inoculated plants. Laboratory analyses showed that some fungal isolates produced a black streaking of the wood in both directions from the point of inoculation, the size of which varied according to the inoculated fungus and the cultivar. All inoculated fungi were re-isolated. The production of black streaks suggests that the fungi used for inoculation are pathogenic. *L. lignicola*, *P. aleophilum*, *P. rubrigenum*, *P. mortoniae*, *P. inflatipes* and *P. angustius* caused to olives symptoms similar to those associated with Petri disease (or young Esca disease) of grapevines.

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STUDY ON PATULIN METABOLISATION BY A POSTHARVEST BIOCONTROL YEAST. R. Castoria¹, L. Mannina², F. De Curtis³, L. Mauro², A. Sobolev⁴, A. Ritieni⁴, R. Ferracane⁴ and A. Spina⁴.¹Dipartimento di Scienze Animali, Vegetali e dell’Ambiente, Università degli Studi del Molise, Via F. De Sanctis snc, 86100 Campobasso, Italy. ²Dipartimento di Scienze e Tecnologie Agro-Alimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Via F. De Sanctis snc, 86100 Campobasso, Italy. ³Istituto di Metodologie Chimiche, CNR, Area della Ricerca di Roma 1, 00016 Monterotondo Stazione, Italy. ⁴Dipartimento di Scienze degli Alimenti, Università di Napoli “Federico II”, Parco Gausone, 80055 Portici (NA), Italy. E-mail: castoria@unimol.it

Patulin contaminates apple juice following Penicillium expansum attack to stored apples. EU has established the highest tolerable level of patulin in fruit-based foods. Our recent findings show that the biocontrol yeast Rhodotorula glutinis LS11 prevents apple decay caused by P. expansum and lowers patulin accumulation in infected apples. LS11 apparently delays fungal development in infected apples (in vivo) and metabolizes patulin in vitro, forming two major metabolites. However, the mechanisim(s) of the in vivo patulin decrease by LS11 is to be fully elucidated. For this purpose, it is necessary to identify the toxic metabolites, to detect their formation in vivo, to assess how deeply LS11 penetrates in decaying tissue to be closer to patulin produced by the invading fungus. NMR-based characterization of the molecular structure of the more stable patulin metabolite produced by LS11, and scanning electron microscopy observations of LS11-treated and infected apples showed that: (i) the metabolite is (4-oxo-2,3-dihydro-2H-pyran-5-yl)acetic acid, known to be much less toxic than patulin; (ii) the surviving cells of LS11 do not follow P. expansum hyphae in apple tissues. The lower toxicity of (4-oxo-2,3-dihydro-2H-pyran-5-yl)acetic acid encourages further studies on the mechanisms of patulin metabolisation by LS11. On the other hand, the absence of LS11 penetration suggests that the metabolism of patulin by the yeast does not take place in vivo. However, this process cannot be ruled out yet, since patulin diffusion in healthy apple tissue has been reported. Methods for patulin metabolite detection in vivo are under development.

EFFECT OF PLANT RESISTANCE ACTIVATORS ON HAZELNUT POWDERY MILDEW. C. Giambella, G. Chilosi, P. Magro. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: magro@units.it

Hazelnut (Corylus avellana) is one of the main crops in the province of Viterbo (Central Italy). In the fall of 2005, powdery mildew caused by Phyllactinia gattata (Wallr. ex Fr.) Lév. was observed on hazelnut plants in some orchards where tests were being done with the plant resistance activators aclibenzolar-S-methyl (ASM), b-aminoobituric acid (BABA), and potassium phosphate monobasic (KH₂PO₄). Phyllactinia gattata is a biotrophic powder mildew fungus that grows parasitically on a wide range of deciduous trees and a smaller number of herbaceous hosts. Our preliminary investigations have shown that the infection of hazelnut leaves results in an increased activity of invertase, concomitant with an accumulation of glucose in the infected leaves with and a redirection of host assimilates to the fungus. The use of the above mentioned resistance inducers has shown a decrease in the activity of invertase, when treated plants were compared with controls. Moreover, an increase in the activity of pathogenesis-related (PR) proteins, such as glucanase and peroxidase, was associated with the augmented level of resistance of treated plants. The higher level of resistance, achieved by BABA treatments, could delay the premature hazelnut leaf drop.

ANTAGONISM TOWARDS RHIZOCTONIA SOLANI AND PRODUCTION OF A BREVIIOXIME-RELATED COMPOUND BY PENICILLIUM SIZOVAE. M.L. Giavatta¹, M.P. Lopez-Gresa², A. Carella³, E. Manzo¹ and R. Nicoletti¹. Istituto di Chimica Biomolecolare, C.N.R., Via Campi Flegrei 34, 80078 Pozzuoli, Italy. ²Centro di Ecologia Quimica Agricola, Universidad Politecnica de Valencia, Camino de Vera, 46022 Valencia, Spain. ³C.R.A. Istituto Sperimentale per il Tabacco, Via Vittello 108, 84018 Scafati, Italy. E-mail: rosario.nicoletti@entecra.it

Two isolates of Penicillium sizovae Baghdadi recovered from a cropped soil in Copertino (Apulia, Southern Italy) showed antagonistic properties toward Rhizoctonia solani AG-2 and AG-4 in vitro. In fact they were able to overgrow R. solani mycelium when inoculated in plates pre-colonized by the latter, and to inhibit its mycelial growth in dual cultures. P. sizovae isolates grew successfully when crab-shell chitin, curdlan, or even lyophilized R. solani mycelium were used as the major carbon source in the culture medium, which demonstrated their ability to produce chitinases and b-glucosidas. Moreover, concentrated culture filtrates (CCFs) produced by growing P. sizovae isolates on Capek-Dox broth were also able to completely inhibit the plant pathogen. The latter observation was indicative of a possible production of fungitoxic metabolites. We have therefore extracted and fractionated CCF of isolate PL10B, obtaining 2-(hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4H-pyrrolo[2,1-b]-1,3-oxazine, a brevioxime-related compound recently characterized from Penicillium brevicompactum. When added to the culture medium at a concentration of 0.2 mg/ml, the extrolute was able to partially inhibit mycelial growth of R. solani. Therefore, it may be regarded as one of the biochemical determinants of the antagonistic properties of P. sizovae toward this widespread soil-borne plant pathogen.

SOME MYCOPARASITES ASSOCIATED WITH MYCELIA AND CARPOPHORES OF PHELLINUS AND FOMITIPORIA. C. Ciccarone. Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Via Napoli 25, 71100 Foggia, Italy. E-mail: c.ciccarone@unifg.it

While examining samples of Fomitiporia and Phellinus from various hosts or matrices, the occurrence of an uncommon fungal community was observed. It is known that some anamorphic ascomycetes are often associated with wood decay by species of Phellinus, Fomitiporia, Inonotus or Schizoplybllum. Various environmental factors, or living vectors including insects such as eusocial ants, could be involved in the dispersal of the above fungi, especially during the late phases of their cycle. Carphophore excudates seem to be attractive for such vectors and they may play a role in triggering the wood-decay processes.

OCCURRENCE OF SOME HYMENOCHAETALES ON CULTIVATED AND WILD PERENNIAL HOSTS IN THE ENVIRONMENT OF THE GARGANO PENINSULA. C. Ciccarone. Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Via Napoli 25, 71100 Foggia, Italy. E-mail: c.ciccarone@unifg.it

The role of wood-decaying basidiomycetes in the development of some diseases of woody plants has received considerable attention in the last decades. Considering the epidemiology of trunk diseases of some Mediterranean crops in the perspective of possible transmission from heterologous hosts, a wide range of potential bridge-plants has been taken into consideration with a peculiar statistical reinforcement on the Gargano area where I
have recently done a detailed mycological survey. *Tamarix gallica*, various species of *Fagaceae*, *Asaica* and *Prunus* were found to host similar agents of white rots on living or dead woody matrices. Several species of *Phellinus*, *Pomitiporia* and other closer genera of *Hymenochaetales* were isolated and compared with isolates from cultivated crops, e.g. grapevine. Carpophores were not always found on symptomatic trees, especially in urban ornamental stands since, often, the formation of fruiting bodies takes place many years after infection. Even if olive trees should be involved, neither carpophores nor detectable infections of the above fungi on this host were found in the area. Information on association or exclusion of ascomycetes and basidiomycetes is also given.

**PHYSIOLOGICAL, BIOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF NOVEL TRICHODERMA STRAINS WIDELY APPLIED IN COSTA RICA.** R. Ciliento1, S.L. Woo1, P. Marinelli2, B. Navarra1, M. Siena1, R. Marra1, F. Vinal1, S. Ferrioli1, P. Ambrosino1, I. Soriente1, M. Ruocco2, D. Turrà1, S. Lanzuise1, M.A. Obregon Gomez2 and M. Lorito1-2.

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Trichoderma spp. are cosmopolitan in all soil types and frequently represent one of the main components of the soil microflora. This is attributed to their diverse biological activities, aggressiveness and competitive nature. We characterized physiologically, biochemically and biologically seven *Trichoderma* strains isolated in Costa Rica and widely used in Central America as biosticides. In all tests, the strains were grown on PDA or minimal medium containing sucrose as the only carbon source, at temperatures of 25° or 30°C in comparison with the strain P1 of *T. atroviride*. We measured mycelial growth, sporulation time and quantity of conidia produced. Strains Bulgvir, Harz, TF, PB17 grew faster and with more spores productions than P1 when grown at 30°C. Biochemical analysis indicated that TF and PB17 in comparison to P1 accumulated in culture filtrates higher endo- and exochitinase and similar glucanase and xylanase activity. In vitro confrontation assays were performed against the pathogens *Rhzizoctonia solani* and *Botrytis cinerea*. Strains Bulgvir, Harz, Exc, TF, PB17 were able to control both pathogens more effectively than P1. In summary, our results demonstrate that the tested strains from Costa Rica exhibited a much higher fitness, enzyme production and antifungal activity, especially when grown at temperature of 30°C instead of 25°C in comparison to the well-known strain P1 of *T. atroviride*. Species identification, proteome analysis and application of these strains as biosticides in a mediterranean climate zone are being carried out.

**BURKHOLDERIA GLADIOLI, A BIOCONTROL AGENT AGAINST GREEN AND BLU MOLD OF FRUITS.** G. Cirvillere1, A. Bonaccorsi1, G. Scuderi1, S. Stefani2, M. Santagati1, A. Vitale1, I. Castello1 and G. Polizzi1.

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Burkholderia gladioli (formerly named *Pseudomonas gladioli*) has attracted attention as an antagonist in the biocontrol of *Botry-

**MOLECULAR STUDIES OF ROSELLINIA NECATRIX ISO-LATES FROM SOUTHERN ITALY.** L. Colatruglio2, I. Camele1, M.L. Raimondo1, A. Carlucci1, E. Lops1, C. Marcone1 and S. Frisullo1.

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Rosellinia necatrix is a soil-borne plant pathogen that causes white root rot disease on a wide range of plant species. In this work, *R. necatrix* isolates, which had previously been identified in southern Italy by traditional methods, were characterized using PCR assays with species-specific primers as well as sequence analysis of PCR-amplified nuclear rDNA. Seventy-one isolates of *R. necatrix*, maintained in pure culture, were examined. The universal primer pair ITS5/ITS4 which amplifies a ribosomal fragment that extends from the 3′-end of the 18S rRNA gene to the 5′-end of the 28 rDNA, thus including the ITS1 and ITS2 regions and the 5.8 rDNA, from many eukaryotes and the primer pair R2/R8 that specifically amplifies rDNA of *Trichoderma* strains, was used. For sequencing, the ITS5/ITS4 PCR products obtained from seven isolates were electrophoresed. Fragments with sizes corresponding to the expected amplified sequences were excised from the gel and eluted. With universal and species-specific primer pairs mentioned above, all isolates tested positive and yielded an amplification product of about 600 and 500 bp, respectively. Nucleotide sequence analysis showed that the southern Italian isolates (among these one was deposited in GenBank under accession number AJ972672) shared a sequence similarity at ITS1, 5.8S and ITS2 region level which ranged from 99 to 100%, with two isolates of *R. necatrix* and one of *R. arcuata*, all from Japan. Although detection and characterization of *R. necatrix* isolates in southern Italy through PCR assays using species-specific primers have already been reported, this is the first evidence of sequence analysis of Rosellinia rDNA from Italy and/or Europe.
**RECOVERY AND CHARACTERIZATION OF TOMATO YELLOW LEAF CURL SARDINIA VIRUS AND ITS VECTOR IN THE BASILICATA REGION.** S. Comes1, A. Faniglìulo1, R. Pacella1, G. Parrella2, L. Scassillo1 and A. Crescenzi1, 1Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: crescenzi@unibas.it. 2Istituto per la Protezione delle Piante, Via Università 133, CNR, 80055 Portici (NA), Italy. E-mail: giuseppe.parella@ipp.cn.it. 3Dipartimento di Entomologia e Zoolo-gia Agraria, Via Università 100, 80055 Portici (NA), Italy

Tomato yellow leaf curl disease (TYLCD) is one of the most damaging diseases of tomato worldwide. Several virus species belonging to the genus Begomovirus, family Geminiviridae, have been reported to cause TYLCD. Two TYLC virus species are known to cause TYLCD in Italy: Tomato yellow curl virus (TYCV, formerly TYLCV-Israel) and Tomato yellow leaf curl Sardinia virus (TLCSV, formerly TYLCV-Sardinia). During autumn 2005 a serious disease was observed in protected tomato crops in the Ionian coast of Basilicata (Southern Italy). Symptoms consisted in marginal leaf yellowing, leaf curling, stunting and flower abortion. The disease was detected in a group of greenhouses with an incidence of infection up to 100%. Since the symptoms were similar to those described for TLCCSV and TYLCV, ELISA and molecular detection assays for these viruses were used. A total of 50 tomato samples were tested for virus infection and for the presence of vector. A couple of synthetic oligonucleotides allowing the amplification of the whole coat protein gene was used for PCR. RFLP analysis of the PCR product showed the presence of only TLCCSV. A PCR-amplified 1008 bp fragment from one of the symptomatic plants was cloned and sequenced, and was found to share a 99% similarity with the Italian TLCCSV isolate (accession number Z228390). The molecular characterization of the COI gene (citochrome oxidase I) from the populations of Bemisia tabaci associated with the TLCVD showed that B biotype was associated with TLCCSV epidemics.

**PRESENCE OF CERATO-PLATANIN-HOMOLOGOUS PROTEINS IN FUNGAL STRAINS OTHER THAN CERATOCTY-S TIS FIMBRIATA F. SP. PLATANI: SEQUENCING OF THE CP- HORTOLOGOUS GENES AND STUDY OF DEDUCED PROTEINS.** C. Comparini1, L. Carresi1, F. Sebastiani1, I. Petroncinì1, N. Luchi1, P. Capretti1, L. Pazzagli2, G. Cappugi2 and A. Scala1. 1Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Piazzale delle Cascine 28, 50144, Firenze, Italy. Laboratorio Genexpession, Via della Lstruccia 12, 50019 Sesto Fiorentino, Università degli Studi di Firenze, Italy. 2Dipartimento di Scienze Biobimiche, Università di Firenze, Viale Morgagni 50, 50134 Firenze, Italy. E-mail: aniello.scala@unifi.it

Cerato-platanin (CP) is a 120 amino acid protein, secreted by the Ascomycete Ceratoctys fimbriata f. sp. platani (Cfp), the causal agent of plane canker stain. CP is able to self-aggregate, and is located in the cell walls of Cfp ascospores, hyphae and conidia. It contains four cysteine (S-S bridged) and is moderately hydrophobic. In the EMBL data bank CP is reported as the first member of a new fungal protein family: the Cerato-platanin family. Moreover, it elicits phytoalexin synthesis and/or cell necrosis in host and in non-host tissues. The complete sequence of the cDNA was obtained; the genomic sequence of the coding region contained an intron of 59 bp. C. fimbriata attacks various other plants of considerable relevance for agriculture, forestry and for their ornamental value. As a rule, one strain isolated from one species is not virulent on the other species, and conversely, susceptible hosts are resistant to C. fimbriata strains if they come from host other than themselves. Results so far obtained by immunotechnical experiments on a total of 17 strains (9 of C. fimbriata, as well as 1 isolate each of C. moniliforme, C. allantospora, C. fagacearum, C. laricicola, C. ambrosia, Microscae cirrosum, Ophiostoma ulmi and O. novo-ulmi) indicate that a CP-homologous protein occurs in all strains of C. fimbriata and in some other species of Ceratoctys. For some strains of C. fimbriata the coding sequences of the cp-hortologous genes have been obtained, and thus the sequences of the deduced proteins.

**EFFECT OF YEASTS INVOLVED IN WINE FERMENTATION ON GROWTH AND OCHRATOXIN A PRODUCTION BY ASPERGILLUS CARBONARIUS.** L. Cubai1,2, S. Giobbe1, M.A. Demonitis1, B. Scherm1, M. Budroni2 and Q. Miglieli1. 1Department of Plant Protection, Center for Biotechnology Development and Biodiversity Research, University of Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. 2Dipartimento di Scienze Ambientali e Biotecnologie Agroalimentari, University of Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: gmgiobi@uniss.it

Aim of this study was to evaluate whether yeast strains involved in wine fermentation may have a biocontrol potential against Aspergillus carbonarius, a fungal contaminant responsible for the accumulation of ochratoxin A (OTA) in grapes and wine. Yeast isolates belonging to two species (Saccharomyces cerevisiae and Kloeckera apiculata) were isolated from the epiphytic flora of grape berries and from must or wine, and identified at the species level using PCR-RFLP. Over 40 yeast isolates were tested in a preliminary screening on different substrates (YPD, CYA and YES agar) to select isolates showing antagonistic activity against A. carbonarius. The most effective isolates were then assayed in in vitro nutritional competition tests for their antagonistic capacity towards two ochratoxigenic strains of A. carbonarius and A. ochraceus. Yeasts were able to inhibit fungal growth when co-cultured in CYA and YES. Growth inhibition was significantly higher on YES than on CYA. Furthermore, A. carbonarius and A. ochraceus were cultivated in CYA and YES and evaluated for their ability to produce OTA after 7, 14 and 21 days of growth at 25°C. OTA concentration in the culture filtrates was determined by high-pressure liquid chromatography. Some yeasts isolates were able to reduce OTA content in the culture filtrates when co-cultivated with ochratoxigenic strains. Yeasts were finally tested on wounded grape berries for their ability to inhibit infection by ochratoxigenic moulds. When applied at a concentration of 10^6 CFU/wound, yeasts reduced significantly A. carbonarius colonization on artificially inoculated grape berries.

**EMERGING ANTIVIRAL DRUGS IN PLANT CHEMOTHERAPY.** F. D’Anna, A. Panattoni and E. Triolo. Dipartimento di Coltivazione e Difesa delle Specie Legnose ‘G. Scaramuzzi’, Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: fdan-nia@agr.unipi.it

In medical research, the strategic design of drugs directed to specific viral targets appears an expanding chemotherapeutic approach. Neuraminidase (NA) and inosine monophosphate dehydrogenase (IMPDH) are two key enzymes, whose inhibition may be expected to affect viral RNA. Oseltamivir (3′,4′,5′-acetylamido-5-amino-3-(1-ethylpropoxy)-1-cyclobexane-1-carboxylic acid), OS, an NA inhibitor representing the innovative compound well known for its activity against Influenza A virus, and Avian influenza virus, and selenenazole (2-D-ribofuranosyleselena-zole-4-carboxamide, SE), belong to IMPDH inhibitors whose an-
tiviral action is extensively reported against many human viruses. Referring to encouraging therapeutic results obtained with some IMPDH inhibitors against Cucumber mosaic virus (CMV) on Nicotiana tabacum cv Xanthi, the aim of this research was to continue this chemotherapeutic experience with SE treatment and to assay antiviral activity of OS on the same in vitro system. The drugs were administered individually for four consequent subcultures. ELISA was used on treated explants after each therapeutic cycle and biological assays were done at the end of the trials to control the sanitary conditions on N. benthamiana plants. Both tests showed that 100% of the explants were sanitized following sele-nazole and oseltamivir administration. We provided compelling evidence that IMPDH and NA inhibitors eradicated CMV infection in N. tabacum. Now would like to point out the SE activity, which provided the highest percentage of sanitation as compared with other IMPDH inhibitors like tiazofurin, benzamide riboside and mycosphenolic acid that had been previously tested.

OCCURRENCE AND CHARACTERIZATION OF TOMATO YELLOW LEAF CURL VIRUS IN THE RAGUSA PROVINCE. S. Davino1, C. Napoli2, M. Davino1 and G.P. Accotto2. 1Dipartimento di Scienze e Tecnologie Fitosanitarie (DISTEF), Sezione di Patologia vegetale, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. 2Istituto di Virologia Vegetale, CNR, Strada delle Cac-ce 73, 10135 Torino, Italy. E-mail: wudavino@unitc.it

Tomato yellow leaf curl virus (TYLCV) has been reported for the first time in Italy in 2002. From 2002-2004 we have followed its spread in Sicily, where the other related begomovirus, Tomato yellow leaf curl Sardinia virus (TYLCSV) is endemic and has caused severe damages since 1989. The presence of both viruses was monitored in the main tomato growing area, the Ragusa province, by tissue print assays followed by hybridization with species-specific probes. At the first screening (spring-summer 2002) both viruses were found in mixed infections, but in 2003 and 2004 18-35% of plants were infected by TYLCSV alone and 8-28% by TYLCV alone, with 41-69% carrying both viruses. In three years from its first appearance the new virus (TYLCV) has spread quickly in the area, demonstrating, as in other parts of the world its high virulence and invasiveness. The DNA genome of an infectious clone of TYLCV from Sicily (TYLCV-IT) was sequenced and showed 97% similarity with the severe type found in Israel and in many countries worldwide. Our data indicate that TYLCV has quickly invaded an area already colonized by TYLCSV, but, at least for the moment, has not displaced the old species completely. In many plants both viruses appear to coexist, causing symptoms more severe than single infections.

MOLLEcular characterization of cucumber mosaic virus isolates infecting ornamental species cultivated in the botanical garden of the university of Bologna. S. Davino1, M. Davino1 and M.G. Bellardi2. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. 2Dipartimento di Scienze e Tecnologie Agroambientali e Patologia Vegetale, Alma Mater Studiorum. Università di Bologna. Via G. Fanin 42, 40127 Bologna, Italy. E-mail: wudavino@unitc.it

During an epidemiological survey carried out in the Botanical Garden of the University of Bologna Cucumber mosaic virus (CMV) was detected by PAS-ELISA in some ornamental species exhibiting a severe symptomatology of the leaves. Datura innoxia showed mosaic and leaf curling; Globularia nudicaulis produced narrow leaves with yellow mosaic and/or variegation; Eupatorium cannabinum showed a systemic chlorotic and/or yellow mosaic and stunting. RT-PCR and single strand conformation polymorphism (SSCP) were used to characterise these CMV isolates. Total RNA was extracted from symptomatic leaf samples with a Qiagen RNeasy Plant Minikit (Qiagen, Milan, Italy) according to the manufacturer’s instructions. RT-PCR was carried out using specific primers for the movement protein gene of CMV RNA-3 (forward MP+ CATGGCTTCCAAAGTGACG, genomic position 118nt to 138nt, and reverse CTAAAGACCGTTAACCAC- CCG, genomic position 938nt to 959nt). All samples from the three ornamental species yielded DNA fragments of the expected size (841 bp). PCR products were then analysed by SSCP to identify specific sequence variants and compare genetic relationships with CMV isolates from other ornamental species present in the same Botanical Garden (Tibetva nereifolia and Nandina domestica). The results showed a different sequence variant for each CMV isolate, indicating that these three isolates may have come from the country of origin of the hosts.
CONTROL OF RHIZOCTONIA SOLANI AND SCLEROTIUM ROLFSII DAMPING-OFF OF TOMATO PLANTS: PRELIMINARY RESULTS. F. De Curtis, D. Vitullo, D. Piedimonte, A.M. Spina and G. Lima. Dipartimento di Scienze Animali, Vegetali e dell’Ambiente, Sezione di Patologia Vegetale, Via De Sanctis, 86100 Campobasso, Italy. E-mail: decurtis@unimol.it

Rhizoctonia solani and Sclerotium rolfsii are fungal pathogens responsible for severe crown and stem rot of several horticultural crops in greenhouse and open field. With a two-year survey in tomato (Lycopersicon esculentum) crops of Central and Southern Italy, a high and unusual incidence of the two pathogens was observed. Because of technical, economical and environmental problems, chemical control against this pathogens is difficult. Thus the activity of some antagonistic bacteria (T1A-2B and T4B-2A) isolated from suppressive soils against these pathogens, was tested in experiments carried out on tomato plants grown under controlled and field conditions. The potential antagonists were compared with two commercial biofungicides (Bacillus subtillis, BSF4 and Trichoderma viridae, TV1) and four synthetic fungicides (Tolclofos-metile, Azoxystrobin, Fosetil-Al and Propamocarb+Fosetil-Al). The antagonists as well as other control treatments were applied in the soil near the plant crown and main root and 24 h later plants were artificially inoculated with the pathogens. In both controlled and field conditions the antagonists significantly reduced the incidence and severity of the pathogens. In both controlled and field conditions the antagonists significantly reduced the incidence and severity of R. solani symptoms. The antagonistic bacteria were as effective as Trichoderma viridae TV1, better than Bacillus subtillis BSF4 and comparable with synthetic fungicides, except for Tolclofos-metile which was more effective. Results are discussed also in relation to the optimization of the new potential antagonists for application aimed at an eco-compatible control of crown and root rot of horticultural crops.

PRELIMINARY STUDIES ON A MIMOSA TREE WILTING IN APULIA. T. de Gioia1, C. Ciccarone2, and G.L. Rana3. 1Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. 2Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Via Napoli 25, 71100 Foggia, Italy. 3Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via Ateneo lucano 10, 85100 Potenza, Italy. E-mail: teresa.degioia@agr.uniba.it

Albizia julibrissin is one of the most widely cultivated ornamental trees in Apulia (Southern Italy) because of its colourful flowers. In spring 2002, plants showing wilting of apical branches, small cankers and a sooty darkening of the inner bark layers, were observed in a private garden in Molletta (BA). In 2003 and 2004, many sporophores of Schizophyllum commune appeared on two of the diseased plants. The micromycetes isolated were the following: Alternaria ziniae, Nectria haematococca and Fusarium oxysporum, from bark cankers, and Kaskanvia gleditsiaca and Diaportha leipihemia from internal darkened bark tissue and fungal stromata, respectively. The main etiological agent of the disease could be considered Fusarium oxysporum f. sp. permicosum F01. S. commune colonized the ornamental when already dead. The other micromycetes found in external and inner bark layers, except for K. gleditsiaca (which can cause depressed cankers on decaying mimosa-tree branches) and D. leipihemia (which infects some Quercus species), are saprophytic or opportunistic fungi. Pathogenicity tests with the F01 of F. oxysporum are under way.

STUDIES ON THE AETHEOLOGY OF WARTS OF THE CULTIVATED MUSHROOM PLEUROTUS ERYNGII. T. de Gioia1, G. Gifiulno2, G.L. Rana3, D. Sisto1, and M. Milordo1. 1Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. 2Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via Ateneo lucano 10, 85100 Potenza, Italy. 3Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: teresa.degioia@agr.uniba.it

Pleurotus eryngii (D.C. Fr.) Quél. is a mushroom the intensive cultivation of which is aleatory due to the scarcity of knowledge available on the control of adversities that can reduce its production. One of these, known as warts, does not alter the organoleptic basidiozyme quality, but modifies so much its aesthetics to impede marketing as a fresh product. Therefore, it seemed useful to study the ethiology of these abnormalities. In particular, it was investigated if they were due to soil type used to cover incubated substrate at the beginning of cultivation; temperature dropping during basidiozyme growth; scratches or superficial wounds of basidiozyme pileus cuticle, or if they were related to the high nitrogen content of sporophores or to morphometric features of specific commercial strains of the mushroom. Warts were not elicited by any of the above causes. Nevertheless, a weak but significant relationship was observed between their incidence and basidiozyme weight and size reduction. In conclusion, warts appeared to be enough strain-specific and, consequently, inheritable.

MORPHO-PHYLOGENETICAL ADAPTATIONS STIMULATED BY TOXIC METALS IN CULTIVATED MICROFUNGI. L.N. Delvecchio and C. Ciccarone. Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Via Napoli 25, 71100 Foggia, Italy. E-mail: c.ciccarone@unifg.it

This work was based on the analysis of the physiological and morphological changes in colonies of Aspergillus niger, A. flavus, Gloeocadium roseum and Rhizopus stolonifer, after exposure to toxic heavy metals, like copper, tin and lead in metallic and salt forms and in different concentrations added to synthetic substrates. After incubation for a month we have registered macroscopic and microscopic changes in all fungal colonies, i.e. the mycelium had a lower growth, and was encrusted with oxalate crystals, chemically synthesized by metallic ions and oxalic acid, produced in a higher quantity for changes in metabolic pathways and secreted by hyphal cells. We observed a dense and compact mycelium grown on media containing metals. There were also morphological differences in conidiphores and in the marginal zones of the mycelium, in which we observed mycelial cords, synnematal structures, hyperproduction of organic acids and increase of cellular breathing. Interesting changes in fungal morphology and physiology demonstrate a great capacity of adaptation, also in extremely adverse habitat conditions. However, cellular vitality results, depressed.

PRODUCTION AND CHARACTERIZATION OF LABORATORY MUTANTS OF BOTRYOTINIA FUCKELIANA RESISTANT TO THE NEW FUNGICIDE BOSCALID. R.M. De Miccolis Angelini1, W. Habib2, C. Rotolo3, S. Pollastrello4 and F. Faretra4. 1Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. 2Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano, Bari, Italy. E-mail: faretra@agr.uniba.it

Botryotinia fuckeliana (de Bary) Whetze. (Botrytis cinerea Pers.), the causal agent of grey mould of numerous crops, is well known
for its broad adaptability and its ability to acquire resistance to fungicides, that often hampers crop protection strategies. Boscalid (Cantus, BASF Agro) is a new fungicide with a novel mode of action, the inhibition of the mitochondrial electron transport chain that is effective against B. fuckeliana. Six monosporous strains of B. fuckeliana were used in mycelial growth and conidial germination tests to evaluate the baseline sensitivity to boscalid. The activity of the fungicide was higher when acetate instead of glucose was used as carbon source in different media. The response of fungal strains to boscalid was EC50 = 1·3 mg ml⁻¹ and MIC = 10 µg ml⁻¹ in colony growth test and EC50 = 0.01-0.03 mg ml⁻¹ and MIC = 1 µg ml⁻¹ in conidial germination assay. Boscalid-resistant laboratory mutants were obtained from UV irradiated or non-irradiated conidia of the wild-type reference strains SAS56 and SAS405 plated on acetate minimal medium and acetate water agar medium amended with 1 or 3 mg ml⁻¹ of boscalid. UV-induced mutants were obtained only from SAS56 in all the tested conditions; the mutation rate was of 1·10⁻⁵ survivor conidia. One spontaneous mutant was obtained from 1·10⁸ plated conidia of SAS405. Mycelial growth and conidial germination of all putative mutants were unaffected or only slightly inhibited by 300 mg ml⁻¹ of boscalid. Sexual crosses of mutants with suitable reference strains have been started. Investigation on the mode of inheritance of resistance traits in meiotic progeny will allow to clarify the genetic basis of resistance to boscalid and to predict the risk of acquired resistance to the fungicide.

**CUCUMBER MOSAIC VIRUS INFECTION OF TOBACCO PLANTS COSTITUUTIVELY EXPRESSING EITHER POLYAMINE OXIDASE OR COPPER AMINE OXIDASE.** E. Di Nicolao-Negri1, P. Tavladoraki2, L. Salandri1, A.D. Palumbo3, S. Ciferri2 and V. Iardì1. 1CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. 2Dipartimento di Biologia, Università "Roma Tre", Viale G. Marconi 446, 00146 Roma, Italy. 3CRA, Istituto Agronomico, Via Ulpiani 5, 70125 Bari, Italy. E-mail: v.ilaridi@ispave.it

Cucumber mosaic virus (CMV) is one of the most economical-ly important plant viruses in the world and has one of the largest host range of any plant virus (ca.1000 plant species). Different approaches are currently under way to find suitable control measures for CMV. The polyamines are low-molecular weight organic compounds found in both eucaryotic and prokaryotic cells. Plant polyamines have been implicated in defense mechanisms during biotic and abiotic stress. Furthermore, H2O2 production through polyamine oxidation has been correlated with defense responses occurring during defense responses. Copper amine oxidase (CuAO) and polyamine oxidase (PAO) are enzymes involved in polyamine oxidative catabolism. In order to investigate the influence of polyamines catabolism on plant-virus interaction and to identify a strategy to control CMV infection, transgenic tobacco plants, constitutively expressing maize polyamine oxidase and pea copper amine oxidase, have been utilized in CMV resistance tests. As inoculum source two different CMV isolates were used. CMV infection was evaluated by monitoring symptoms and by ELISA on both inoculated and non inoculated leaves. The results obtained comparing three typologies of transgenic tobacco plants (CuAO and PAO expressed in the cell wall and PAO expressed in the cytoplasm) and wild type control plants will be presented.

**EFFECT OF HEAVY METAL IONS ON THE GLUTATHIONE TRANSFERASE ACTIVITY OF TRICHODERMA HARZIANUM.** R. Faedda1, A.R. Lo Piero1, S.O. Cacciola2 and G. Petrone1. 1Dipartimento di Scienze Agronomiche, Agrochimiche e delle Produzioni Animali, Università degli Studi di Catania, Via S. Sofia 98, 95123 Catania, Italy. 2Dipartimento di Scienze Entomologiche, Fitotopologiche, Microbiologiche agarie e Zootecniche, Università degli Studi di Palermo, Viale delle Scienze 2, 90128 Palermo, Italy. E-mail: gpetrone@unito.it

Glutathione S-transferases are a family of multi-functional enzymes involved in cellular detoxification processes that catalyze the glutathione conjugation to a variety of xenobiotics. Moreover they have also been involved in metal tolerance by different strains of fungi. In this study the effect of different heavy metal ions (Cd, Hg, Pb and Zn) on glutathione transferase (GST) activity of Trichoderma harzianum (IMI 393899) has been evaluated. GST activity of T. harzianum, grown in a liquid substrate (Czapek) supplemented with the different heavy metals at 1 and 10 ppm respectively, resulted to be significantly higher with respect to the control. The highest GST activity was observed with 10 ppm of Hg (155 nmol min⁻¹/mg) and 10 ppm of Cd (76 nmol/min/mg), which is respectively 17 and 8.4 folds higher than the control enzyme activity (9 nmol/min/mg). Preliminary data on the in vitro growth rates at different concentrations of heavy metals seem to indicate that T. harzianum is extremely tolerant to heavy metals, which, in some cases, could even stimulate mycelial growth. These very promising results would indicate that GST activity of the fungus is correlated with its ability to withstand the effect of heavy metal contamination. Therefore T. harzianum could be a very useful microorganism in the environmental decontamination from heavy metals.

**SOLANUM NIGRUM, DATURA STRAMONIUM AND SONCHUS ASPER AS RESERVOIRS OF TOMATO YELLOW LEAF CURL SARDINIA VIRUS IN SOUTHERN ITALY.** A. Fanigliulo, R. Pacella, S. Comes and A. Crescenzi. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: crescenzi@unibas.it

From August 2004 to June 2005 a serious tomato yellow leaf curl epidemic was observed in protected tomato crops in Castrovillari (Calabria, Southern Italy), in a group of greenhouses where tomato is grown hydroponically. An extensive survey for Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato yellow leaf curl virus (TYLCV) reservoir hosts was conducted during summer period (July-September) in order to identify where the two viruses persist during the host-free period, in an area covering a radius of 500 m around the group of greenhouses. About 300 samples were collected from symptomless and symptomatic plants of the following botanic families: Gramineaeae, Compositaeae, Solanaceae, Portulacaceae, Malvaceae, Chenopodaceae, Aamaranthaceae, Convolvulaceae, Brassicaceae, Labiatae, Plantaginaceae, and Asteraceae. Virus presence was evaluated by DAS ELISA, using a “broad-spectrum” reagent combination able to detect different begomoviruses including TYLCSV and TYLCV. Samples positive in ELISA underwent PCR and RFLP analysis for confirmation. Solanum nigrum, Datura stramonium (Solanaceae) and Sonchus asper (Compositaeae) were the only weeds found to be infected by TYLCSV. Similarity analysis performed between the PCR amplified fragment from each sample and the TYLCV isolate recovered within the greenhouse and responsible for the epidemics with a TYLCV isolate gave a 100 %, value, thus indicating that there was no variability in TYLCV population in the surveyed
area. S. nigrum, D. stramonium and S. asper, as alternative hosts of TYLCV and nutrient plants for the virus vector, Bemisia tabaci, appear to play an important role in virus ecology and epidemiology in the studied tomato ecosystem.

ESSENTIAL OIL ACTIVITY AGAINST POSTHARVEST PATHOGENS AT LOW TEMPERATURES. G. Farina, M. Moretti, M. Saracchi, P. Sardi and G. Venturini. Istituto di Patologia Vegetale, Facoltà di Agraria, Università degli Studi di Milano, Vai Celoria 2, 20133 Milano, Italy. E-mail: marco.saracchi@unimi.it

Essential oils extracted from different plants, belonging to Geraniaceae, Graminaceae, Lamiaceae, Lauraceae and Mirtaceae, were able to inhibit the growth of Botrytis cinerea, Alternaria spp. and Penicillium spp. strains, isolated from strawberry and apple postharvest rots, when grown at 24°C. The aim of this research was to verify if the same compounds maintain similar level of activity when tested at lower temperatures (5 and 10°C). Tests were carried out following the protocol proposed by Raboso et al. (Plant Disease 79: 294-296, 1995) to assess fungicide activity, as so to assay many compounds and concentrations at the same time. Of the tested pathogens, B. cinerea showed good growth also at low temperatures, while for Penicillium and especially Alternaria the growth rate was strongly reduced, thus increasing the time scheduled for result recording. Results confirmed that at 24°C the most effective oils were those extracted from Cynelopogon martiniti (palmarosa), Leptospermum scoparium (manuka), Pimenta dioica L. (allspice) and Syzygium aromaticum (clove). They maintained and in some cases increased the activity against pathogens at 5 and 10°C. The more constant results were obtained with B. cinerea, with the exception of palmarosa oil that reduced its activity at low temperatures. The results were compared with those obtained by amended agar plate and paper disc techniques which, on the whole, confirmed the data. Observations with a scanning electron microscope of colonies of the pathogens, grown on polycarbonate membranes in the presence of different concentrations of oils, frequently showed peculiar morphological alterations.

SOME POSSIBLE MECHANISMS DEPLOYED BY FUNGAL PATHOGENS TO PREVENT THE EFFECTS OF PLANT PR-PROTEINS. F. Favaron1, L. Sella1, S. Odorizzi1, M. Marongoni1 and M. Lucchetta1. 1Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell’Università 16, 35020 Legnaro, Italy. 2Dipartimento di Biotecnologie Agrarie, Università degli Studi di Padova, Viale dell’Università 16, 35020 Legnaro, Italy. E-mail: francesco.favaron@unipd.it

Expression and accumulation of PR proteins is part of the defense machinery developed by plant to contrast plant pathogen attacks. Different classes of PR-proteins accumulate locally, near the site of infection, and systemically along the entire plant. It is supposed that plant pathogens have evolved mechanisms to avoid or prevent the harmful effects of PR-proteins. We have studied the effects of PR-proteins from grapevine on the fungal necrotrophic pathogens Botrytis cinerea and Sclerotium rolfsii. Mature berries and wine were chosen as useful sources of PR-proteins, especially because enriched in chitinase and thiamatin-like proteins. When the fungi were grown on berry juice, PR-proteins precipitated probably because complexes with plant oxidized polyphenols occur, a process catalyzed by fungal polyphenoloxidase. When berry or wine proteins were added to the medium, B. cinerea produced appreciable proteinase activity and showed a noticeable mycelium fragmentation not observed when the fungus was grown in culture containing heat-denatured proteins. Instead, S. rolfsii grew in a normal way in the presence of native PR proteins and secreted only trace amounts of proteinase activity in the medium. The abundant glucan sheath, externally secreted by S. rolfsii, appears as the principle mechanism deployed by this fungus to defend itself from the toxic effects of grape PR-proteins.

ENHANCEMENT OF TRICHODERMA ATROVIRIDE DISEASE CONTROL ABILITY BY APPLICATIONS OF “BIO-CONTROL INDUCERS”. S. Ferraioli1, I. Soriente1, S.L. Woo1, R. Ciliento1, R. Marra1, F. Vinale1, P. Ambrosino1, M. Ruocco2, D. Turri1, S. Lanzuise1, A. Sodano1, V. Fogliano1, F. Scala2 and M. Lorito2. 1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. 2Istituto per la Protezione delle Piante (IPP), CNR, 80053 Portici (NA), Italy. 1Dipartimento di Scienza degli Alimenti, Università di Napoli Federico II, 80055 Portici (NA), Italy. E-mail: lorito@unina.it

Fungi of the genus Trichoderma able to parasitize pathogenic fungi are commonly used to control a variety of plant diseases. During the interaction between antagonist, plant and pathogen, different molecules that may induce the biocontrol gene expression cascade are produced, released and/or detected by the antagonist. To isolate and characterize these compounds acting as “biocontrol inducers”, we prepared and fractionated culture filtrates (CFs) from T. atroviride strain P1 grown in the presence of different plant or pathogen tissues. The CF fractions of <3 kDa MW were tested for their ability to affect biocontrol by using Trichoderma mutants containing the marker genes GFP or GOX with two mycoparasitic promoters. The highest production of the biocontrol-related enzymes endochitinase and exochitinase was obtained with “biocontrol inducers” prepared by growing Trichoderma P1 in a salt medium enriched with Rhizoctonia solani cell walls, tomato plant live tissues or extracts. The CFs obtained by growing P1 in the presence of the “biocontrol inducers” showed to reduce the in vitro spore germination of the pathogens Botrytis cinerea and Alternaria alternata. In vivo assays on tomato plants showed that co-incubations of these “biocontrol inducers” with Trichoderma P1 spores strongly enhanced biocontrol activity against Botrytis disease development. When the same inducers were infiltrated into tomato leaves and spores of both Trichoderma and Botrytis inoculated on the same or a different leaf, an induced systemic resistance (ISR) effect was obtained. Mass spectrometry analysis (ESI-MS) of the mixtures of “biocontrol inducers” indicated the presence of hexose oligomers, likely cellobiose.

CHARACTERIZATION OF PSEUDOMONAS SYRINGAE pv. TABACI STRAINS COLLECTED IN CENTRAL ITALY. P. Ferrante, C. Moretti and R. Buonaurio. Dipartimento di Scienze Agrarie e Ambientali, Sezione Arboricoltura e Protezione delle Piante Patologia Vegetale, Università degli Studi di Perugia, Via Borgo XX Giugno, 74, 06121 Perugia, Italy. E-mail: patferrante@itsclus.it

Forty nine Pseudomonas syringae pv. tabaci strains, isolated in 2005 in Central Italy and previously identified by pathogenicity, morphological, biochemical, physiological and nutritional tests,
were characterized determining copper sensitivity and ability to produce tabtoxin in vitro, and verifying the presence of the tblA and tabA genes required for tabtoxin production. A further characterization of the strains was carried out by rep-PCR, using the following sets of primers: BOX, ERIC, REP, KRP2 and KRP. Italian strains were compared with 10 P. syringae pv. tabaci strains: LMG 5192, 5393, 5394, 5526 and 5527; NCPPB 1427 and 1918; ICMP 2835; 113R; 15D41. All strains tested were copper resistant as they grew on nutrient agar added with 0.5% glucose and amended with 200 mg ml⁻¹ of copper sulphate (0.8 mM). Bioassay for tabtoxin production using Escherichia coli strain BL21 as indicator organism revealed that among all the strains tested only NCPPB 1918 and 113R produced tabtoxin. These results were confirmed by the amplification of the tblA and tabA genes, except for the strains LMG 5393, NCPPB 1427, ICMP 2835 and 15D41 that, even if they have the tblA and tabA genes, they do not produce tabtoxin. Spontaneous mutations frequently occurring in the gacS and gacA regulator genes, which control the tblA and tabA expression, could explain this discrepancy. Rep-PCR made with the primers BOX, ERIC and REP showed that the Italian strains generated fingerprints identical to each other. By contrast, when KRP2 and KRP were used, polymorphisms were observed, which were not correlated with the geographical origin of the strains.

**DETECTION OF ENDOPHYTIC MICRORGANISMS WITHIN ROOTSTOCK MOTHER VINES GROWN IN THE MARCHE.** L. Flamini¹, E. Rossini¹, S. Toni², E. Biondi², S. Sandalo², F. Bini², P. Nipoti² and C. Bazzi². ¹ASSAM, Agenzia Servizi Settore Agroalimentare Marche, Servizio Fitosanitario Regionale, Via Alpi 21, 60131 Ancona, Italy. ²Dipartimento di Scienze e Tecnologie Agronomi Ambientali, Alma Mater Studiorum, Università di Bologna, Viale Fasan 40, 40127 Bologna, Italy. E-mail: flamini.lucio@assam.marche.it

An increase in the number of reports on infectious diseases of young grapevines have characterized the last twenty years. The replacement of old vineyards was a chance to check the phytosanitary state of mother vines. A plethora of microorganisms can be isolated with a variable frequency (approx. 3-15 %). Results from this preliminary survey showed the suitability of the analytical method for the detection not only of bacteria but also of fungal endophytes.

**ANTIFUNGAL ACTIVITY OF EXTRACTS FROM WILD EDIBLE HERBACEOUS SPECIES.** M.A. Gatto¹, D. Di Venere², V. Linsalata³, S. Vanadia³, V.V. Bianco¹ and A. Ippolito¹. ¹Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. ²CNR-Istituto di Scienze delle Produzioni Alimentari (ISPA), Via Amendola 122/O, 70126 Bari, Italy. ³Dipartimento di Scienze delle Produzioni Vegetali, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: donato.divenere@ispa.cnr.it

The use of natural antimicrobial substances could be a useful alternative to synthetic pesticides for controlling postharvest pathogens of fresh fruit and vegetables. In the present work, the in vitro antifungal activity of extracts from some wild edible herbaceous species containing a phenolic fraction, was evaluated. Their activity was assessed on both conidial germination and germ tube elongation. The efficacy of extracts from Borago officinalis, Orobanchus crenata, Plantago coronopus, P. lanceolata, P. major, Sanguisorba officinalis, Silene vulgaris, Sonchus asper, S. oleraceus, and Taraxacum officinale was tested against some important post-harvest pathogens: Botrytis cinerea, Monilia laxa, Penicillium digitatum, P. expansum, P. italicum, Aspergillus carbonarius, and A. niger. The most effective extracts were those obtained from S. officinalis and O. crenata. In particular, extracts of S. officinalis completely inhibited conidial germination of M. laxa, P. digitatum, P. italicum and A. niger, strongly reduced conidial germination of B. cinerea and P. expansum, and slightly affected A. carbonarius. The extract of O. crenata showed a good efficacy against B. cinerea and M. laxa and, to some extent, also against P. digitatum, P. expansum, P. italicum, and A. niger. Moreover, the extracts of both species were effective in reducing germ tube elongation also when a slight inhibition on conidial germination was observed. Further studies are in progress to test the activity of the extracts on stored fruit and vegetables and to identify the active compound(s).

**GENETIC VARIABILITY IN A POPULATION OF ARMILLARIA GALlica FROM A DECLINING OAK STAND IN SOUTHERN ITALY.** A. Gatto, G. Sicoli, N. Luisi. Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. E-mail: luisin@agr.uniba.it

The genus Armillaria includes harmful fungal pathogens causing root rot and wood decay in a broad range of host plants throughout the world. In southern Italy declining oak stands have been frequently found infected by A. gallica, a well-known weak pathogenic species. The aim of this study was to detect, by means of RAPD-PCR markers, the level of intraspecific variability within isolates of a A. gallica population sampled from the “Difesa Grande” Quercus spp. stand located at Gravina (Apulia, Southern Italy). UPGMA cluster analysis of RAPD profiles generated by the decamer primers (OPA) grouped the isolates in subclusters showing low intraspecific genetic variability. Moreover, the RAPD pattern analysis yielded clusters, which did not correspond, to the groups discriminated by the somatic incompatibility (SI) tests performed in a previous investigation on the same population. The findings of this research pose the question of whether SI, which involves an undefined number of genes and alleles per gene, might still be considered an effective tool for the study of the epidemiology of A. gallica rather than RAPD-PCR analysis of the entire genome of the fungus.
BIOCONTROL ACTIVITY OF WINE YEASTS AGAINST POSTHARVEST ROT ON APPLE FRUIT CAUSED BY PENICILLIUM EXPANSUM AND MONILINIA FRUCTIGENA. S. Giobbie1, L. Cubaiu1,2, B. Scherm1, M. A. Demontis1, M. Budroni2 and Q. Miglieli1. 1Department of Plant Protection, Center for Biotechnology Development and Biodiversity Research, University of Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. 2Dipartimento di Scienze Ambientali e Biotecnologie Agroalimentari, Università di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: qmiglieli@uniss.it

Penicillium expansum and Monilinia spp. cause severe rot of apple fruit during storage and shelf life. Aiming at the development of new antagonistic yeast active in controlling multiple postharvest pathogens, over 100 wine isolates representing 32 species were tested from our collection in biocontrol experiments carried out under laboratory conditions. Three isolates of Pichia fermentans, Metschnikowia pulcherrima and Kloeckera javanica were selected for their high biocontrol efficacy against infection caused by P. expansum, and were used in further experiments against Monilinia spp. Strain 726 of P. fermentans, isolated from wine must, was chosen for its remarkable antagonistic properties against blue mould and brown rot of apple. In trials carried out on the cvs Golden Delicious and Renetta, the yeast reduced both apple rot caused by P. expansum and M. fructigena with up to 100% efficacy. Killed yeast cells and culture filtrates had no or very low biocontrol activity. The role of killer toxins and of the competition for space and nutrients in the biological activity of the most effective strains is reported.

PRELIMINARY CHARACTERIZATION OF PHOMA TRACHEIPHILA ISOLATES FROM ITALY AND GREECE BY DNA-BASED TYPING METHODS. F.M. Grasso1 and V. Catarà1. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: vcatar@unito.it

Many attempts have been carried out in the past to characterize isolates of Phoma tracheiphila, the causal agent of citrus mal secco based on the fact that chromogenous and non-chromogenous variants were distinguished in culture and strains differing in virulence were found to occur in nature. Nevertheless isolates are still undistinguishable on the basis of electrophoresis of mycelial proteins and isozymes, ITS sequences and a number of molecular markers. With the aim of investigating possible variability of P. tracheiphila populations from Italy and Greece we conducted a survey in different citrus groves in both countries. Ninety isolates were obtained from 57 Italian citrus groves in Sicily, Calabria, Basilicata, Campania and 24 Greek citrus groves from Rhodes, Chios, Crete and Corinth, and from different citrus hosts (lemon, sour orange, lime and cedar). Fungal identification was based on morphological and biometric characteristics. ITS sequencing and the PCR-based fingerprinting techniques, random amplified polymorphisms (RAPD) and amplified fragment length polymorphisms (AFLP) were evaluated as methods to assess intraspecific variation. Sequences of rDNA ITS regions obtained after amplification with primer ITS1 and ITS4 did not show any difference as well as random amplified polymorphic DNAs, as previously reported by others. On the contrary, preliminary AFLP fingerprints produced by restriction with EcoRI/McoI enzymes, the use of preamplification primer mix II (Invitrogen), and 2-base extension McoI primers with 2-base extension EcoRI primer in the selective amplification disclosed the presence of polymorphic bands between the Greek and the Italian isolates.

TOXIN PRODUCTION OF FUNGI ASSOCIATED WITH ACTINIDIA WOOD DISEASE. A. Haegi, M. Valsassori, G. Di Giambattista, L. Riccioni. Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: luca.riccionitispave.it

Actinidia delicosa var. delicosa cv. Hayward and Actinidia chinesis var. chinesis ‘Hort16A’ (yellow fleshed kiwifruit) are affected by wood decay causing die-back and death of the plants. Several fungal species have been isolated from wood lesions. In order to study factors involved in pathogenicity, in this work some fungi were analyzed for phytotoxin production: Cadophora luteo-olivacea, Cadophora sp., Phialophora mustea, Pheacromenium aleophilum, Cryptosporiopsis actinidiae, and Paraconiothyrium spp. Culture filtrates were infiltrated on leaves of actinidia species and non-host plants with an Hagborg device. Several isolates induced necrotic symptoms and among them isolates of Cadophora luteo-olivacea and Cadophora sp. gave the strongest response. The toxic activity was stable after treatment at 121°C for 15 min. and absent after dialysis with 10.000 MWCO membranes, suggesting that the Cadophora toxin has low molecular weight. Extraction and/or precipitation were attempted with several organic solvents to determine both solubility and polarity, and to set up a purification procedure. TLC analysis was also done with different solvent systems to determine the nature of the compound.

BEHAVIOUR OF OLIVE CULTIVARS TOWARDS THE KNOT DISEASE CAUSED BY PSEUDOMONAS SAVASTANOI. N. Iannotta, M.E. Noce, S. Scalerio and V. Vizzarri. C.R.A., Istituto Sperimentale per l’Olivicoltura, Contrada Li Rocchi-Vermicelli, 87036 Rende (CS), Italy. E-mail: nino.iannotta@enteca.it

To control olive knot disease, preventive measures are essential, among which the use of low susceptibility cultivars seems to be promising. Previous investigations have shown a large variability in severity of Pseudomonas savastanoi (Smith) attacks. This study was carried out in spring 2005 by evaluating responses to the pathogen of 262 Italian and 43 foreign cultivars, grown in a germplasm conservation field, under the same environmental and growing conditions. The response to the pathogen was assessed by rating the quantity of tubercles present on branches, arranged in classes of infection. During the survey adverse meteorological events took place, including low temperatures that influenced the onset of the disease. A different behaviour of olive cultivars to P. savastanoi attacks was observed in relation to their susceptibility to the pathogen. Among Italian cultivars, 61% showed an infection rate ranging from 0 to 20%, 22.5% from 20 to 40%, 11.1% from 40 to 60%, 5% from 60 to 80% and 0.4% from 80 and 100%. Eighty-six cultivars showed no symptoms and none had infection rate of 100% was observed, thus indicating that the Italian germplasm comprises genetic resources that may be exploited for olive knot disease prevention. Among foreign cultivars, 41.9% showed a percentage of infection between 0 and 20%, 23.3% between 20 and 40%; 4.7% between 40 and 60%; 4.7% 60 and 80%; 23.3% between 80 and 100%. cvs Drobnica, Korneiaki and Vasilakada showed an infection rate of 100%, whereas five cultivars (Bardhi, Chetani, Hjotiblanka, Lucques, Salonenque) were apparently not infected. This different susceptibility, evident under the same agro-environmental conditions, confirms a different response to the pathogen in relation to the plant/parasite ratio and appears strictly dependent on tolerance to low temperatures.
STUDY OF BIOLOGICAL AND TECHNOLOGICAL PARAMETERS OF STEAM APPLICATIONS FOR THE CONTROL OF SOIL-BORNE FUNGAL PATHOGENS. Infantino A.1, D. Luison1, R. Tomason2, C. Cedrola2 and G. Colorio2. 1CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. 2C.R.A., Istituto Sperimentale per la Meccanizzazione, Via della Pascolare, 16, 00016 Monterotondo Scalo, Rome, Italy. E-mail: alessandro.infantino@enteca.it

The use of steaming has become one of the most attractive alternatives to methyl bromide for the control of soil-borne pathogenic fungi since the ban of this chemical for soil fumigations. The recent development of a self-propelled machine for direct steaming represents a further option for soil disinfection in greenhouses and nurseries. Steam is applied to the soil via implements (blades) that move and enter the soil horizontally to an established depth. The machine is endowed with the “BIOFLASH” patented system, using steam in association with an inorganic non-toxic substance (calcium oxide, potassium hydroxide, etc.) that causes an exothermic reaction when mixed with soil. The efficiency of two modified steam diffusing blades and the use of CaO in controlling soil-borne fungi was evaluated in several experiments, done with a small-scale experimental apparatus that reproduces machine field treatments. Verticalismium dahliae and Pyrenochaeta lycopersici were chosen as target pathogens. Fungi were grown on autoclaved pearl millet and placed into polyethylene bags (20 micron mesh opening), buried into the soil at two depths (5 and 15 cm) immediately after steaming. Heating profiles were recorded by the use of temperature probes inserted into the soil at multiple levels. The survival of the inoculum was evaluated in the laboratory by plating 100 kernels of each bag in selective media for each fungal species. The efficiency of the treatment was inversely correlated with the distance of the vapour source, being very high in the horizontal plane through which the steam-diffusing organ passes. No differences in survival rate were observed between the two distribution implements and the use of CaO. These results are consistent with those obtained with the machine operating in open field tests.

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WORK-RELATED ACTION OF DURUM WHEAT KERNELS IN ORGANIC FARMING IN ITALY. A. Infantino1, A. Santori1, G. Conca1, A. Belocchi2, G. Aureli2, G. Avantaggiato3, A. Visconti2 and F. Quaranta2. 1CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. 2C.R.A., Istituto Sperimentale per la Meccanizzazione, Via della Pascolare, 16, 00016 Monterotondo Scalo, Rome, Italy. 3Istituto di Scienze e Tecnologie Alimentari e Microbiologiche, Sezione di Nutrizione Umana, Università di Milano, Via Salernitana 20, 20133 Milano, Italy. 2Istituto di Scienze e Tecnologie Alimentari e Microbiologiche, Sezione di Nutrizione Umana, Università di Milano, Via Salernitana 20, 20133 Milano, Italy. E-mail: marcello.iriti@unimi.it

The in vivo and in vitro effects of UV-C exposure on arresting and selecting durum wheat kernels. Kernels were analyzed by the deep-freezing blotter test, while Deoxynivalenol (DON) levels were measured with a 0.039 watt·m−2s for 2 h) of currant tomato (Lycopersicon pimpinellifolium), a species native to Peru and Ecuador, were assayed. DON deposits, dead cells and DNA damage were localized, 12/24 h after irradiation, mainly in perivascular parenchyma of the 1st and 2nd order leaf veins, and before the appearance of visible symptoms, which occurred 48 h after irradiation. Cell death index was of 43.5% in exposed leaf tissues, 24 h after treatment. In currant tomato protoplasts, the percentage of viable cells dropped 1 h after UV-C irradiation from 97.42% to 43.38%. Afterwards, protoplast viability progressively decreased to 40.16% at 2h, to 38.31% at 4 h, and to 36.46% at 6 h from exposure. The genotoxic impact of UV-C radiation on protoplasts was assessed with single cell gel electrophoresis (SCGE, or comet assay). UV-C treatment greatly enhanced DNA migration, with a 75.37% of DNA in tail versus 7.88% in the case of untreated nuclei. Oxidative stress by H2O2, used as positive control, induced a similar damage on non-irradiated protoplasts, with a 71.59% of DNA in tail, whereas oxidative stress imposed to UV-C irradiated protoplasts slightly increased DNA damage (85.13%). According to these results, SCGE performed in protoplast systems could be an alternative to extraction of nuclei directly from leaf tissues, with the great advantage of harvesting a much larger number of nuclei, and in turn, of ameliorating the statistical significance of the assay.

STUDIES ON THE BIOLOGICAL AND MOLECULAR DIAGNOSIS OF APRICOT LATENT VIRUS. S. Jarra1,2, D. Boscia1, A. Myrta1, A. Minafra1, G. Loconsole1 and V. Savino1. 1Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari e CNR, Istituto di Virologia Vegetale, Sezione di Bari, Via Amendola, 165/A, 70126 Bari, Italy. 2Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy. E-mail: d.boscia@ba.ivv.cnr.it

Apricot latent virus (ApLV), a member of the genus Foveavirus, infects latently apricot in nature. The viral genome has been par-
tially sequenced, and primers for its detection by RT-PCR have been designed. However, very little is known on the biological properties of the virus and the spatial-temporal variation of its titer in infected trees. Objects of this study were ApLV tracking in the host plant throughout the year and collection of data on its incidence in stone fruit accessions from several geographical areas. Contrary to the well-established timing for optimal detection of major stone fruit viruses, the results of this study clearly showed the hottest months of the year to be the optimal period for ApLV detection. No significant differences were observed when different parts of the canopy and type of tissues (leaves or bark) were comparatively analyzed. Testing of 550 samples, mainly apricots, coming from different Mediterranean countries revealed a significant incidence (34%) of ApLV in 137 apricot accessions from Palestine, and its occasional presence in the other countries. Starting from July 2006 an infected apricot tree of cv. Tyrinthos from an Italian collection exhibited symptoms (chlorotic blotching, spotting, and deformation of the leaves) similar to those shown by a tree of cv. Haward artificially inoculated with the Palestinian isolate ApLV-APR 47. This is the first observation ever reported of symptoms associated with ApLV infection in apricot.

TRANSFORMATION OF PHAEOMONIELLA CHLAMYDOSPORIUM WITH THE SYNTHETIC GREEN FLUORESCENT PROTEIN (sGFP) GENE. L. Landi, G. Romanazzi. Department of Environmental and Crop Science, Marche Polytechnic University, Via Brecce Bianche 10, 60131 Ancona, Italy. E-mail: g.romanazzi@unitpm.it

Petri disease is one of the most serious fungal diseases of grapevine, since it can reduce the economic life of a vineyard. The disease is caused by the fungus Phaeomoniella chlamydospora and commonly occurs on vines aged from 1 to 5 years, which can suffer a sudden dieback. To track the fungal spread in ecological studies, a non-invasive marker, the synthetic gene for green fluorescent protein (sGFP) of the jellyfish Aequorea victoria, was introduced into the wild-type strain (CBS 229.95) of P. chlamydospora. The pCT74 plasmid (kindly provided by L. Guffetti, Oregon State University, OR, USA) was used for transformation via a PEG-mediated system contained both an sGFP (Blue-sGFP-TRY) variant of the gfp gene under the control of the ToxA gene promoter of Pyrenophora tritici-repentis, and the hph gene for hygromycin resistance. Colony regeneration was on PDA containing 0.8 M sucrose, and transformants were selected on PDA supplemented with 100 mg/l hygromycin B. P. chlamydospora transformants were analyzed by PCR using gfp and hph specific primers, and the expression of sGFP was observed by fluorescence microscopy. sGFP transformation of P. chlamydospora will be useful for investigating the ecology of the pathogen in grapevine tissues and in the environment.

MOLECULAR CHARACTERISATION OF ALLEXIVIRUSES FROM GARLIC IN ITALY. C. Lanzoni1, C. Ratti1, M. Turina2, A. Pisi1, P. Tedeschi1 and C. Rubies Autonell1. 1Dipartimento di Scienze e Tecnologie Agroambientali e Patologia Vegetale, Viale Panin 40, 40127 Bologna, Italy. 2Istituto di Virologia Vegetale, Strada delle Cacce 73, 10135 Torino, Italy. 1Dipartimento Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17, 44100 Ferrara, Italy. E-mail: crubies@agrisci.unibo.it

Most garlic plants grown in the world are infected with various virus species, including members of the genera Potyvirus, Carlavirus, Fijivirus and Allexivirus, which give rise to mosaic or streak symptoms and reduction of the size of bulbs and cloves. During 2005 and 2006, garlic plants were collected from different provinces of Italy (Ferrara, Rovigo, l’Aquila, Imperia). ssRNAs were extracted from leaf tissues and tested by RT-PCR. Eight samples resulted positive when tested using allexivirus specific primers. Nucleotide sequence analysis of PCR products (554 bp) showed the presence of Garlic virus C (GarV-C) and Garlic virus D (GarV-D) infection. To confirm these results, specific primers for coat protein (CP) amplification of GarV-C and GarV-D were designed and complete nucleotide sequence (780 and 753 bp respectively) determined by RT-PCR. The nucleotide sequences identity among our isolates varies between 56.0 and 99.1%. On the basis of deduced CP amino acid sequences, high homology (97.2 to 99.6%) was observed between a Korean isolate of GarV-D (accession number AF519572) and seven Italian isolates from Ferrara and Rovigo. One sample, collected from Imperia, showed the highest homology (95.4%) with a Japanese isolate of GarV-C (accession number AB010302). Low amino acid sequence identity, ranging from 49.6 to 77.6%, was observed among Italian isolates and others allexivirus species. These results provide experimental evidence of the occurrence of GarV-C and GarV-D in the garlic accessions tested. To our knowledge this is the first molecular characterization of these allexivirus species in Italy.

MORPHOLOGICAL AND PATHOGENIC CHARACTERIZATION OF BOTRYOSPHAERIA ISOLATES ASSOCIATED WITH DECLINING CORK-OAK TREES IN SARDINIA. B.T. Linaldeddu, A. Franceschini and F. Marras. Dipartimento di Protezione delle Plante, Sezione di Patologia vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: afrun@uniss.it

Cork-oak (Quercus suber L.) is an evergreen oak species typical of the western Mediterranean basin. In the last two decades, a progressive decline affects cork-oak forests in this region. Various fungi have been found associated with declining trees, some of them belonging to the genus Botryosphaeria. Several species of Botryosphaeria and related anamorphs are known as causal agents of cork dieback diseases of different oak species, while others are reported as opportunistic pathogens or saprophytes. Therefore, a careful identification and pathogenic characterization of these species are essential in order to understand their effective role in the aetiology of cork-oak decline. In this paper, we report the results of surveys carried out for characterizing the species of Botryosphaeria associated with branch cankers of declining trees in a cork-oak forest in North Sardinia (Italy). Symptomatic branches from 132 declining trees were analysed. Three Botryosphaeria species were isolated and identified on the basis of their morphological characteristics, namely: B. corticola Phillips, Alves et Luque, B. obtusa (Schwein.) Shoe-maker, and B. parva Pennycook et Samuels. B. corticola was the most frequently detected species. Pathogenicity trials on cork-oak seedlings showed the high virulence of B. corticola and B. parva, which induced the collapse of stem cortical tissues associated with dark-brown discolorations and vascular necroses. By contrast, necrotic lesions caused by B. obtusa were small and limited around the inoculation site. These results suggest that B. corticola and B. parva, but specially B. corticola, are more directly involved in the tree decay.
ACTIVITY OF NATURAL AMENDMENTS AND COMPOSTS AGAINST SOIL-BORNE PLANT PATHOGENS. G. Lima, F. De Curtis, D. Vitullo and D. Piedimonte. Dipartimento di Scienze Animali, Vegetali e dell’Ambiente, Sezione di Patologia Vegetale, Via De Sanctis, 86100 Campobasso, Italy. E-mail: lima@unisinol.it

In recent years considerable research efforts were made to find more eco-compatible and safer plant diseases control methods for organic and integrated agriculture systems. Among the new alternative approaches, natural amendments and composted biomass are being intensively studied not only for beneficial agro-nomic effects, but also for their potential suppressive effects against different plant pathogens. In the last years, our investigations were aimed at evaluating the inhibitory activity of some natural-derived substrates and composts for their suppressive activity against important fungal pathogens of crops. Assays in vitro on solid and liquid media amended with water extracts (10% w/v) of natural substrates showed high inhibition rates of mycelial radial growth as well as the reduction of conidial germination and elongation of some fungal pathogens (Fusarium spp., Botrytis cinerea, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum and Verticillium dahliae). In experiments conducted under controlled conditions on pot-grown plants of olive, eggplant and tomato (plants artificially inoculated or soil contaminated with fungal inoculum), some compost was tested at different concentrations in mixtures with standard substrates. A concentration of 15% (w/v) of an experimental compost reduced the inoculum density (microsclerotia) of V. dahliae in the rhizosphere of olive and eggplants and the severity of S. rolfsii and S. sclerotiorum symptoms on tomato and lettuce plants, respectively. Biochemical and microbiological investigations are in progress to characterize mechanisms of suppressiveness and to optimize the potential application of amendments and composts as alternative disease control means.

AFLP ANALYSIS OF A POPULATION OF EUROPEAN PHYTOPHTHORA PSEUDOSYRINGAE ISOLATES. R. Linzer1, D. Rizzo2, M. Garbelotto1, A. Chimento3 and S.O. Cacciola3. 1Department of Environmental Science, Policy and Management, Ecosystem Science, University of California, Berkeley, 151 Hilgard Hall, Berkeley, CA 94720 USA. 2Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616 USA. 3Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche Agrarie e Zootecniche, Università degli Studi di Palermo, Viale delle Scienze 2, 90128 Palermo, Italy. E-mail: caccio la@unipa.it

Phytophthora pseudosyringae, a recently described homothallic species, has been reported in central Italy to be responsible for trunk cankers and root rot of beech and oak, respectively. In California and Oregon forests this species has an overlapping geographic distribution with both P. nemorosa, a closely related species occurring only in the USA, and P. ramorum, the causal agent of sudden oak death. Amplified fragment length polymorphism (AFLP) analysis was used to compare the genetic structure of a population of 19 European P. pseudosyringae isolates, including 16 Italian isolates from beech, with the genetic structure of a population of 29 American P. pseudosyringae isolates encompassing most of the host and geographic ranges of this species in western USA. Although 17 distinct AFLP genotypes were found among the 48 isolates examined, 9 from Europe and 8 from USA, both populations showed a very limited genetic variability. Jaccard similarity coefficient (Sj) values for the whole dataset ranged from 0.9 to 1.0. The Sj distribution indicated fewer clonal individuals and higher genetic dissimilarity in the European population. No AFLP genotype was shared between the two populations. However, the distance phylogram did not show a distribution of the isolates in separate clades corresponding to the geographic origin. Most American isolates, in fact, were in a sub-clade nested within the European isolates. The low level of genetic diversity of P. pseudosyringae populations, which is consistent with the hypothesis of recent introduction of this pathogen to both America and Europe, could be the result of homothallism.

BACTERIAL SOFT ROT OF CAULIFLOWER CAUSED BY PSEUDOMONAS FLUORESCENS IN APULIA. P. Lo Cantore, N. S. Iacobellis. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via dell’Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: iacobellis@unibas.it

Recent investigations in some commercial cauliflower fields of Apulia (Southern Italy) have shown severe alterations of inflorescences, almost ready to be harvested, which turned brown and rotted. Crop loss could be serious because rotting developed also post harvest. The alterations interested either the whole inflorescences or, occasionally, only a few florets. Longitudinal sections of the symptomatic inflorescences or the single floret showed that the internal tissues were discolored and rotten. Bacterial isolation from altered tissues yielded almost pure cultures with the LOPAT profile (++++) of the group Vb of fluorescent pseudomonads. Only from one of ten specimens a fluorescent pseudomonad with the LOPAT profile (++++) of the group IVb was isolated. Pathogenicity assays by spraying or dipping detached cauliflower florets with 10⁶ UFC/ml bacterial suspensions showed that the above pseudomonads reproduced the natural symptoms (i.e. brown discoloration and rotting of florets) observed in the field. The same symptoms were obtained in internal tissues when the above suspensions were injected into the floret peduncle. The nutritional analysis of virulent isolates with the computerised system Biolog lead to the identification of representative isolates with the LOPAT profile (+++++) as strains of Pseudomonas fluorescens and of the isolate with the LOPAT profile (++++) as a strain of Pseudomonas spp.

IMPROVEMENT OF OLIVE LEAF YELLOWING-ASSOCIATED VIRUS DETECTION USING MOLECULAR HYBRIDISATION. G. Loconsole1, M. Saponari2, G. Mondelli3, V. Savino1 and G.P. Martelli1. 1Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126, Bari, Italy. 2Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: giuliana.loconsole@agr.uniba.it

Olive leaf yellowing-associated virus (OLYaV), an unassigned virus to the family Closteroviridae, is one of the most widespread viruses in commercial olive groves of several Mediterranean countries. Its absence is required for the production of propagating material of both “certified” and CAC (Conformitas Agricola Communitatis) categories. OLYaV detection is mainly based on RT-PCR made on total RNA (TNA) plant extracts, which, however, are biased by the inconsistency of the results when a large number of samples is processed. Virus detection by dot-blot hybridisation using RNA was unsuccessful, unless dsRNA extracts were used as template. Because preparation of dsRNA extracts is time- and reagent-consuming, thus inappropriate for routine OLYaV detection, a mini-dsRNA procedure was developed, whereby 100 mg of tissue from different sources (cortical scrap-
ings, herbaceous shoots, in vitro-grown plantlets) were ground in extraction buffer, treated with phenol-chloroform and dsRNAs recovered by microchromatography on CF-11 cellulose. All steps were done in 1.5 ml centrifuge tubes. dsRNAs samples were spotted on membranes for dot-blot hybridisation. To validate this protocol a total of 90 olive samples were comparatively processed by RT-PCR and mini-dsRNA hybridisation: 42 samples reacted positively with both techniques, and 16 additional samples were positive by dot blot hybridisation only, totalling 58 (64%). Thus, hybridisation of purified mini-dsRNAs is more sensitive than RT-PCR and represents a further approach to a fully reliable OLYA detection. The small amount of olive tissue required for mini-dsRNA allows the processing of a large number of samples and the possibility of detecting the virus when a limited quantity of tissue is available (i.e. in vitro-grown plantlets).

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EFFECTIVENESS OF SOLARIZATION TO CONTROL THE MAIN TELLURIC FUNGAL PATHOGENS OF CUCURBITS. F. Lops, A. Carlucci, G. Guifreda, M. Mucci, L. Colatruglio, C. Lazzizera, M.L. Raimondo, V. Gentile and S. Frisullo. Dipartimento di Scienze Agro-ambientale, Chimica e Difesa Vegetale (DISACD), Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy. E-mail: f.lops@unifg.it

Two year experiments in the open field were carried to control the main telluric pathogens of cucurbits, i.e. Acremonium cucurbitacearum, Plectosporium tabacinum, Rhizopycnis vagum, Fusarium spp., by solarization using three plastic films, EVA, Polydak and Mater-B (bio-degradable films made of maize starch). In the years that followed the treatment, the residual effect or solarization by itself or combined with the use of Trichoderma viride was also evaluated. The fungal populations of the soil and on the roots of vegetable marrow and “carosello” (Citulhus lanatus) were monitored before and after solarization for several years. Whereas solarization with EVA and Polydak films reduced significantly disease incidence on vegetable marrow and “carosello” and in the following year, on melon and “carosello”, solarization with Mater-B film increased disease gravity in all crops. In the year following the treatment a significant positive effect was observed when T. viride was added to solarized soil.

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STEAM AND EXOTHERMIC REACTIONS FOR THE CONTROL OF RHIZOCTONIA SOLANI ON RADISH AND ROCKET BY USE OF A SELF-PROPELLED SOIL-STEAMING MACHINE. A. Luvisi and E. Triolo. Dipartimento Coltivazione e Difesa delle Specie Legnose “G. Scaramuzza”, Sezione Patologia Vegetale, University of Pisa, Via del Borgoetto 80, 56124 Pisa, Italy. E-mail: ettrolo@agr.unipi.it

The search for non-chemical methods to control soil-borne pathogens was recently intensified in view of the forthcoming phasing out of methyl bromide. We tested pre-planting treatments done with a self-propelled soil-steaming machine designed for the release of steam after incorporation in soil of a substance that causes an exothermic reaction. Two trials were conducted during 2005 in a glasshouse in Florence for assessing the effectiveness of treatments with steam and calcium oxide against Rhizoctonia solani on radish (Raphanus sativus) and rocket (Eruca sativa). Thermal values reached in the soil exposed to steam changed when energy-releasing exothermic reaction chemicals were applied to soil before treatment, thereby generating a temperature peak. Plots were prepared in sandy soils, and random layouts were used, performing two consecutive trials during summer. Mycelial disks from 7-day-old cultures of an isolate of R. solani were mixed with soil and used for artificial soil inoculation. The equipment used was able to spread and incorporate CaO (1000 kg ha⁻¹) into the soil, then to inject the steam. Radish and rocket were sown two days after treatment. The combination of steam and CaO was more efficient in reducing the incidence of R. solani attacks than the steam alone. Combining steam with CaO a disease reduction was obtained of 92.2% on rocket and 90.5% on radish, while disease reduction given by steam alone was of 82.0% on rocket and 80.2% on radish. Our conclusion is, therefore, that the combination of steam and products inducing an exothermic reaction can be included in integrated pest management programs.

PROTEOMIC ANALYSIS OF THE COMPLEX THREE-WAY INTERACTION OCCURRING BETWEEN PLANT, FUNGAL PATHOGENS AND TRICHODERMA ATROVIRIDE STRAIN P1. R. Marra 1, P. Ambrosino 1, V. Carbone 2, F. Vinale 3, S.L. Woo 4, M. Ruocco 5, R. Ciliento 5, S. Lanzuise 6, S. Ferraioli 7, I. Soriente 1, D. Turra 8, V. Fogliano 9, F. Scala 1, 3 and M. Lorito 1, 3. 1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. 2Centro di Speziometria di Massa Proteomica e Biomolecolare, Istituto di Scienze dell’Alimentazione del CNR, Avellino, Italy. 3Istituto per la Protezione delle Piante (IPP), CNR, 80053 Portici (NA), Italy. 4Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. E-mail: lorito@unina.it

We have used a proteomic approach to analyse the molecular factors involved in the complex three-way interaction occurring between plant (bean), fungal pathogens (Botrytis cinerea or Rhizoctonia solani) and the antagonist Trichoderma atroviride strain P1. Interactions were studied on water agar plates, where the fungal mycelia, grown separately on cellophane, were layered over plant tissues (roots or leaves). Proteomes were obtained individually from each partner and separated by 2-D gel electrophoresis. Differential proteins were partially identified using tryptic digestion, MALDI-TOF mass spectrometry and in silico analysis. In the plant proteome, specific pathogenesis-related proteins and other disease-related factors (i.e. potential resistance genes) were found to accumulate in the presence of the antagonist alone or together with the fungal pathogens. In the B. cinerea proteome, an overall view of the proteins involved in the infection process was obtained and many of the proteins could be identified (i.e. cyclophilins, superoxide dismutase, cutinase, etc.). Many differential proteins obtained from the proteome of T. atroviride showed interesting homologies with fungal hydrophobins, ABC transporters, stress-related proteins (heat shock proteins), chitin synthase, Hx1 proteins, etc. For example, a fungal hydrophobin accumulated by T. atroviride strain P1 in the presence of R. solani showed homology to the NIP1 avirulence factor. This finding supports the hypothesis that the molecular cross-talk between the plant and Trichoderma involves a gene-for-gene (avr-R) interaction.
DIFERENT CHITINASES EXPRESSION IN SUGAR BEET SEEDLINGS INDUCED BY CHEMICAL OR BIOLOGICAL CONTROL AGENTS. S. Marinello, P.L. Burzi, S. Galletti, E. Sala, C. Cerato. Consiglio per la Sperimentazione e Ricerca in Agricoltura (CRA), Istituto Sperimentale per le Culture Industriali, Via Corticella, 133, 40129 Bologna, Italy

Chitinases are hydrolytic enzymes (EC 3.2.1.14) that catalyse the hydrolysis of chitin, a polymer of N-acetyl-D-glucosamine. Plant chitinases belong to relatively large gene families subdivided in classes that suggest class-specific functions. Chitinases are also classified as pathogenesis-related proteins, which are mainly activated in a salicylic acid dependent manner leading to the expression of systemic acquired resistance (SAR). Trichoderma spp. are known as effective biocontrol agents towards a number of pathogens, due to different mechanisms of action, such as competition, antibiosis, mycoparasitism, induction of defence response and other adjuvant mechanisms which can positively affect plant response to diseases, such as growth promotion. The ability of the Trichoderma koningii isolate B12 to induce chitinase expression in sugar beet seedlings was tested in comparison with the chemical inducer acibenzolar-S-methyl (ASM, Bion, Syngenta). Sugar beet seeds (cv. Aaron) were pre-treated by washing under tap water for 3 h at 20-25°C. After drying at room temperature, 270 seeds were treated with B12 cultural sterile filtrate (4.5 ml) or ASM (4.5 ml, 60 mg L-1 a.i.). Water treated seeds served as control. Three, 4 and 5 days after treatment RNA was extracted from germinating seeds using the total RNA purification kit (Macherey-Nagel). The expression of target genes was studied by RT-PCR using degenerate primers for chitinases I and IV based (Macherey-Nagel). The expression of target genes was studied by RT-PCR using degenerate primers for chitinases I and IV based on the cDNA sequence alignment of the protein regions from different dicots. In the seeds treated by B12 and ASM the activation of transcription of chitinases already started at the first sampling time and was maintained over time, while in the untreated control the expression was only observed 4 and 5 days after treatment. In any case the level of induction of chitinase by T. koningii was lower than ASM.

MOLECULAR CHARACTERIZATION OF RESISTANCE BREAKING STRAINS OF TOMATO SPOTTED VILT VIRUS CARRYING A DELETION IN THE NSs CODING REGION. P. Margaria and M. Turina. Istituto di Virologia Vegetale, Sezione di Torino, CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: m.turina@ivv.cnr.it

The most effective and environmentally sound control strategy for Tomato spotted wilt virus (TSWV, Tospovirus, Bunyaviridae) relies on the availability of resistant cultivars. All known resistant pepper cultivars possess a single dominant resistance gene, Tsw, which is easily overcome by TSWV resistance-breaking (RB) strains. We provide evidence that the NSs protein of TSWV-RB strains on pepper is the avirulence factor, with reassembling experiments and full-length sequencing of a series of S genome segments cloned from a collection of TSWV-RB strains. Two of the RB strains carried a deletion in the NSs coding region. Strain p170RB was obtained from a Sicilian virus isolate through successive inoculations on resistant Capsicum chinensis PI152225 in a controlled environment. Strain p202 is an RB isolate from an infected plant from a field in the province of Palermo (Southern Italy). Host range and symptoms of these RB strains were compared with the wild type field isolates from Sicily (p170), which produces an NSs protein of 467 aa, while the S segment of p202 showed a two-base deletion at position 1171 producing a protein of 399 aa. p170RB showed a deletion of 25 nt at position 684, encoding a putative protein of 238 aa. The in vivo presence of the deleted forms of NSs protein was checked by Western blotting. Moreover, wild type NSs protein and the two deleted forms were transiently expressed through agroinfiltration in Nicotiana benthamiana C16 leaves in order to assess their silencing-suppressor potential.

ANTIMICROBIAL ACTIVITY OF CARVACROL AND THYMOL AGAINST ERWINIA AMYLOVORA AND ASPERGILLUS OCHRACEUS. P. Minardi, S. Mucini, U. Mazzucchi. Dipartimento di Morfofisiologia Veterinaria e Produzione Animali, DI-MORFIPA, Università degli Studi di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy. Dipartimento di Scienze e Tecnologie Agroambientali, DiSTa, Università degli Studi di Bologna, Viale Fanin 42, 40127 Bologna, Italy. E-mail: paola.minardi@unibo.it

Essential oils are naturally occurring terpenic mixtures with well-known antimicrobial properties against some important plant pathogens. Plant secondary metabolites have potential in medical procedures and relevant applications in the cosmetic, food and pharmaceutical industries. The growing interest in the substitution of "traditional food preservatives" with "natural preservatives" fostered research on plant sources, with the aim of identifying new active compounds. On the other hand, prevention of fungal growth is becoming increasingly interesting in connection with the prevention of mycotoxin contamination in several foods, and these natural compounds represent possible alternatives to most food additives currently in use. Along these lines, the main objective of this study was to determine the inhibitory effects of carvacrol and thymol, the main components of the essential oils of oregano and thyme, on the growth of the causal agent of fire blight disease, Erwinia amylovora, and of the ochratoxigenic Aspergillus ochraceus that causes food spoilage. The antimicrobial activity was investigated by the disc diffusion and dilution (agar and broth) methods. The effects on spore germination and radial growth rates were also evaluated. Carvacrol and thymol showed antimicrobial activity against the micro-organisms tested. The effect of composition of media, exposure time, and other environmental factors (i.e. temperature, pH) on the antimicrobial activity was investigated. Finally, the present study evaluates whether these antimicrobial compounds may be suitable candidates for enhancing the protection of pear plants damaged by hail towards E. amylovora, and inhibiting ochratoxin production by A. ochraceus.

TRIALS FOR THE CONTROL OF SOIL-BORNE DISEASE AGENTS WITH ECOCOMPATIBLE PHYSICAL METHOD AND BIOCONTROL AGENTS. A. Mirotti, M. Sportelli and S. Gennari. C.D.F. s.r.l., Via Amendola 40, 48022 Lugo (RA), Italy. E-mail: cdf@cdfugo.it

Alternatives to methyl bromide with the same degree of activity are difficult to find. Thus, studies were carried out in economically and ecologically compatible way in the Emilia Romagna region (Northern Italy) in which methods with low environmental impact, such as solarization and soil steaming coupled with an exothermic reaction substance, were integrated with the use of biocontrol agents such as Trichoderma spp., the non pathogenic Fusarium oxysporum strain FO47, and mycorrhizae. Three trials were conducted in horticultural farms of the province of Bologna for controlling: (i) Verticillium spp. and Rhizoctonia spp. in egg-plant; (ii) Fusarium spp. in tomato; (iii) Monosporascus cannonballus in melon. Soil steaming with the additions of CaO was suc-
succesful for plants grew better and had a longer productive cycle as compared with untreated controls. Positive results were also obtained with mycorrhizal applications, which augmented plant vigour notwithstanding the fact that a slight decrease of activity was caused by steaming. The efficacy of Trichoderma and F. oxysporum strain FO47 on plant growth and control of telluric diseases was also confirmed. The results obtained emphasize the importance of an integrated strategy for controlling diseases induced by soil-borne agents.

Work supported by the Emilia Romagna region (L.R. 28/98).

NITRIC OXIDE RELEASING COMPOUNDS SYSTEMATICALLY PROTECT ARABIDOPSIS THALIANA PLANTS FROM TOBACCO NECROSIS VIRUS. C. Moretti, S. Silvestri and R. Buonanario. Dipartimento di Scienze Agrarie e Ambientali, Sezione Arboricoltura e Protezione delle Pianta e Patologia Vegetale, Università degli Studi di Perugia, Via Borgo XX Giugno, 74, 06121 Perugia, Italy. E-mail: chialuceru.moretti@unipg.it

The ability of nitric oxide releasing compounds, sodium nitroprusside (SNP) and S-nitrosocysteine (CysNO), to induce resistance in Arabidopsis thaliana plants (Col-0) to Tobacco necrosis virus (TNV) was evaluated. SNP and CysNO were able to systemically and significantly (P<0.05) protect Arabidopsis plants against TNV, with protection levels of 63 and 71%, respectively. A protection level of 66% was obtained with the SAR inducer acibenzolar-S-methyl as comparison. To verify whether the protection exerted by SNP and CysNO is mediated by salicylic acid, transgenic A. thaliana plants expressing the nahG gene were used. Reduced protection levels (38%) were observed in NahG Arabidopsis plants treated with SNP or CysNO in comparison with wild-type plants. It is noteworthy that untreated NahG plants are less susceptible to TNV infection than wild-type plants. Our results suggest that the protection induced by the nitric oxide releasing compounds used are in part mediated by salicylic acid.

VEGETATIVE COMPATIBILITY IN CERCOSPORA BETICA. M. Moretti, M. Saracchi, G. Farina. Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. E-mail: maddalena.moretti@unimi.it

Complementary nitrate non utilizing (nit) mutants have been widely used to test vegetative compatibility between strains with many phytopathogenic fungi. In this study different kinds of media were assayed to generate nit mutants in Cercospora betica. For most of the strains potato dextrose agar amended with 3% potassium chloride produced most nit mutants, which became visible in about 3 weeks at 26°C as fast growing strains at the margin of the colonies. Generally, most of the nit mutants were stronger cercosporin producers, compared to their respective wild types, when grown on a medium containing nitrate as the only nitrogen source. The characterization of the physiological phenotype (nit 1, 3 or M) was done based on their growth on nitrogen source different from nitrate. About 70% of the mutants were nit 1 or 3, while only 30% were nit M. One nit 1 (when possible) and one nit M mutant were obtained for each strain and they were paired on a medium containing only nitrate as a nitrogen source to test their vegetative compatibility, which was readily seen by the heterokaryon formation. Preliminary results on a limited number of strains showed that this technique provides a useful tool to test population structure in C. betica.

INCREASE OF TRANSCRIPT LEVEL OF PAL AND CHS GENES IN OLIVE PLANTLETS FOLLOWING VERTICILLIUM DAHLiae INFECTION AND ACIBENZOLAR-S-METHYL APPLICATION. F. Nigro, I. Pentimone, M. Mammella, A. Ligorio, A. Ippolito and M.G. Salerno. Dipartimento di Protezione delle Pianta e Microbiologia Applicata,Università degli Studi di Bari, Via Amendola 163/A, 70126 Bari, Italy. E-mail: nigro@agr.uniba.it

Resistance can be activated in plants by a series of substances. Among these acibenzolar-S-methyl (ASM) was found to behave as an effective resistance elicitor, facilitating protection under field conditions from a wide range of pathogens in several crops. Phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) are key enzymes in the phenylpropanoid/flavonoid pathway, leading to production of compounds, including lignin, flavonoids and phytalexins, extremely important in the plant defence strategy. A molecular method for monitoring PAL and CHS gene expression, in olive plantlets (cv. Leccino), in response to Verticillium dahliae infection and ASM application, was developed. Specific primers, amplifying a 233 and 188 bp amplicons, suitable for the development of a real time PCR detection method, were designed to quantify the relative transcript level of PAL (Olea europaea accession number AY738639) and CHS (O. europaea accession number AF384049) genes by quantitative RT-PCR. A portion of O. europaea beta-actin (act1) mRNA gene (accession number AF545369) was used as housekeeping gene to normalise results. Results indicated that pathogen induces a low and transient increase of PAL transcripts as compared with ASM. Moreover, olive plantlets showed a good tolerance toward different amounts (0,25÷2,5 g/l a.i.) of ASM. The study also indicated that PAL expression was rapidly and strongly induced by low levels of ASM (0,25 g l-1 a.i.) associated with a notable increase of enzyme activity. CHS expression, instead, was enhanced later than PAL, especially by higher ASM concentration. In either case enzyme activity was induced systemically.

ANTAGONISTIC ACTIVITY OF EPIPHYTIC GRAPEVINE YEASTS ON OCTROXOTOGENIC ASPERGILLUS CARBONARIUS ISOLATES. C. Oliveri1, M. Fardella2, V. Grimaldi1 and A. Catara1. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. 2Parco Scientifico e Tecnologico della Sicilia s.c.p.a., Viale Lancia, 95030 Catania, Italy. E-mail: c.oliveri@unicat.it

Fifteen epiphytic grapevine yeasts (Candida magnoliae, C. vini, Debaryomyces Hansenii, Hanseniaspora vinacea, Kloeckera apiculata, Metschnikowia pulcherrima, Pichia anomala, P. guilliermondii, P. membranifaciens, Saccharomyces cerevisiae, S. bayanus, Schisosaccharomyces pombe, Torulospora delbreuki, Zygosaccharomyces bailii) were screened in vitro against A. carbonarius in order to evaluate their relative ability to reduce fungal growth and OTA production. Green coffee beans were used as a natural substrate for the assays. In each treatment, 100 berries of autoclaved green coffee were dipped in yeast suspensions (107 cfu/ml), drained, and placed inside Petri dishes (20 beans/plate). Berries were incubated with 10 ml spore suspension (106 conidia/ml) of two different strains of A. carbonarius (MUCL 1981 and MV/CP/CT 325). After 10, 20 and 30 days of incubation, all the preincubated yeast strains gave a strong inhibition effect, up to 100%, on mycelial growth and, especially, on conidia production of A. carbonarius. The strong inhibitory effect of yeast preincubation against A. carbonarius on coffee was confirmed as estimated following the development of rot and the OTA content on apple fruits. OTA determination in berry extracts by ELISA showed the different effi-
cacy of preinoculations. Results suggest that some yeast applications reduce the percentage of infected coffee beans and the sporulation of A. carbonarius.

CHARACTERIZATION OF OTA-PRODUCING ASPERGILLUS SPP. STRAINS ISOLATED FROM SICILIAN VINEYARDS. C. Oliveri1, M. Fardella2, V. Grimaldi3 and V. Catar2, 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. 2Parco Scientifico e Tecnologico della Sicilia s.c.p.a., Viale Lancia, 95030 Catania, Italy. E-mail: c.oliveri@unict.it

Mycological monitoring of air, soil and grapes was done in eight Sicilian vineyards during 2005 to investigate the occurrence and the population density of the most frequently occurring species of Aspergillus, at pea berry stage, early veraison and harvesting. Aspergillus strains were present in all sampled vineyards (soil and air) starting from the pea berry stage. Their incidence was higher at early veraison and harvesting time. A. niger and A. carbonarius were the main fungi isolated from grapes. A total of 393 isolates belonging to Aspergillus section Nigri were isolated. According to macro- and micro-morphological characteristics, the isolates were mainly referred to A. niger aggregate and A. carbonarius. The ability of 133 isolates to produce OTA was tested by using the AgraQuant Ochratoxin Assay (Romer Labs, Singapore) on extracts of 10 day-old colonies grown on YES medium at 27°C in the dark. A. carbonarius isolates were strong OTA producers with 68% of them producing high concentration of OTA (>40 p.p.b.). Most of the A. niger isolates were very weak OTA producers and only few of them produced high amounts of the toxin (>40 p.p.b.). Only one strain of A. japonicus (MPVCT150) was able to produce OTA (>40 p.p.b.). PCR assays using six pairs of species-specific primers allowed to discriminate species within the black Aspergillii group (43 A. niger, 22 A. carbonarius, 3 A. japonicus). Genomic profiles generated by RAPD and AFLP allowed strain differentiation at the intraspecific level.

HIGH INCIDENCE OF TOMATO MOSAIC AND CUCUMBER MOSAIC VIRUSES IN OPEN FIELD CROPS OF TOMATO ECOTYPES IN CAMPANIA. M. Parisi1, G. Parrella2, M. Zac- cardelli3 and I. Giordano1. 1C.R.A., Istituto Sperimentale per le Colture Industriali, Sezione di Battipaglia, 84091 Battipaglia, Italy. 2Parco Scientifico e Tecnologico della Sicilia s.c.p.a., Viale Lancia, 95030 Catania, Italy. E-mail: c.oliveri@unict.it

In 2005, a virus-like disease was observed in commercial globe artichoke fields in Campania (Southern Italy). Symptoms consisted of stunting and discoloration of the leaf blade (yellow blotches, rings and line patterns). Disease incidence was around 50-60% with peaks of almost 100% in some areas and was present again in 2006, with a similar appearance and incidence. To identify the possible causal agents, symptomatic plant samples were collected from different locations (Scafati, Pompei). Observations of leaf-dip preparations by electron microscopy showed the presence of a filamentous virus ca. 730-740 nm long and of an isometric virus ca. 30 nm in diameter. Ultra-thin sections of Nicotiana benthamiana tissues inoculated with leaf extracts from symptomatic artichokes showed typical inclusion bodies caused by potyviruses. Based on the aspect of cylindrical inclusions (CI) the potyvirus was assigned to the subdivision-I, which induces type-2 CIs, consisting of pinwheels and more or less straight laminate aggregates. The filamentous virus was clearly decorated by an antiserum to Artichoke latent virus (ArLV). Isometric virus particles were readily recognized in ultrathin sections, especially when they were arranged to form circular arrays identical to those typically induced by Broad bean wilt virus (BBWB). Attempts to decorate the isometric particles with antisera against the subgroup B of nepoviruses failed, but no serological identification of BBWV was made. The outbreaks of bright yellow mosaic of globe artichoke observed in Campania, caused by mixed infections of ArLV and putative BBWV resembled those reported earlier from Apulia, a neighbouring region.

HIGH INCIDENCE OF BRIGHTEST MOSAIC ASSOCIATED WITH MIXED INFECTIONS OF ARTICHOKE LATENT VIRUS AND AN ISOMETRIC VIRUS IN GLOBE ARTICHOKE IN CAMPANIA. G. Parrella1, A. De Stradis2 and C. Vovlas3, 1Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. 2Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. 3Istituto di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: parrella@ipp.cnr.it

In 2005, a virus-like disease was observed in commercial globe artichoke fields in Campania (Southern Italy). Symptoms consisted of stunting and discoloration of the leaf blade (yellow blotches, rings and line patterns). Disease incidence was around 50-60% with peaks of almost 100% in some areas and was present again in 2006, with a similar appearance and incidence. To identify the possible causal agents, symptomatic plant samples were collected from different locations (Scafati, Pompei). Observations of leaf-dip preparations by electron microscopy showed the presence of a filamentous virus ca. 730-740 nm long and of an isometric virus ca. 30 nm in diameter. Ultra-thin sections of Nicotiana benthamiana tissues inoculated with leaf extracts from symptomatic artichokes showed typical inclusion bodies caused by potyviruses. Based on the aspect of cylindrical inclusions (CI) the potyvirus was assigned to the subdivision-I, which induces type-2 CIs, consisting of pinwheels and more or less straight laminate aggregates. The filamentous virus was clearly decorated by an antiserum to Artichoke latent virus (ArLV). Isometric virus particles were readily recognized in ultrathin sections, especially when they were arranged to form circular arrays identical to those typically induced by Broad bean wilt virus (BBWB). Attempts to decorate the isometric particles with antisera against the subgroup B of nepoviruses failed, but no serological identification of BBWV was made. The outbreaks of bright yellow mosaic of globe artichoke observed in Campania, caused by mixed infections of ArLV and putative BBWV resembled those reported earlier from Apulia, a neighbouring region.

BIOLOGICAL AND GENETIC VARIABILITY OF PARIETARIA MOTTLE VIRUS ISOLATES. G. Parrella Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. E-mail: parrella@ipp.cnr.it

Parietaria mottle virus (PMoV, genus Ilarvirus, family Bromoviridae) has emerged as pathogen of solanaceous crops in the last 10 years in some Mediterranean countries (Spain, France, Italy and Greece). PMoV was also recently detected in Mirabilis jalapa plants in Italy. Its origins and mechanisms for evolution and spread represent important traits for understanding plant virus emergence. Biological and molecular characterization of a collection of nine PMoV isolates differing in natural host and geographic origin was done. Clustering analysis of the coat protein nucleotide sequences discriminated two major groups of isolates.
These two groups appear to correlate with their pathogenicity and year of collection rather than with the geographic origin of isolates. Within the larger cluster, comprising seven isolates, two major subsets of isolates, with some divergent ones, were defined showing no correlation with geographic origin or biological properties. The smaller cluster grouped only two PMoV isolates both from Italy and collected in the same region. These isolate were also the oldest in terms of year of collection. These data support the idea of a rapid molecular evolution of PMoV isolates related with the widening of their natural and experimental host ranges.

**MOLECULAR CHARACTERIZATION OF ALFALFA MOSAIC VIRUS ISOLATES FROM OFFICIAL PLANTS. G. Parrella1, M.G. Bellardi2, 1Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. 2Dipartimento di Scienze e Tecnologie Agroambientali, Patologia Vegetale, Alma Mater Studiorum, Università di Bologna, Via G. Fanin 42, 40127 Bologna, Italy. E-mail: parrella@ipp.cnr.it**

Seven new Alfalfa mosaic virus (AMV) isolates from official plants have been characterized at the molecular level. Isolates were from symptomatic plants of *Lavandula hybrida* (three varieties of lavandins), *Lavandula x alardii* (one hybrid) and single plants of *Lippia citriodora*, *Ocimum basilicum* and *Symphytum tuberosum*. In all instances symptoms consisted in a more or less extended yellow bright mosaic of the leaves. The nucleotide and amino acid sequences of the coat protein (CP) and movement protein (MP) were determined. Phylogenetic analysis of CP and MP sequences showed that all isolates belonged to AMV subgroup I except for that from the “Sumiens” variety of *L. hybrida*, which was related to subgroup II. Data were confirmed by RFLP of amplicons from CP and MP. Since subgroup II isolates have been detected so far only in France, the finding of one of its member in Italy could be explained with the accidental introduction of infected propagating materials form France where the cv. “Sumiens” was constituted several years ago. The possibility of spreading of subgroup II isolates in Italy and related consequences are discussed.

**DETECTION OF 16SrI-B SUBGROUP PHYTOPLASMAS ASSOCIATED WITH VIRESCENCE, PHYLLODY AND SEVERE GROWTH ABNORMALITIES IN RANUNCULUS IN CAMPANIA.. A.M. Picco1, 1Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. 2Dipartimento di Scienze e Tecnologie Agroambientali, Patologia Vegetale, Alma Mater Studiorum, Via Università 133, 80055 Portici, Italy. E-mail: apicco@et.unipv.it**

In the late 2005 ranunculus hybrids with phytoplasma-like symptoms were observed in two protected cultivations in Campagna (Southern Italy). Before flowering, symptoms included severe growth abnormalities, shoot proliferation and decline; later on virescence and phyllody of the flowers was observed. PCR using phytoplasma-specific primer pairs confirmed the presence of phytoplasma DNA in the infected plants. Sequence analysis of the 16S rDNA amplicon obtained with P1/P7 primer pair (ca. 1800 bp), revealed high homology (99%) with phytoplasma DNAs in the database. Based on phylogenetic and RFLP analysis of this amplicon carried out with *TruI*, *Tsp509I*, *SpI*, *RsaI* and *HhaI* the ranunculus phytoplasma was identified as an isolate of *Candidatus Phytoplasma asteris* (aster yellow), subgroup 16SrI-B. Three leafhopper species (*Homoptera, Cicadellidae*) captured with yellow sticky traps were associated with ranunculus cultivations: *Empoasca decipiens* (Paoi), *Eupteryx zelleri* (Kirschbaum) and *Zygrina* sp. Batches of ten individuals per species were tested for the presence of phytoplasmas by nested-PCR/RFLP analyses using primers P1/P7 followed by primers P1/B6. Only *E. decipiens* samples were positive and RFLP analysis of the F1/B6 PCR products (ca. 1700 bp), revealed the association to subgroup 16SrI-B phytoplasma in two batches, whereas in another batch, a restriction profile referable to the 16SrI group was obtained. Although *E. decipiens* already been reported to be associated to phytoplasmas, its the role as vector must be proven. This is the first report of 16SrI-B subgroups phytoplasmas in ranunculus in Italy.

**NOTES ON MYCOTIC INFECTIONS WHICH PRODUCE CHANGES IN THE INSIDE OF BARRELS AND CORK OF CAPS. L. Petruzzi, S. Bertulino and C. Ciccarone. Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Via Napoli 25, 71100 Foggia, Italy.**

Oak wood has traditionally been used in the construction of barriques for the aging of wine because of both its mechanical properties and its extractable compounds, which induce changes in wine composition and flavor. The importance of mycotic wood infections is a matter discussed as the main theme of several studies made to establish how these agents can affect the qualitative characteristics of the barrels. Our work mainly consisted in isolating fungi from barriques, in order to study their metabolic characters, and to identify possible synergies among their activities. Different species belonging to the genera *Aspergillus*, *Cladosporium*, *Penicillium*, *Ulocladium*, *Acremonium*, *Chaetomium*, and *Trichoderma* were taken into consideration. We also present evidence of some biochemical partnership between fungi and cellular environmental conditions in which different species are present. The second part of this work takes into consideration the presence of mycelia in the cork of caps. The profile of the wood is modified by fungal enzymatic activity and also its organoleptic properties may be spoiled. Several samples analyzed for the presence of mycotic infections of the cork proved to contain different species to the genera *Chaetomium*, *Aspergillus* and *Penicillium*.

**WARM - A NEW MODEL FOR PADDY RICE SIMULATIONS: PRODUCTIVE ASPECTS AND ADVERSITIES. A.M. Picco1, M. Rodolli1, R. Confalonieri2, M. Acutis3, L. Mariani3, M. Donatelli4. 1Istituto di Ecologia del Territorio e Ambienti Terrestri, Sezione di Micologia, Università degli Studi di Pavia, Via S. Epifanio 14, 27100 Pavia, Italy. 2Joint Research Centre of the European Commission, AGRIFISH Unit, MARS-STAT Sector, 21020 Ispra, Italy. 3Dipartimento di Produzione Vegetale, Sezione di Agronomia, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. 4Consiglio per la Ricerca e Sperimentazione in Agricoltura, Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, 40128 Bologna, Italy. E-mail: apicco@et.unipv.it**

WARM is a simulation model for flooded rice designed by an open research group in order to manage all the main aspects influencing crop production (e.g. crop development and growth, pests, weeds, management). WARM is a model with innovative features aiming at reproducing the peculiar conditions of mid-latitude paddy fields (e.g. floodwater effect on vertical thermal profile). In order to facilitate its use, the model has amodular struc-
DIVERSITY OF PSEUDOMONAS AND PANTOEA SPECIES ISOLATED FROM DISCOLOURED RICE GRAINS IN ITALY.

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Rice grain discolouration is responsible for reduced quality and yield losses. Disease etiology is controversial. Although Cochiobolus miyabeanus (Ito et Kurib.) Dresch. ex Dast. was isolated from discoloured rice, no correlation was found between brown spot and grain discolouration incidence, whilst fungicides effective on brown spot epidemics did not reduce grain discolouration incidence. Recently, the isolation of Pseudomonas and Pantoea species from discoloured grains strengthened the hypothesis of a bacterial etiology of the disease. The aim of this study was to assess the genetic diversity and pathogenicity of the Pseudomonas and Pantoea populations. Analysis of 16S rDNA sequences within the Pseudomonas population showed that it was subdivided in two major clusters: one composed of P. psychrotolerans strains and the other comprising P. putida, P. straminea and P. fulva. Within the P. fulva strains, Amplified Ribosomal DNA Restriction Analysis (ARDRA) and BOX fingerprinting revealed 5 restriction types and 13 BOX-haplotypes, whereas P. psychrotolerans group was characterized by a low genotypic diversity. Within the Pantoea population, we distinguished three major phenotypic groups, matching the BOX-fingerprints clusters. Two strains, identified as P. ananatis were sprayed on the rice cvs Gladio and Loto. They induced the typical symptoms of palesa browning, but not grain discolouration. However, two strains of Pantoea sp. and two of P. fulva, inoculated on the cultivar Selenio at the booting stage, determined grain discolouration incidence significantly higher than the control.

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VALIDATION OF PROTOCOLS FOR THE QUANTITATIVE DETECTION OF THE OCHRATOXIGENIC FUNGUS ASPERGILLUS CARBONARIUS IN GRAPE AND MUSTS. S. Polastro1, R.M. De Miccolis Angelini1, M. Romito1 and F. Faretra1.
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Risk assessment in vineyards of ochratoxin A (OTA) contamination is crucial for wine and/or other grape-derived beverages production, due to the maximum tolerable level of OTA established in 2.0 mg kg-1 by the Reg. (CE) N. 123/2005 of 26.1.2005. Aspergillus carbonarius (Bainier) Thom. is the main responsible of OTA contamination in musts and wines. The development of a semi-selective medium and a molecular protocol based on real-time Scorpion PCR PCR accelerated and improved the detection and quantification of the fungus in grapes and musts. A. carbonarius was quantified in 583 must samples obtained from bunches collected in 60 vineyards of 9 grapevines cultivars from verasion to vintage time. The results obtained by comparing the two methodologies yielded a correlation coefficient as high as 0.8. About 4 ng of A. carbonarius DNA (corresponding to about 0.8·103 conidia) were detectable with the molecular protocol. A. carbonarius confirmed to be rare at verasion (detected at low density in about 5% of samples), and the number of its propagules increased till harvest time, when it was detected in more than 70% of the samples with a number of propagules even higher than 106 conidia ml-1 of must. OTA was quantified through...
HPLC analysis in the same samples, resulting close to the maximum tolerable level in more than 20% of samples. The positive relationship between the number of propagules of A. carbonarius and the level of OTA contamination was confirmed by a correlation index as high as 0.8. Generally, a contamination of A. carbonarius of at least 10³ conidia ml⁻¹ of must was associated to a high risk of exceeding the maximum tolerable level of OTA.

**MONITORING OF FUNGAL POPULATION IN BREAD AND DURUM WHEAT WITH DIFFERENT SUSCEPTIBILITY TO SBCMV.** A. Prodi¹, A. Pisi¹, C. Rubies Autonell¹, S. Toni¹, S. Sandalo¹, C. Lanzoni¹, D. Rovito² and P. Nipoti¹, ¹Dipartimento di Scienze e Tecnologie Agroambientali, Alma Mater Studiorum, Università di Bologna, Viale G. Fanin 40, Bologna Italy. ²Ente Nazionale Sementi Elette, Via Cà Nova Zampieri 37, 37057 S. Giovanni Lapopoto (VR), Italy. E-mail: paola nipoti@unibo.it

Soilborne cereal mosaic virus (SBCMV) is quite widespread in Italy especially in the central and northern regions. It may cause yield reduction by 50% to 70% on the most susceptible Italy especially in the central and northern regions. It may cause correlation between fungal colonization and susceptibility to molecularly identified. The preliminary data show that there is no fungi causing Fusarium crown rot and FBH. Fusaria species were detected (CFU/g) in the rhizosphere soil. All plant parts at the Italy), were tested for fungal infections Mycoflora presence was infected soil in an experimental field near Bologna (Northern with different susceptibility to SBCMV , grown in a SBCMV infected plants. Bread wheat cvs Artico, Trofeo, Agadir and Isengrain, and (BYDV)-infected plants than in BYDV-free plants. Bread wheat cvs Artico, Trofeo, Agadir and Isengrain, and durum wheat cultivars Neudur, Provenzal, Claudio and Orobel, plants. Bread wheat cvs Artico, Trofeo, Agadir and Isengrain, and durum wheat cultivars Neudur, Provenzal, Claudio and Orobel, with different susceptibility to SBCMV, grown in a SBCMV infected soil in an experimental field near Bologna (Northern Italy), were tested for fungal infections Mycoflora presence was detected (CFU/g) in the rhizosphere soil. All plant parts at the end of their life cycle and seeds were analyzed for the presence of fungi causing Fusarium crown rot and FBH. Fusaria species were molecularly identified. The preliminary data show that there is no correlation between fungal colonization and susceptibility to SBCMV of the withering plants. Further studies are in progress.

**CHARACTERIZATION OF A QUORUM SENSING CONTROLLED PROMOTER: THE PHENAZINE OPERON PROMOTER IN PSEUDOMONAS AUREOFACIENS STRAIN 30-84.** G. Puopolo¹, A. Raio², A. Zoina¹ and L.S. Pierson³, ¹AR-BOPAVE, Università di Napoli “Federico II”, Via Università, 100, 80055 Portici (NA), Italy. ²Istituto per la Protezione delle Piante, CNR, Via Università, 133, 80055 Portici (NA), Italy. ³Dipartimento di protezione delle Piante, Università degli Studi di Napoli, Via Università, 133, 80055 Portici (NA), Italy. E-mail: zoina@unina.it

Phenazines are broad host range antibiotics produced by several bacterial species, including Pseudomonas and Streptomyces. These compounds play a key role in the antagonistic activity of the biocontrol strain P. aureofaciens 30-84 towards Gaecumano-myces graminis var. tritici, the causal agent of wheat take all disease. Strain 30-84 contains a seven-gene operon (phzXYPABCDF) responsible for the biosynthesis of three phenazines. Expression of the phenazine operon is dependent on the PhzR/PhzI quorum sensing system. The gene phzI encodes an AHL synthase responsible for the production of the signal molecule hexanoyl homoserine lactone (HHL). Once HHL reaches a threshold density it is believed to bind and activate PhzR, the transcriptional factor encoded by phzR, resulting in PhzR binding to the phenazine operon promoter and consecutive phenazine production. However, little is known regarding the functional phenazine promoter in strain 30-84. To further investigate the phenazine promoter, the region between phzX and phzR was characterized. Subclones of the phenazine promoter were cloned upstream of promotorless reporter genes in order to identify the smallest and most active region sufficient for phenazine transcription. Results showed that a 20 bp inverted repeat region (the phz box) proximal to the potential -35 position of the phenazine promoter is required for transcription. This work represents a first insight in the characterization of the quorum sensing controlled promoter of the phenazine operon in strain 30-84. Understanding phenazine gene regulation may be useful for the improvement of biological control in organic agriculture.

**FUSARIUM VERTICILLIOIDES AND RELATED SPECIES OF THE GIBBERELLA FUJIKUROI COMPLEX SECRETE AN EN-DOPOLYGALACTURONASE NOT INHIBITED BY MONOCOT PGIPs.** A. Raiola¹, L. Sella¹, C. Castiglioni³, V. Balmas², A. Tomassini² and F. Favaron², ¹Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Via dell’Università 16, 35020 Legnaro, Italy. ²Dipartimento di Protezione delle Piante, Università degli Studi di Sassari, Viale E. De Nicola, 07100 Sassari, Italy. E-mail: francesco.favaron@unipd.it

Fusarium verticillioides, like other fungal species of the Gibberella fujikuroi complex, is a toxigenic and pathogenic species able to produce disease on important monocotyledonous crops. In order to understand the involvement of the endopolygalacturonase (endo-PG) of F. verticillioides in the infection process we have purified the endo-PG produced in culture by four different isolates of this fungus and cloned the corresponding genes. The purified endo-PGs showed similar biochemical and molecular properties. In particular, all endo-PGs were not inhibited by polygalacturonase-inhibiting proteins (PGIPs) extracted from monocot host plants (like asparagus or maize) and were partially inhibited by bean PGIP, but only at extraordinarily high dose. Deduced amino acid sequences of endo-PGs from F. verticillioides were similar to those obtained from endo-PGs of seven other species of the G. fujikuroi complex (F. sacchari, F. fujikuroi, F. proliferatum, F. subglutinans, F. rhaponticum, F. nivagmi, F. circinatum). These endo-PGs, like that of F. verticillioides, were not inhibited by monocot PGIP whilst were inhibited, at various degrees, by the bean PGIP. Thus, since many species of the G. fujikuroi complex are pathogens of monocot plants, their endo-PG appears particularly suitable to overcome the hindrance of the host PGIPs. The multiple alignments of the endo-PG sequences allow identifying the few amino acid substitutions of the different fungal enzymes that are likely involved in the binding with the bean PGIP.

**UTILIZING ANTAGONISTIC FUNGI AGAINST DIPLODIA CORTICOLA.** A. Ruscelli, M. Nigro, G. Campanile and N. Luisi, Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari, Via G. Amendola, 163/A, 70126 Bari, Italy. E-mail: Luisin@agr.uniba.it

Diplodia corticola is the causal agent of cankers, vascular necrosis and dieback on various oak species, although many epidemiological and pathogenetic aspects of its relation with the host still need to be clarified. Several studies have proved its pathogenicity, but scant information is available on the biological control of D. corticola. The aim of this research was to assess the activity of cultural filtrates of some fungal isolates of Trichoderma viride Pers.Fr., Epicoccum nigrum Link., Fusarium tricinctum (Corda) Sacc., Al-
ternaria alternata" Fries von Keissler and Sclerotinia sclerotiorum (Libert) de Bary to inhibit the growth of D. corticola, as well as of Cytospora (teleomorph: Valsa sp.) genera which are also present in epigeous declining oak tissues. For the antagonistic tests, 10 ml of PDA fluid were uniformly poured into Petri dishes. Before solidification, 5 ml of warm cultural filtrate of the antagonists were added. After solidification, a 0.5-cm disk cut from the margin of 2-day-old cultures of D. corticola was placed on each of the plates. Data were analyzed statistically by analysis of variance (ANOVA) and Duncan's test. The activity of antagonistic cultural filtrates on the growth of D. corticola was low. In particular, F. tricinctum filtrates inhibited the growth of D. corticola by 16.6%; in A. alternata, Cytospora sp., S. sclerotiorum and E. nigrum the activity was statistically similar. By contrast, T. viride filtrates did not inhibit the growth of D. corticola isolates. ANOVA of the average colony-growth data between the antagonistic isolates confirmed that the differences were highly significant (P = 0.01), whereas the differences between T. viride isolates were not.

SOIL-BORNE DISEASES OF MELON IN CENTRAL ITALY: FUNGAL AGENTS AND RELATIONSHIP WITH CULTURAL PRACTICES AND ENVIRONMENTAL CONDITIONS. R. Reda, M.P. Aleandri, P. Magro and G. Chilosi. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: chilosi@unittus.it

Melon is one of the major source of revenue in the out-season horticultural growing area of the province of Viterbo (Central Italy). Soil-borne diseases of fungal origin have become in the last few years the yield-limiting factors of this crop in a number of farms, probably due to changes in cultural practices in greenhouse cultivations. As a consequence of the rapid spread of these melon diseases in the area, their constant monitoring is strictly necessary along with epidemiological studies and setting up of effective means of prevention and control. The aim of this work was to survey melon fields for the occurrence of soil-borne diseases under field and greenhouse conditions. The fungal pathogens most frequently isolated from infected plants were Fusarium oxysporum f.sp. melonis (FOM) race 1,2, Sclerotinia sclerotiorum, Pythium sp., Fusarium sambucinum, Verticillium dahliae, Rbizoctonia solani, Dildynema bryoniae and Plectosporium tabacinum. In particular, the recent rapid spread of collapse caused by Monosporascus cannonballus represented, together with FOM, the main cause of losses. Aboveground symptoms become evident just prior to harvest resulting in rapid wilt of plants, premature fruit ripening and low sugar content. The frequency of isolation of the different fungal pathogens varied with seasonal variation, type of melon genotype and cultural practices, such as irrigation regime and sanitation practices.

INFECTION OF PEAR LEAVES BY PLEOSPORA ALLII ASCOSPORES. V. Rossi, E. Pattori and S. Giosué. Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it

Pleospora allii (teleomorph of Stemphylium vesicatorium) is the causal agent of brown spot of pear, but its role in the life-cycle of the pathogen is not clear. The fungus forms pseudothecia in the pear leaf litter and in the weeds present in the orchards. Ascospores mature between February and May, but very few or none of them are air-borne in June, when disease symptoms first appear. The ability of ascospores to elicit infection to pear has not been sufficiently demonstrated, and no information is available on the environmental conditions favouring ascospore germination and infection. In the present work these ecological aspects of P. allii ascospores were investigated under environment-controlled conditions. Dynamics of germination were observed between 0.5 and 48 h of incubation at different temperatures (5 to 35°C), in water or in dry conditions, with relative humidity (RH) between 100 and 67%. Highest germination occurred after 48 hours at 21-23°C in water; few ascospores germinated below 15°C and 30-35°C. At 100% RH germination decreased by about one third and no germination was observed below 80%. Ascospores were inoculated on the leaves of three pear varieties (‘Abate Fétel’, ‘Conference’, and ‘William’ in decreasing order of susceptibility under field conditions), incubated at different temperatures (5 to 35°C), 100% RH, and observed daily for the appearance of necrotic spots. Ascospores caused heavier infection on ‘Abate Fétel’ than ‘Conference’, while sporadic symptoms were observed on ‘William’. Highest disease incidence occurred at 25°C. Mathematical relationships between ascospore germination or infection and environmental variables were developed and used to elaborate a risk index for ascosporic infections.

CLONING AND FUNCTIONAL SCREENING OF FOUR SOLANUM TUBEROSUM ABC TRANSPORTER GENES IN RESPONSE TO INFECTION BY PHYTOPHTHORA INFESTANS AND ABIOTIC STRESSES. M. Ruocco1, P. Ambrosino2, G. del Sorbo2, M. Lorito1,2 and F. Scala1,2. Istituto per la Protezione delle Piante (IPP), CNR, 80055 Portici (NA), Italy. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. E-mail: scala@unina.it

Pleiotropic drug resistance (PDR) proteins are a group of the ABC (ATP Binding Cassette) superfamily transporters found only in fungi and plants. Although ABC transporters in fungi are known to be involved in transport of xenobiotics and virulence factors, their physiological functions in plants are still largely unexplored. We cloned four genes encoding ABC transporters from potato, named StABC1 (Solanum tuberosum ABC transporter 1), StABC2, StABC3 and StABC4, respectively. Gene expression studies were made by RT-PCR both in plant organs and in vitro-grown potato cell suspensions after exposure to different compounds. Results indicated that StABC2 is strongly up-regulated after treatment with plant defence compounds (terpenoid antifungal sclareol), pathogen-derived materials (partially purified Botrytis cinerea cell wall preparations), herbicides (2,4-dichlorophenoxyacetic acid and sulometuron methyl), and salts (NaCl). The possible involvement of the four ABC transporters in plant-pathogen interactions has been studied in the potato-Phytophthora infestans system. Using real-time quantitative PCR, StABC2 expression was found to be up-regulated in the whole leaf following infection with the fungus. Our data suggest that the StABC2 protein is involved in the plant response to both biotic and abiotic stresses.

A PUTATIVE NEW MEMBER OF THE FAMILY FLEXIVIRIDAE ISOLATED FROM PHLOMIS FRUCTICOSA IN EPIRUS. P. Saldarelli, D. Boscia, A. De Stradis, C. Vovlas. 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. E-mail: vovlas@agr.uniba.it

Phlomis fructcosa (Lamiaceae) is a native perennial Mediter-
MYCOLOGICAL STUDIES ON DECLINING OAKS IN LOMBARDY. M. Saracchi and F. Rocchi. Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. E-mail: marco.saracchi@unimi.it

Oak decline was first described in Lombardy in 2003, following which a research programme was initiated for assessing disease incidence and distribution in different protected areas. The presence of various kinds of symptoms was recorded on 150 oaks representing various degrees of disease expression. Samples were collected from symptomatic twigs, branches, trunks, and collars and analyzed in order to isolate and characterize the potentially pathogenic fungal population. Different types of symptoms were found such as bark necrosis, cortical cankers, discoloured and rotted wood, necrotic xylematic tissues, dieback on twigs and branches, and detached bark near collars. Fungal fruiting bodies and mycelial felts were also collected. Two to 10 samples representative of each kind of symptom were collected from 17 trees growing in different woods and processed within 24 h of collection. More than 250 fungal strains were isolated and phenotypically characterized. They were related to 21 different genera: some of them were typical saprophytic fungi whereas others, i.e. Armillaria, Fusarium, Graphium, Phoma, and Phomopsis were pathogens. 132 strains did not differentiate reproductive structures in vitro and were identified on the basis of ITS nucleotide sequences. The majority of these micelia sterili were related to Botryosphaeria dothidea, B. stevensii, B. parva, and Amphiporthe humuli. Others were identified as Colpoma quercinum, Phoma caea, and Valsa sordida, while a few isolates remain still unidentified.

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RESULTS OF A FOUR-YEAR INVESTIGATION ON OAK DECLINE IN LOMBARDY. M. Saracchi, F. Rocchi, M. Lantica, M. Vailati, V. Parco and E. Caronni. Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. 2Studio Associato EcoLogico, Via Larnarmora 12, 20013 Magenta (MI), Italy. 3Parco Lombardo della Valle del Ticino, Via Isonzo 1, Pontevioccio di Magenta, 20013 Magenta (MI), Italy. E-mail: marco.saracchi@unimi.it

During last 10 years sporadic cases of dead or declining oaks (Quercus robur) were observed in the Ticino River Regional Park. In 2003 a project initiated to verify the distribution and development of oak decline in this protected area 20,000 ha wide, was later extended to other three regional parks. Over 120 different localities were inspected, recording twice a year the frequency of epicormic shoots, withered branches and diebacks, crown thinning, microphyllia, leaf yellowing, and mucilaginous bleeding oozing from bark. The four-year data confirmed the presence of oak decline in Lombardy, especially along the Ticino river, where the disease incidence was particularly high. In the other parks more than 45% of the plots comprise symptomatic plants. The periodic observations showed the spread of the problem towards healthy localities and a generally increasing intensity in previously monitored areas. Only in a few cases a reduction of symptom intensity was observed. Since the first oak decline observations, the disease was shown to be rapidly increasing. Disease-progress curves, based on symptoms, seem to reach the maximum intensity in 4-5 years, afterwards oaks begin show a slow decline characterized by a reduction of new vegetation, increased frequency of diebacks and epicormic shoots resulting in the death of some trees. In some woods decline progress was faster so that, in 2 or 3 years, most of the crowns consisted only of the epicormic shoots

ACTIVITY OF SELECTED PHYTOALEXINS IN POSTHARVEST CONTROL OF PENICILLIUM EXPANSUM AND PATULIN PRODUCTION IN APPLES. S.M. Sanzani, A. De Girolamo, L. Schena, M. Solfrizzo and A. Ippolito. 1Dipartimento di Protezione delle Piane e Microbiologia Applicata, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. 2Istituto di Scienze delle Produzioni Alimentari, CNR, Via G. Amendola 122/O, 70126 Bari, Italy. E-mail: simona.sanzani@agr.uniba.it

Penicillium expansum, the agent of blue mold, causes considerable postharvest losses of apples and other pome fruits. This pathogen is of health significance, since it produces patulin, a mycotoxin with genotoxic properties, known to cause immunological, neurological and gastrointestinal toxic effects in animal models. The use of natural compounds has been proposed as a possible alternative to chemical fungicides. In the present study phytoalexins, such as dihydroquercetin, umbellifereone, ferulic acid, esculetin, scopoletin, scoparone, 6-methoxymelline and resveratrol, were tested to control P. expansum and patulin production in in vivo and in vitro trials. In in vivo trials surface sterilized apples (variety Golden Delicious) were wounded, treated with each phytoalexin, inoculated with a toxigenic strain of P. expansum and incubated at 16°C in the dark. Lesion diameters were recorded every 2 days. After 14 days, tissue samples from untreated and treated lesions were collected and analyzed by HPLC for their patulin content. In in vitro trials fungal growth and patulin production were evaluated on agarized apple juice medium. Among the tested phytoalexins, umbellifereone and dihydroquercetin were the most effective in reducing fungal infections in apples, although no reduction in patulin accumulation was observed. In particular, umbellifereone reduced lesion diameters by 100%, 68% and 38% and dihydroquercetin by 97%, 71% and 27% after 6, 10 and 14 days of incubation, respectively. When tested in vitro, umbellifereone and dihydroquercetin reduced patulin accumulation by 53% and 48%, respectively, although no inhibition of fungal growth was observed. These results suggest that the control activity of umbellifereone and dihydroquercetin towards P. expansum may be related to their ability to enhance defence responses in the host. Further studies are in progress to confirm this hypothesis or to identify other possible mechanisms of action.

ranecan plant widespread in the Greek Ionian Islands and Epirus. Plants with mosaics and yellow flecking of the leaves and occasional bushy growth were observed in several Epirus locations. Mechanical transmission from leaf tissues of symptomatic plants was successful in Nicotiana occidentalis, which reacted with a systemic chlorotic mottling, ringspots, and necrosis. Electron microscopy observations of ultrathin sections, leaf dips, and partially purified preparations from infected N. occidentalis leaves, showed the presence of flexuous virus-like particles with distinct cross banding, 800-850 nm in length, resembling those of members of some genera of the family Flexiviridae. To check this hypothesis, a set of degenerate primers designed on conserved domains of the RNA dependent RNA polymerase (RdRp) of flexiviridos, capable of recognizing several members of this family, were tested on total RNA extracts from infected N. occidentalis plants. RT-PCR using this set of primers amplified a DNA fragment with the expected of ca 270 nt, which was cloned and sequenced. This DNA fragment encoded a protein with homology to members of the family Flexiviridae. In particular, BLAST analysis showed that the virus under study had the highest homology with Banana virus X (BVX), an unassigned member to the family Flexiviridae. Further studies are in progress for a thorough characterization of the virus in question to which the tentative name of Phomis mottle virus (PhMV) is provisionally assigned.
and many trees died. Symptom development was monitored in 150 representative oaks in particular. Also these results are discussed.

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**NEW STRATEGY TO IDENTIFY MICROSATELLITES IN THE GENUS PHYTOPHTHORA.** L. Schena1 and D.E.L. Cooke2, 1Department of Plant Protection and Applied Microbiology, Via Amendola 165/A, 70126 Bari, Italy. 2Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA Scotland, UK. E-mail: leonardo.schena@agr.uniba.it

Microsatellites or simple sequence repeats (SSR) are short DNA sequences repeated many times occurring frequently in the genomes of all eukaryotes. Their high mutation rate and distribution across the genome makes microsatellites powerful molecular tools to evaluate inter- and intra-specific variability and to study evolution and population genetics of a number of organisms including *Phytophthora* species. However, the identification of microsatellites for each species is challenging and markedly limits their application. The aim of the present study was to develop a strategy to identify microsatellite loci across a number of *Phytophthora* species by comparing genome sequence data from the projects of *P. infestans* (http://www.sanger.ac.uk/Projects/Fungi/) and *P. sojae* and *P. ramorum* (http://genome.jgi-psf.org/). Experiments were conducted using 16 *Phytophthora* species selected to represent the breadth of diversity in the genus. Available unigenes from the genome projects were screened for SSR's and compared by BLAST analyses to identify conserved regions flanking SSR's in at least 2 of the 3 species. Twenty-four target regions containing SSR's were selected and amplified using 79 different primers designed against such conserved flanking regions. A total of 283 single PCR bands where amplified and for 182 of these, direct sequencing was possible using the same primers utilised for amplifications. Among these sequences 109 contained SSR’s of at least 4 tandem repeats but in many other cases the SSR motif was absent. Key studies on the inter- and intra-specific variation of the identified SSR’s remain. However, the research has already yielded a vast dataset and laid a foundation for future research.

**FLUORESCENT IN SITU HYBRIDIZATION ON GRAPEVINE AND NICOTIANA TISSUES INFECTED BY GRAPEVINE VIRUSES A AND B.** A. Sciancalepore, A. De Stradis, A. Minafra, A. Campanale and G.P. Martelli. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi e Istituto di Virologia Vegetale, CNR, Sezione di Bari, Via Amendola, 165/A, 70126 Bari, Italy. E-mail: a.minafra@ba.ivv.cnr.it

In grapevine, multiple natural infections by vitiviruses are relatively common since mealybug transmission and grafting favours the spread of both *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB). The two viruses share a relatively high sequence similarity (average of 50% nucleotide identity on the whole genome). Thus, close or identical replication sites in infected cells may increase the chance of homologous recombination, producing viable chimeric RNAs. Since potential recombination events between low titer virus RNAs in infected grapevines, studied by nested RT-PCR, are difficult to detect, an accurate analysis of virus replication sites is needed. Fluorescent in situ hybridization was designed with oligo-labelled DNA or PCR-amplified fragments of CP genes with tetramethyl-rhodamine or Alexa Fluor-488 dUTP (Invitrogen), for GVA and GVB, respectively. This system was chosen because post-hybridization processing like use of antibodies or RNase A incubation was not needed. The method was first validated on single infections by GVA in *Nicotiana benthamiana* and GVB in *N. occidentalis*. Hybridization was successfully obtained at 65°C in 50% formamid, after a pretreatment of sectioned tissues with proteinase K. In infected tobacco, viral RNAs were clearly identified as fluorescent spots on the plasma membranes. Comparable results were obtained when in vitro-grown shoots from two double-infected grapevines, were hybridized. In this case, however, the intrinsic fluorescence of polyphenols interfered with a clear-cut detection of viral RNAs.

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**RESIDUE LEVELS AND EFFECTIVENESS OF PYRIMETHANIL AND TRIFOXYSTROBIN VERSUS IMAZALIL AFTER POSTHARVEST DIP TREATMENTS AT 20 AND 50°C TO CONTROL PENICILLIUM SPP. ON CITRUS FRUIT.** M. Schirra1, S. D’Aquino1, A. Palma2, A. Angioni2, P. Cabras2, S. Giobbe1 And Q. Miglieli3. 1C.N.R. Istituto di Scienze delle Produzioni Alimentari, Sezione di Sassari, Via dei Mille 4, 07100 Sassari, Italy. 2Department of Tossicologia, Università di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy. 3Department of Plant Protection and Center for Biotechnology Development and Biodiversity Research, University of Sassari, Via E. De Nicolai 9, 07100 Sassari, Italy. E-mail: maria.schirra@ispa.cnr.it

The effectiveness of postharvest dip treatments at 20 or at 50°C with pyrimethanil (PYR) and with the quinone outside inhibitor (QoI) fungicide trifloxystrobin (TFX) in controlling green and blue mold (incited by *Penicillium digitatum* and *P. italicum*, respectively) on different citrus fruit was investigated in comparison with the standard fungicide imazalil (IMZ). Fungicide residues retained by fruit were determined as a function of treatment time, dip temperature and fruit storage conditions. Residues of PYR, TFX, and IMZ were significantly correlated with dip temperature and described a non-linear expression that was sigmoidal in trend. A 3-min dip treatment at 50°C produced a deposition of fungicide ca 2-fold higher than when treatments were carried out at 20°C. Results obtained on wounded, non inoculated ‘Miho’ satsumas revealed that when treatments were performed at 50°C, the PYR or IMZ concentration needed to achieve complete control of decay were 8- and 16-fold less than by treating at 20°C. The lowest concentration of PYR, TFX, or IMZ required to achieve almost total protection of fruit against decay in artificially wounded ‘Di Massa’ lemons, ‘Tarocco’ oranges and ‘Monreal’ clementines during seven days of storage at 20°C was 100 mg l-1 at 50°C and 400 mg l-1 at 20°C, respectively. When fruits were inoculated with either *P. digitatum* or *P. italicum*, application of 400 mg l-1 of either fungicide at 20°C or 100 mg l-1 at 50°C similarly reduced green and blue mold development. These results were confirmed on non-wounded oranges cv. Tarocco and grapefruits cvs. Marsh Seedless and Star Ruby during 3 weeks of simulated quarantine conditions at 1°C, plus standard storage (5 weeks at 8°C, oranges or 8 weeks at 11°C, grapefruits) and an additional week of simulated marketing conditions (SMP) at 20°C. Noteworthy, while PYR was effective at both tested temperatures, TFX could explicate its curative activity when applied at 50°C but not at 20°C. Treatments did not affect fruit external appearance, flavour and taste. It is concluded that postharvest treatment with PYR or with TFX represents an effective option to control green and blue mold in citrus fruit, and that integrating fungicide application and hot water dip may
reduce the possibility of selecting fungicide-resistant populations of the pathogen, as a consequence of the increased effectiveness of the treatment at 50°C.

A BACTERIAL DISEASE CAUSED BY XANTHOMONAS SP. ON LAVANDA OFFICINALIS. L. Sigillo1, T. Iorio1, F. Campanile2, R. Bravi1, R. Zottola3 and M. Zaccardelli2. 1Ente Nazionale delle Sementi Elette, SS 18, Km 77,700, 84091 Battipaglia (SA), Italy. 2CRA, Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091 Battipaglia (SA), Italy. 3Azienda Agricola Giandomenico Consolato, Via Lago Lucrino, 84098 Pontecagnano (SA), Italy.  E-mail: ense-battipaglia@ense.it

In spring 2005, a disease of lavender (Lavanda officinalis), a perennial plant grown for its aromatic and officinal properties, was observed in a greenhouse in Southern Italy. Diseased plants showed brown spot of the leaves mainly in their basal part, which heavily compromises the quality and marketability of the plants. Bacterial strains were consistently isolated from symptomatic tissues on selective and generic media. The colonies were yellow, mucoid, produced a brown pigment on YDCA and YPGA and were identified as Xanthomonas sp. by morphological and biochemical methods. Bacterial isolates gave a hypersensitivity reaction when infiltrated on bean pods and were non pathogenic when inoculated to cauliflower and a number of Lamiales. They did not hydrolyse starch, produced acids from arabinose, utilised glycerol and did not grow on SX medium. The isolates were not identified at a significant level by BIOLOG analyses. Amplicons of 1.4 kb were obtained from the Hrp region using the primers DLH109/DLH112 designed on Xanthomonas campestris 16S rDNA sequence but no amplicons were obtained using the Hrp-C/D and Hrp-B related primers designed on the comparable sequence of X. campestris pv. vesicatoria. By sequence homology analysis of 16S rDNA, a 100% homology was observed with the sequence of several pathovars of X. campestris, especially X. campestris pv. zanthedeschiae and X. campestris pv. campestris, and with X. axonopodis pv. dieffenbachiae and X. vesicatoria. All biochemical tests distinguished the lavender isolates from X. campestris pv. campestris but not from X. vesicatoria. Pathogenicity tests with different isolates are in progress on pepper, tomato and other host plants.

INTEGRATED USE OF TRICHOSTERMA HARZIANUM STRAINS T22 OR T39 AND CELL WALL DEGRADING ENZYMES TO CONTROL PLANT DISEASES. I. Soriente1, S. Ferrari2, S.L. Woo1, R. Gliiento1, R. Marra1, F. Vinale1, P. Ambrosino1, M. Ruocco2, D. Turrà1, S. Lanzuise1, F. Scala1-2 and M. Lorito1-2. 1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy. 2Istituto per la Protezione delle Piante (IPP), CNR, 80055 Portici (NA), Italy.  E-mail: lorito@unina.it

Fungi of the genus Trichoderma are effective against numerous plant pathogens and their biocontrol activity depends on different factors, including production of cell wall degrading enzymes (CWDEs). We tested the efficacy as antifungal agents of different CWDE mixtures produced in various conditions by the commercially used strains T22 and T39 of T harzianum, selected because of their high antagonistic activity against pathogens. The most powerful CWDE mixtures were obtained by growing these fungi in a salt medium containing Botrytis cinerea cell walls (1%). The culture filtrates were concentrated, dialyzed (cut-off 3500 Da) and biochemically characterized. Culture and medium conditions were optimised in order to obtain metabolite mixtures with the highest enzyme (endo- and exo-chitinase, glucanase, protease) and inhibitory activity. Antimicrobial effect of the CWDE mixtures were also tested in combination with spore applications of T22 and T39 in bioassays on tomato and lettuce leaves. The enzyme solutions sprayed or applied as a drop on leaves together with Trichoderma and B. cinerea spores significantly reduced the percentage and area of lesions of developing disease on the leaves. Seed-coating treatment with CWDEs + Trichoderma spores increased the percentage of seed germination in Rhizoctonia solani- infested soil as compared with controls. Tests with CWDEs mixtures used in combination with some of the most commonly used fungicides are ongoing.

GENETIC DIVERSITY OF CRYPHONECTRIA PARASITICA POPULATION IN SICILY. D. Spica1, G. Sammarco1, S.O. Cacciola1 and P. Cortesi2. 1Dipartimento di Scienze Entomologiche, Fitopatologiche, Agrobiologiche, Università degli Studi di Palermo, Viale delle Scienze, 2 90128 Palermo, Italy. 2Istituto di Plant Pathology, State University of Milan, Via Celoria 2, 20133 Milan, Italy. E-mail: mirmospica@libero.it

The chestnut blight is the most serious and widespread fungal disease of chestnut in Sicily, where it endangers trees survival in parks, orchards and forests. Biological control of the disease with hypoviruses infecting the chestnut blight fungus, Cryphonectria parasitica, proved to be effective. However, the release of hypovirulent isolates must be supported with data concerning the genetic diversity of the fungal population. The objectives of this study were to determine: (i) the multilocus population structure of C. parasitica in Sicily; (ii) the diversity of vegetative compatibility types; and (iii) the incidence of CHV-1 virus infected isolates within this population, as potential biological control agent. More than 220 single-conidial isolates were obtained from bark samples collected in 20 localities within three provinces: Catania, Messina and Palermo. CHV-1 infected isolates were 8 out of 20
subpopulations. Two vc types were found in Sicily, EU2 and EU12. EU12 isolates were the most frequent in 18 subpopulations, whereas they were absent in two small subpopulations in the province of Messina. Molecular analysis of the MAT alleles frequency, assessed by multiplex PCR, showed that isolates of both mating types are present in Sicily, however MAT2-2 isolates were found exclusively in two populations in the province of Messina. Multilocus analysis of population structure is not consistent with the hypothesis of random mating and a few subpopulations were genetically differentiated. These results indicate that biological control with hypovirulent isolates must be carefully planned to prevent a possible increase of genotypic diversity of C. parasitica.

MOLECULAR VARIABILITY OF A POPULATION OF FUSARIUM POAE, THE MAIN CONTAMINANT OF WHEAT IN NORTHERN-ITALY. S. Somma1, A. Moretti1, C. Alvarez2 and A. Susca1. 1ISPA, CNR, Via Amendola 122/0, 70126 Bari, Italy. 2De- partment of Biodiversidad y Biologia Experimental, Universidad de Buenos Aires, Buenos Aires, Argentina. E-mail: antonio.moret- ti@ispa.cnr.it

Fusarium head blight is caused by a complex of Fusarium species some of which produce harmful metabolites for plants, humans and animals. A survey of wheat fields in the Ferrara area (Northern Italy) at pre-harvest time of the last season, led to the collection of 40 ear samples of wheat each from a single field.KERNELS, analyzed for the main occurring Fusarium species, resulted highly contaminated, predominantly by F. poae (from 1% to 34% of contaminated kernels), a toxigenic species able to produce several trichothecenes, which are potent inhibitors of protein synthesis. Around 100 strains of F. poae were isolated and morphologically identified, showing a high level of variability. Molecular studies were carried out in order to confirm such variability by using DNA analysis: AFLP and β-tubulin and elongation factor loci sequencing were made confirming a high level of variability within this species. Studies to relate this variability to other traits such as toxin production or pathogenicity are in progress.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN ETROG CITRON IN RESPONSE TO CITRUS VIROID III INFECTION. M. Tessitori1, V. Cargina1c, G. Maria1, S. Rizza1, G. Catar1, C. Capasso1, A. Capasso1 and A. Catar1.1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. 2Istituto della Biochimica delle Proteine, CNR, Via P. Castellino 111, 80131 Napoli, Italy. 1Parco Scientifico e Tecnologico della Sicilia, Blocco Palma I, Z. I., 95131 Catania, Italy. E-mail: mtessitori@unict.it

Citrus trees are natural hosts of several viroids responsible for different specific symptoms as stuntting and leaf epinasty in some indicator hosts. The relationhip of metabolic and structural changes induced by viroidal infection is little investigated, but it was demonstrated that the host response can be specific at the gene expression level. Citrus viroid III (CVd-III) can reduce the growth rate of some rootstocks, without inducing apparent disease. Therefore, its use in commercial groves has a potential economical value. In this study, differential display reverse transcription-PCR (DDRT-PCR) was used to detect differentially expressed genes in Etrog citron (Arizona 861-S1) leaves showing symptoms of mild leaf bending, from plants grafted on sour orange rootstock, as result of inoculation of an isolate of C. viroid IIIb (CMC-CVd-IIIb). Young, almost fully expanded leaves, at an early stage of symptoms display, were collected and processed with standard procedures in comparison with symptomless non inoculated controls. The technique was able to identify eighteen genes based on their amino acid sequences, two of which coded for proteins with unknown function. Thirteen genes were up-regulated while five were down-regulated in response to infection. The identified genes were mainly involved in plant defence/stress response, signal transduction, amino acid transport, and cell wall structure. Among the up-regulated genes, of particular interest appears to be a regulator of host RNA silencing reported as involved in viroid and RNA virus pathogenicity.

MOLECULAR DETECTION OF CAPER VIRUSES. L. Tomassoli and A. Tiberini. Istituto Sperimentale per la Patologia Vegetale, Sezione di Virologia, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: l.tomassoli@ipave.it

Caper (Capparis spinosa) cultivated in the minor Sicilian islands (Italy) is suffering a severe and progressive decline caused by pests and viruses. Three viruses have been reported from this host, i.e. Caper latent virus (CapLV, genus Carlavirus), Eggplant mottled dwarf virus (EMDV, genus Nucleorhabdoviruses) and Cucumber mosaic virus (CMV, genus Carlaviruses). Molecular diagnostic techniques can be usefully applied for virus detection in view of an effective strategy for the control of infections and the production of virus-free plants of caper. Specific RT-PCR methods were therefore developed for CapLV and PVYV detection in naturally infected plants. In particular: (i) one-step RT-PCR allowed the identification of CapLV in total RNA extracts from immature buds, leaves and fruits of capers growing in different isolated Sicilian areas. The amplification of a 730 bp product was obtained using a primer set designed in a ORF1 region encoding a polypeptide that shares a high degree of similarity with other carlaviruses; (ii) RT-PCR amplification of an EMDV sequence was obtained from total RNA extracted from capers showing typical leaf vein yellowing symptoms, using degenerate primers designed on the L-protein gene after the alignment of the corresponding genomic region of four different nucleorhabdoviruses. The molecular analysis of the RT-PCR amplicon confirmed its high level of similarity with a nucleorhabdovirus sequence. Specific primer sets, used in one-step RT-PCR assays, allowed the amplification of a product with predicted size of 358 bp in all plants showing EMDV symptoms.

PRODUCTION OF SELENATE-RESISTANT MUTANTS OF WILD TYPE AND GFP TRANSFORMED TRICHODERMA VIRENS ISOLATES AND THEIR EXPLOITATION IN VEGETATIVE COMPATIBILITY TEST. G. Vannacci1, C. Cristiani2, P. Kaisaris1 and S. Sarrocco1. 1 Dipartimento di Coltivazione e Difesa delle Specie Legnose “Giovanni Scaramuzza”, Sezione Patologia Vegetale, Via del Borghetto 80, 56124 Pisa, Italy. 2 Dipartimento di Biologia delle Piante Agrarie, Via delle Pugge 23, 56100 Pisa, Italy. E-mail: g.vannacci@age.unipi.it

The genus Trichoderma comprises a large set of asexual fungal strains, which are heterogeneous in genome structure and behaviour. Despite the importance of the genus, there are still many problems concerning taxonomical classification of its constituent species. Several attempts have been made to use molecular techniques for Trichoderma spp., flourishing the microscopic approach on which Rifai’s classification is based. By contrast, very little information is still available on the interactions or hyphal fusion be-
between different isolates, similar to those described for vegetative compatibility (VC) in other fungi. VC has been profusely studied in many fungal genera using auxotrophic mutants, frequently related to nitrogen and sulphur cycle such as nit and sul mutants, respectively. In this work, a wild type and some green fluorescent protein (GFP) transformants of a T. virens isolate (strain 110) were investigated for the ability to grow in the presence of different concentration of potassium-chlorate and sodium-selenate. Despite the use of increasing concentration of chlorate, up to crystallization of the salt in the growing medium, no sensitivity towards this compound was detected, resulting in no production of nit mutants. Instead, several potential sul mutants, both from the wild type and the GFP strains, were obtained and partially characterized. Mutants were submitted to complementation tests in order to detect intra-specific complementation.

CHARACTERIZATION OF THE GREEN COMPOST FUNGAL COMMUNITY. Vettraino1 A.M., S. Franceschini1, F. De Cesare2, A. Vannini3, 1Dipartimento di Protezione delle Piane, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. 2Dipartimento Agrobiologia e Agrochimica, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. 3Dipartimento di Patologia Vegetale, Università degli Studi di Milano and Istituto di Virologia Vegetale, CNR, Via Celoria 2, 20133 Milano, Italy. Email: vannini@unitus.it

Among the residential community of microorganisms of composts, fungi play an important role in the decomposition of many complex plant polymers. Source materials determine differences in the microbial community composition of mature compost. Monitoring of the residual fungal community is needed to detect species hazardous to humans, animals and plants and to define potential applications. In this study the fungal composition of mature green compost is reported. Samples were randomly collected every 2 months from the same compost. The diversity in the fungal microbial communities was assessed by plating compost dilutions on eight different media, among which PARP, NP10 and SFA, semi-selective for Phytophthora spp., Verticillium spp. and Fusarium spp., respectively. Traits, such as colony type on PDA at different concentration, production of pigments, and morphology of vegetative and reproductive structures were used to group the isolates in morphotypes. ITS sequences of two isolates for each morphotype were compared with molecular data from the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). A total of 35 morphotypes were detected. Statistic index of richness, evenness and diversity of the fungal community was used to assess the biodiversity of compost samples. The relationships among fungal community composition and enzyme activities (phosphatase, β-D-glucosidase, sulphatase, protease) were also analyzed.

PHYTOPHTHORA SPP. ASSOCIATED TO AGRO-FOREST ECOSYSTEMS IN WESTERN HYMALAYA. A.M. Vettraino1, A. Brown2, C. Brasier2, E. Patrizi1 and A. Vannini1. 1Dipartimento di Protezione delle Piane, Università degli Studi della Tuscia, Via Camillo de Lellis, 01100 Viterbo, Italy. 2Forest Research Alice Holt Lodge, Farnham Surrey, UK. Email: vannini@unitus.it

Phytophthora species are pathogens to most economically important crops grown throughout the world. Probably they originated in Asia and were transferred to the rest of the world through human activities. To investigate whether these pathogens might be present in Himalayan forest ecosystems an expedition was organised in October 2005 in Western Nepal. An intensive soil sample survey was conducted during a round trip from Kolti (1396 mt asl) to Rara Lake (3500 mt asl). The geographic co-ordinates of the samples were recorded by GPS and used to construct a distribution map. A total of 47 soil samples were collected in the rhizosphere of 17 target broadleaved and coniferous trees and shrubs in three distinct ecological zones: temperate forest, sub-tropical forest, and sub-tropical agriculture. Samples were baited and the bait material plated onto a selective medium. Resulting colonies were identified on the basis of their morphological, behavioural and molecular traits. A total of 89 Phytophthora isolates were obtained belonging to three Phytophthora species. P. citricola was the most frequently isolated species, and was confined to the soil around temperate forest trees including Acer, Aesculus, Juglans, Ulmus and Viburnum. A distinct species of Phytophthora, with some similarities to P. meadii was associated with the sub-tropical forest vegetation including Lithocarpus, Cupressus, Cornus, Carpinus, and Castanopsis. This may be a previously unreported species. A third species, P. palmivora, was only found in the sub-tropical agricultural zone around Ficus, Persea, and Olea. The distinct zoning of the two possibly endemic forest Phytophthoras, P. citricola and the P. meadii-like Phytophthora, may lead to further understanding of the role of the genus in forest ecosystems. Whether the P. meadii-like Phytophthora may present a threat to other forest ecosystems has yet to be determined. The discovery of P. palmivora in association with native Olea trees in Nepal may have significance for proposed commercial cultivation of Olea species in this region and elsewhere.

TRANSMISSION OF GRAPEVINE LEAFROLL ASSOCIATED VIRUS 3 BY THE SOFT SCALE INSECT NEOPULVINARIA INNUMERABILIS. A. Zorloni, S.Prati, S.Chiesa, P. A. Bianco. Istituto di Patologia Vegetale, Università degli Studi di Milano and Istituto di Virologia Vegetale, CNR, Via Celoria 2, 20133 Milano, Italy. E-mail: pietro.bianco@unitn.it

The presence in Italy of the North American soft scale insect Neopulvinaria innumerabilis Rathvon was first reported in 1975, in vineyards of Veneto. Today, N. innumerabilis is present in most viticultural areas of Northern Italy, where high populations of this insect are often observed. N. innumerabilis is responsible for serious damages to vines encompassing reduction of fertility and vigour. Moreover, this insect is known as a vector of the ampelovirus Grapevine leafroll- associated irus 1 (GLRaV-1) one of the agents of grapevine leafroll disease (LR), and of the vitivirus Grapevine virus A (GVA), agent of Kofer stem grooving syndrome from the rugose wood complex. In this work, we report the results of experimental transmission trials conducted with individuals of N. innumerabilis collected in a vineyard where LR is widespread. Adult females were allowed to feed on Grapevine leafroll-associated virus 3 (GLRaV-3)-infected Vitis vinifera rooter cuttings, used as sources of inoculum and young instars were left to feed on the same rootlings for 2 weeks (acquisition access period, AAP), prior to transferring to healthy seedlings of cv. Riesling Italico. Each vine used as test plant was analysed by ELISA every three months after inoculation. GLRaV-3 was transmitted to 2 out of 13 inoculated test plants. This is the first report of the capacity of the scale insect N. innumerabilis to transmit the ampelovirus GLRaV-3 to grapevine.
EFFECT OF COMPOST AND NITROGEN FERTILIZERS ON THE DAMAGE OF CERECOSPORA BRASSICICOLA TO BRASSICA RAPA var. ESCULENTA. M. Zaccardelli, D. Perrone, A. Del Galdo and I. Giordano. CRA, Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091 Battipaglia (SA), Italy. E-mail: m.zaccardelli@tiscali.it

The effect of compost and nitrogen fertilizers on the damage of Cercospora brassicicola to Brassica rapa var. esculenta, ecotype Novantina, was evaluated in a field located in the Sele valley (Southern Italy). At harvesting (January 2006) a damage severity index with an empirical scale from 1 (no damages) to 5 (highest damages) was applied to plants grown in plots manured with compost at three different doses (15, 30 and 45 t ha\(^{-1}\) of dry compost), or treated with chemical fertilizers only, or manured with compost at three different doses (15, 30 and 45 t ha\(^{-1}\) of dry compost plus nitrogen fertilizers at a dose equal to 25\% or 50\% of the highest damages were observed in the plots treated with the highest dose of compost (mean 3.0), in those treated with chemical fertilizers only (mean 2.5) or with the lowest dose of compost plus 50\% nitrogen fertilizer (mean 2.5). Conversely, the highest damages were observed in plots treated with 15 or 30 t ha\(^{-1}\) of dry compost (mean 3.5). These results suggest that a sufficient availability of nitrogen, assured by chemical fertilizers or by very high dose of compost, is required to reduce damages of C. brassicicola to B. rapa var. esculenta.

SUSCEPTIBILITY OF CAULIFLOWER CULTIVARS TO XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS IN THE FIELD. M. Zaccardelli, D. Perrone, A. Del Galdo and F. Campanile. CRA, Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091 Battipaglia (SA), Italy. E-mail: m.zaccardelli@tiscali.it

Xanthomonas campestris pv. campestris (Pammel) Dowson (Xcc), is the causal agent of black rot or vascular blackening of crucifers. During the autumn-winter 2005/2006, symptoms of marginal chlorosis of the leaves and blackening of vascular tissue appeared in an experimental field located in the Sele valley (Southern Italy) cropped with 38 different cultivars of crucifers. Using an empirical severity scale with values from one (no symptoms) to seven (highest damages), the damages were evaluated on all cultivars. The highest severity indexes were observed in the cvs Belot (mean 6.7), Lepini (mean 5.3) and Utopia (mean 5.3) whereas the lowest severity indexes were observed in cvs Rafale (mean 1.7), Pamyros (mean 2.0), Magnifico (mean 2.0), Galileo (mean 2.0), CLX 316 (mean 2.0), CaRon (mean 2.3), and Amistad (mean 2.3). Bacterial colonies isolated from leaf lesions were identified as Xcc using a specific PCR protocol with pathovar-specific primers designed on the brcc gene of the HrpXcc pathogenicity island.

CHARACTERIZATION FOR ANTIBIOSIS ACTIVITY OF SPORE-FORMING BACTERIA ISOLATED FROM COMPOST. M. Zaccardelli, A. Malzone, F. Campanile and F. De Nicola. CRA, Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091 Battipaglia (SA), Italy. E-mail: m.zaccardelli@tiscali.it

Organic matter influences positively the physical and chemical characteristics of the soils. Application of compost generally increases biological activity and confers suppressive property against soil-borne plant pathogens. In this study, the spore-forming bacteria contained in a compost obtained from the organic fraction of municipal solid wastes (OFMSW) were characterized for their antibiosis activity against phytopathogenic fungi. Three compost samples were washed in a buffer and an aliquot of the washing was plated after a thermal treatment. After incubation, sixteen isolates were collected from the ten-fold dilution used to calculate the concentration of spore-forming bacteria in the compost. All the isolates collected were tested, in vitro, for antibiosis activity against Rhizoctonia solani, Pyrenochaeta lycopersici, Fusarium solani, Fusarium oxysporum and Sclerotinia sp. The concentration of the spore-forming bacteria in the compost was 3589 ± 1097 x 10³ UFC g\(^{-1}\) of dry weight and 94\% of the isolates were able to control, in vitro, at least one of the fungi tested; 31\% of the isolates were able to inhibit three species of fungi at the same time; Fusarium solani and Pyrenochaeta lycopersici were inhibited by the highest number of isolates. The fifteen isolate that showed antibiosis activity in vitro were characterized for DNA polymorphism of ITS between the 16S and 23S rRNA gene: eight haplotypes were obtained. These results suggest that compost from OFMSW is a major source of spore-forming bacteria potentially able to control soil-borne pathogens. Tests in vitro using the strains isolated from compost are in progress.

INFECTIONS OF XANTHOMONAS CAMPESTRIS pv. CAMPESTRIS ON CRAMBE ABYSSINICA IN ITALY. M. Zaccardelli, A. Del Galdo and F. Campanile. C.R.A., Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091 Battipaglia (SA), Italy. E-mail: m.zaccardelli@tiscali.it

Xanthomonas campestris pv. campestris (Pammel) Dowson (Xcc) is the causal agent of black rot or vascular blackening of crucifers. Crambe abyssinica is a crucifer used to produce oil, extracted from the seeds, with a high level of erucic acid. The oil with this characteristic is a good lubricant and is also used as an additive in plastic films or for making fibres. Moreover, C. abyssinica shows a great potential for use in phytoremediation of arsenic. During the spring of 2000, symptoms of marginal chlorosis of the leaves and blackening of the vascular tissue appeared in an experimental field located in the Sele valley (Southern Italy). Isolation on YDC-agar showed mucoid and yellow-orange bacterial colonies that induced symptoms on crambe and cauliflower plantlets following inoculation. Using a specific PCR protocol with pathovar-specific primers designed on the brcc gene of the HrpXcc pathogenicity island, the colonies were identified as Xcc. This is the first report of infection of Xcc on Crambe abyssinica in Italy.

SEQUENCE ANALYSIS OF THE VIRULENCE GENE AVR-RPT2 IN ISOLATES OF PSEUDOMONAS SYRINGAE pv. TOMATO AND PSEUDOMONAS SYRINGAE pv. SYRINGAE. M. Zaccardelli, F. Campanile and B. A. Vinatzer. 'C.R.A., Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091 Battipaglia (SA), Italy. 2Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Franklin Biotechnology Center, Blacksburg, USA. E-mail: m.zaccardelli@tiscali.it

avrRpt2 is an avirulence gene that encodes an effector protein secreted by the type III secretion system into host plant cells. It has been demonstrated that avrRpt2 contributes to virulence of Pseudomonas syringae strains inoculated to Arabidopsis thaliana and Lycopersicon esculentum. It has also been shown that avrRpt2 in the P. syringae pv. tomato (Pst) strain JL1065 is located in an integrative conjugative genomic island between a DNA helicase gene and the pilF gene. AvrRpt2 is nearly omnipresent in P. syringae populations. In fact, in our previous studies, this effector gene was amplified from seventy strains of Pst and from twenty-
three strains of *P. syringae* pv. *syringae* (Pss) isolated from different hosts as pepper, pea, bean, sugar beet, lemon, plum, pear, almond, and laurel. The objective of this study was the evaluation of the variability at the *avrRpt2* locus in nine *Pst* and seven *Pss* isolates using PCR with primers designed directly on *avrRpt2* and on the genes that flank *avrRpt2* in *Pst* JL1065. PCR products were sequenced and compared to each other. We found that all, except one, of the analyzed *Pst* strains contain *avrRpt2* in the same locus as *Pst* JL1065, while none of the *Pss* strains contains *avrRpt2* in that locus. Implications of our findings regarding the evolution and the mechanisms of virulence in *P. syringae* are discussed.

**POTENTIAL USE OF ESSENTIAL OIL, EXTRACTED FROM OFFICINAL PLANTS CULTIVATED IN MEDITERRANEAN AREA, TO CONTROL PHYTOPATHOGENIC BACTERIA AND FUNGI.** M. Zaccardelli¹, E. Mancini², F. De Nicola¹, F. Campanile¹, E. De Feo², E. De Falco². ¹C.R.A., Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091 Battipaglia (SA), Italy. ²Facoltà di Farmacia, Università degli Studi di Salerno, 84084 Fisciano (SA), Italy. E-mail: m.zaccardelli@tiscali.it

A lot of plants are cultivated for their aromatic and officinal properties. The interest for these plants is increasing not only in medicine but also in agriculture, for the production of natural eco-compatible substances able to control dangerous biotic agents. In this study, essential oil extracted by distillation in vapour flow from the officinal plants *Carum carvi* L., *Foeniculum vulgare* Mill., *Hyssopus officinalis* L., *Lavandula angustifolia* Mill., *Majorana hortensis* L., *Melissa officinalis* L., *Ocimum basilicum* L., *Origanum vulgare* L., *Pimpinella anisum* L., *Salvia officinalis* L., *Thymus vulgaris* L. and *Verbena officinalis* L., were tested for their ability to control, in vitro, phytopathogenic bacteria and fungi like *Erwinia carotovora*, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *campestris*, *Alternaria* sp., *Botrytis* sp., *Fusarium oxysporum*, *F. sambucinum*, *F. solani*, *Sclerotinia* sp. At 1% concentration, the essential oils extracted from melissa, thyme and verbena were active against all the pathogens tested. Other than melissa, thyme and verbena, only the essential oil extracted from oregano was active against *F. solani*. All the essential oil tested were active against all the phytopathogenic bacteria used in this study.

**SUSCEPTIBILITY OF PEACH FRUITS TO MONILINIA LAXA AND GENE EXPRESSION.** P. Zubini and E. Baraldi. Dipartimento di Protezione Vegetale, Università di Bologna, Facoltà di Agraria, Via Fanin 46, 40127 Bologna, Italy. E-mail: elena.baraldi@unibo.it

Starting four weeks after full bloom until commercial maturity, peach fruits were harvested weekly and assayed for susceptibility to *Monilinia* rot. Susceptibility strongly decreased during the S2 phase in a two weeks time, in correspondence to the pit hardening stage and to accumulation of aromatic compounds. Afterwards, susceptibility increased again until full ripeness. RNA was extracted from the peel of inoculated and non-inoculated fruits and used for studying gene expression alteration during this stage (a 7-weeks period fully comprising the pit hardening). RT-and Real Time PCR were used to monitor alteration in expression of genes encoding enzymes of the phenylpropanoid metabolism, such as phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), cinnamate 4-hydroxylase (C4H), leucoanthocyanidine reductase (LAR), hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase (HQT), and enzymes involved in plant defensive processes, such as lipoxygenase (LOX) and 1-3glucanase (β-gluc). Gene expression profiles of most of these enzymes showed clear alteration during the pit hardening stage.